Effect of osmotic and salt stresses on germination and phenolic compounds of Eruca vesicaria subs. sativa L.

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Research Article

Keywords: Germination, NaCl, PEG6000, Phenolic compounds, Rocket

Posted Date: November 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2256129/v1

Abstract

Rocket is a plant well adapted to the different climates in Algeria. It is known for its ability to grow in marginal conditions. It produces protein- and oil-rich seeds as well as edible leaves. Rocket and other members of the Brassicaceae plant family have been shown to contribute beneficial, health promoting phytochemicals to the human diet. However, little is known about its physiological response to water and salt stress. In this study, we sought to investigate the osmotic and salt stress effect on rocket seeds at the germination level and monitor phenolic compounds accumulation during this process. Our findings show that at an osmotic potential of -0.75 MPa, 33.33% of the seeds exposed to polyethylene glycol germinated which suggests that rocket seeds displayed tolerance to water stress. When decreasing the osmotic potential to -1.44 MPa of sodium chloride, the seed germination rate decreased to 21.33% which indicates that rocket seeds were resistant to salt stress. Quantitative analysis of seed's phenolic compounds showed an increase in polyphenols, flavonoids and anthocyanins content. As expected, the biggest increases were observedin the highest levels of osmotic and salt stress exposures. This accumulation during germination could explain rocket seed's ability to resist drought and salinity.

Introduction

Drought and salinity are amongst the most critical environmental factors limiting plant development (Bouda and Haddiouib 2010). These environmental conditions affect seed germination, plant growth, productivity, ecology, and biodiversity. In Algeria, 95% of the territory is in arid and semi-arid state (Halitim 1988). Moreover, 7.5 million hectares of land are threatened by desertification and can result in the loss of agricultural land (Mate 2002). Soil salinity is especially pronounced in arid and semi-arid regions and threatens about 3.2 million hectares of Algerian arable land (Benmahioul et al. 2009). To improve vegetation cover, it is important to choose plants that are drought and/or salt tolerant. Furthermore, desert plant regeneration must be addressed by improving seed germination, which may be an alternative to revegetating these environments (Bakhshandeh et al. 2013).

The most critical stage in the plant life cycle is germination (Bakhshandeh et al. 2013). This process is the basis of plant success and development, it determines uniformity and crop stand density. The success of seed germination and establishment of a normal seedling are key determinants of plant species propagation, specifically for annual plant growing in arid and semi-arid regions (El Hamdaoui et al. 2021). Germination is the most sensitive stage to abiotic stress (Patade et al. 2011) and environmental stressors such as salinity and drought can have a deleterious effect on this physiological stage (Mohammadizad et al. 2013). Salinity and drought can highly influence the germination and seedling growth of many plant species with non-dormant seeds (Nosratti et al. 2017; Hosseini Sanehkoori et al. 2021). Water and salt stress can reduce germination by limiting seeds water uptake, impairing mobilization of storage reserves, affecting protein synthesis and structural organization of germinating embryos (Aslam et al. 2006).

The study of germination requirements of species and their response to environmental and physical changes can help further our understanding of these species' adaptation strategies and their propagation under extreme environmental stress (Tetsuto and Michio 2011). Seed germination is an important indicator of revegetation success, as it is the initial driver of plant establishment and plant community dynamics (Abbad et al. 2004).

From a physiological standpoint, seed germination stage is host to several biochemical changes. Notably, there is an increase in the accumulation of specific secondary metabolites such as phenylpropanoids. Phenylpropanoids and specifically phenolic compounds participate in many physiological processes such as cell growth, rhizogenesis, seed germination, and fruit maturation (Tanase et al. 2019). These compounds also play a role in the epicotyl elongation during germination and are known antioxidants that help fight abiotic stressors (Tarasevičienė et al. 2019).

Eruca vesicaria ssp. sativa (Rocket) is native of southern Europe and western Asia (Bell and Wagstaff 2014). Rocket leaves are used as salad greens and are known for their unique pungent, spicy taste and contain putative health-promoting compounds such as glucosinolates (Bell et al. 2017; Cocetta et al. 2018). In traditional medicine, the plant is known for its circulatory, diuretic, digestive, tonic, laxative and anti-inflammatory properties (Fuentes et al. 2014). Rocket is a good source of vitamin C and A, carotenoids, polyphenols, and flavonoids (Michael et al. 2011). It grows spontaneously in arid and semi-arid bioclimates which is prevalent in the Algerian Sahara Desert

There is a limited body of literature related to rocket plants and little understanding of their physiology and mechanisms of adaptation to various environmental stressors.

In the work presented herein, we sought to investigate the effect of polyethylene glycol (PEG) and sodium chloride (NaCl) exposure on the germination process of arugula seeds and evaluate the ability of the specie to resist dehydration and salinity. Phenolic compounds in germinated seeds were also quantified under various salinity and drought conditions to better understand their role during the germination stage.

Results

Osmotic stress effects on germination kinetic of Eruca vesicaria ssp. sativa seeds

Figure 1 summarizes the effect of various PEG concentrations on the germination rate's evolution over time. A quick review of the germination curves allows to clearly delineate three phases:

A latency phase which is necessary for the emergence of the first germinations and during which the germination rate is low. The duration of this phase was variable according to the osmotic potential. It was short (6 to 9 hours) for osmotic potentials below or equal to -0.14 MPa, and started to get longer (up to 12 hours) for osmotic potentials higher than -0.14 MPa

An exponential phase, which corresponds to a rapid increase in germination rate. This phase was observed for seeds subjected to a potential of -0.46 MPa or lower. When the osmotic potential decreased,

this period became shorter and disappeared from -0.7 MPa.

A linear phase, which provides the final percentage of germination and indicates the maximum germination capacity. Control seeds and seeds exposed to osmotic potentials of -0.40, -0.14, -0.29, -0.46 MPa had the highest germination rate (about 100 percent). For these osmotic potentials, the amount of free water in the soaking solution is sufficient for optimal seed germination. Water availability decreased at -0.70 MPa and as a result, germination rate decreased to 92.66±3.05% (reduction of 7.34%). At -0.75 MPa germination rate decreased even more to 33.33±1.77 % (reduction of 67.66%). At -0.80 and -0.86 MPa, the germination rates decreased significantly to respectively 4.00 ± 2.33% and 1.33 ± 0.00%, a drop of 96% and 98.67% ($p < 0.001$). Below -0.86 MPa, germination was inhibited because water, a crucial element for germination, did not diffuse to the intra cellular medium. When water potential of the germination medium decreases, water availability to the seeds is lowered and these results in slow and inefficient germination and a drop in the maximum germination rate (Figure 2).

The slowing of germination rate is accompanied with a decrease in speed of germination (expressed by the time at 50 percent germination, $T_{50\%}$).$T_{50\%}$ increased with a decrease in PEG_{6000} induced osmotic potential. The same germination speed was observed up to -0.29 MPa. At -0.75 MPa, the $T_{50\%}$ for stressed seeds was 50% higher than for control seeds ($p<0.001$). Above -0.75 MPa, 50% germination was not reached (Table 1).

The mean germination time (MGT) follows a similar pattern as the $T_{50\%}$ and increases significantly in stressed seeds. At -0.86 MPa, the MGT of the stressed seeds was 86.49% higher than the MGT of the unstressed seeds. Regarding the mean Germination per hour (MGH), the osmotic stress had no effect on stressed seed's MGH up to -0.70MPa. The MGH of stressed seeds decreased significantly (99.6%) when submitted to a potential of -0.86MPa.

(Table 1).

Table 1 Evolution of time to 50% germination (T_{50}%), mean germination time (MGT) and mean germination hours (MGH) of *Eruca vesicaria ssp sativa* seeds germination under different levels of osmotic potential induced with PEG_{6000}

[PEG] MPa	T_{50}%(H)	MGT(H)	MGH
0	12.00 ± 0.50	14.53 ± 1.32	2.56 ± 0.06
-0.04	12.10 ± 1.23	15.40 ± 3.11	2.43 ± 0.02
-0.14	12.43 ± 2.35	20.01 ± 2.69	2.42 ± 0.07
-0.29	19.68* ± 3.33	20.96* ± 1.06	2.37 ± 0.03
-0.46	21.18* ± 3.65	24.40** ± 3.24	2.20± 0.01
-0.70	24.91**± 2.71	28.28** ± 5.25	2.02 ± 0.09
- 0.75	NR	42.10*** ± 6.21	0.18* ± 0.02
-0.80	NR	97.36*** ± 9.10	0.05** ± 0.02
-0.86	NR	107.57***±8.51	0.01**± 0.00
-0.92	NR	0	0
-0.98	NR	0	0

Standard error is represented by mean (n = 6). Asterisks indicated significant difference with control at * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001 by Duncan test

NR=Median time of germination not reached

Germination recovery

After 210 hours of experimentation, ungerminated seeds of each PEG_{6000} pre-treatment were recovered and put to germinate in distilled water condition as the control seeds for 33 hours.

Analysis of the germination recovery experiments showed that seeds were not affected by elevated levels of osmotic stress. Results show that after two days, recovery was complete and quick for all tested osmotic potentials that inhibited germination (-0.75, -0.80, -0.86, -0.92 and -0.98 MPa) (Figure 3).

Effect of osmotic stress on phenolic compounds content

Total phenolics reached a maximum value 8.84 ± 0.18 (mg GAE.g^{-1} seeds) at -0.98 MPa, compared to 1.74 ± 0.15 (mg GAE.g^{-1} seeds) in control seeds (Figure 4a). More specifically, the total flavonoid content increased with decreasing water potential (Figure 4b) and reached a value of 0.310 ± 0.004 (mg QE.g^{-1} seeds), at an osmotic stress of -0.98 MPa, an increase of 68.90% compared with unstressed seed levels (0.074±0.001 mg QE.g^{-1} seeds). Anthocyanin levels were significantly affected with water stress. When

subjected to an osmotic stress of -0.98 MPa, seeds anthocyanin levels reached 4.02 ± 0.01 (mg Cyd-3-glu.g^{-1} seeds), an tenfold increase compared to unstressed seed levels (0.45 ± 0.02 (mg Cyd-3-glu.g^{-1} seeds)) (Figure 4c).

Salt stress effect on germination kinetics of Eruca vesicaria ssp. sativa seeds

Analysis of figure 5 shows that the germination curve includes several growth phases. In the latency phase and for potential inferior to -0.96 MPa, stressed seeds had a lower latency time (6h) compared to the control seeds (9h).

The latency time increased with decreased osmotic potentials reaching a maximum value of 21h for seeds subjected to a -1.68 MPa osmotic potential. In the exponential phase, seeds subjected to the highest potentials of -0.24, -0.46 and -0.72 MPa were not affected and germinated at a similar, fast, pace as the control seeds. The germination rate decreased from the osmotic potential of -0.96 MPa and was inhibited completely at osmotic potential of -1.68 MPa. The third phase is linear and represents the final percentage of germination, indicating maximal germination capacity.

Comparing the germination capacity of control seeds with stressed seeds can help better our understanding of the rocket seeds tolerance to salinity. A high germination capacity was recorded until osmotic stress of -0.96 MPa (>80%). In addition, the seed germination rate remained above 60% at -1.2 MPa salt stress (reduction of 34.22 %). The germination capacity decreased significantly from -1.44 MPa to 21.33±0.39 % (reduction of 78.52%). No seeds germinated at -1.92 MPa (Figure 6).

Table 2 shows the effect of salt on germination speed, measured by 50% germination time ($T_{50\%}$). $T_{50\%}$ increased with increasing salt stress. Comparison of controls and stressed seeds shows that $T_{50\%}$ increases by 90% at a salt stress of -1.20MPa. Above this level, the $T_{50\%}$ was not reached.

The mean germination time (MGT), for stressed seeds, increased with decreasing osmotic potentials of NaCl. Rocket seeds germinated faster if left untreated as shown in (Tab 2). The slowest MGT values were obtained when the seeds were subjected to high salt stress (-1.68 MPa).

Table 2 Evolution of time to 50% germination ($T_{50}^{\%}$), mean germination time (MGT), and mean germination hours (MGH) of *Eruca vesicaria ssp sativa* different levels of osmotic potentials induced with NaCl

[NaCl] MPa	$T_{50\%}$(H)	MGT(H)	MGH
0	12.00 ± 0.50	14.26 ± 1.02	2.54 ± 0.10
-0.24	16.34 ± 3.52	16.21 ± 24.7	1.46 ± 0.08
-0.48	19.18* ± 4.21	19.85 ± 1.96	1.23 ± 0.02
-0.72	30.26** ± 3.28	26.44* ± 2.45	0.66 ± 0.01
-0.96	56.06*** ± 5.63	98.92*** ± 3.59	0.50 ± 0.03
-1.20	118.33***± 5.20	112.71±3.25	0.39 ± 0.01
- 1.44	NR	181.81*** ± 4.26	0.12* ± 0.05
-1.68	NR	1000.00*** ± 5.68	0.02** ± 0.03
-1.92	NR	0	0

Standard error is represented by mean (n = 6). Asterisks indicated significant difference with control at * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001 by Duncan test

NR=Median time of germination not reached

Compared with the control, the solutions with osmotic potentials of -1.20, -1.44 and -1.68 MPa saw their MGT increase by 87.34%, 92.15% and 98.57%, respectively.

Mean germination per hour (MGH) was affected by salt stress. MGH decreased gradually over the course of the experiment, with 40% and 124% decreases recorded in stressed seeds in NaCl solutions of -0.96 and -1.68 MPa, respectively.

Germination recovery

After 210 hours of experimentation, ungerminated seeds of each salt pre-treatment (-0.24, -0.46, -0.72, -0.96, -1.20, -1.44, -1.68, -1.92 MPa) were put to germinate in the same conditions as the control seeds (distilled water for 96 hours). Germination was recovered entirely (100% germinated seeds) for seeds that have undergone NaCl exposure of up to -0.96 MPa. However, for values of -1.20 and -1.40 MPa recovery decreased to 50% and 43% respectively. No germination recovery was observed for the two higher levels of salinity of -1.68 and -1.92 MPa (Figure 7).

Effect of salt stress on phenolic compounds content

Experimental results show that exposure to various salt concentrations induced the accumulation of secondary metabolites. The highest concentrations of phenolic compounds were found in seeds subjected to an osmotic stress of -1.98 MPa. Their concentration reached 5.19 ± 0.09 (mg GAE.g^{-1} seeds), a threefold increase compared to seeds germinated under normal conditions (1.57 ± 0.03 mg GAE.g^{-1} seeds) (Figure 8a). Total flavonoids increased gradually from 0.074 ± 0.001 (mg QE.g^{-1} seeds) in the control seeds to 0.184 ± 0.002 (mg QE.g^{-1} seeds) at -0.96 MPa of salinity stress. Flavonoids content reaching a maximum value of 0.230 ± 0.008 (mg QE.g^{-1} seeds) at -1.96 MPa. A threefold increase compared to the control (Figure 8b). During germination, the total anthocyanin concentration in sprouted rocket seeds increased and it reached a maximal concentration of 4.02 ± 0.04 (mg Cyd-3-glu.g^{-1}) seeds at a stress value of -1.92 MPa. This is close to a tenfold increase compared to the control seeds (0.42 ± 0.02 mg Cyd-3-glu.g^{-1} seeds) (Figure 8c).

Discussion

Germination is one of the most important stages in plant life cycle. For germination to initiate, seeds need to imbibe water and this process won't take place unless the seed is in the presence of favourable environmental conditions. A lack of water availability will have a deleterious effect on seed germination (Toumi et al. 2017).

The results of this comprehensive study showed that rocket seeds were tolerant to environmental factors such as salinity and drought. Rocket seeds germination rate was reduced, inhibited in some cases, when exposed to the most stressful potentials. Ultimately, germination was still recorded for potentials that would have inhibited most other seeds.

Overall, rocket seeds that were subjected to drought and salinity stress were more affected by water stress compared to salt stress. This can be explained by a more rapid water uptake in NaCl solutions and a higher moisture content, and both factors favour seed germination. Similar results were reported for soyabeans by Khajeh-Hosseini et al. (2003). They suggested that the germination rates in NaCl solutions were due to rapid imbibition of soyabean seeds. More results corroborating this findinghave been reported for sorghum, sunflower and cowpea (Patané et al. 2013). According to Cheen and Arora (2010), the presence of PEG, a non-penetrating solute, severely hampered water uptake in seeds, whereas the penetrating ions (Na^+ and Cl^-) could have contributed to a drop in the internal osmotic potential of the seed. The delay in seed germination can be explained by the time required for the seed internal osmotic pressure to adjust to the extra cellular medium potential (Parihar et al. 2015; Parmoon et al. 2018).

Čanak et al. (2020) reported an estimated desiccation tolerance threshold of -0.75 MPa,based on their findings and our empirical observations we can conclude that *E. vesicaria ssp. sativa* is tolerant to drought stress during germination and therefore can be successfully cultivated in dry and arid lands. Channaoui et al. (2017), while studying different varieties of canola seeds, reported that the osmotic level of -0.9 MPa drastically reduced seed germination and -1.1 MPa completely inhibited it. It was also reported that seed germination of several other species was affected by osmotic stress after application

of PEG_{6000} at -0.46 MPa, like *Anthephora pubescent*, *Heteropogon contortus* and *Themeda triandra* (Van den Berg et al. 2005). In our study, the inhibition caused by the osmotic effect was especially noticeable from -0.8 MPa and lower. The fact that *E. vesicaria ssp. sativa* can adapt to osmotic stress levels up to -0.7 MPa, with final germination percentages similar to the control, strongly indicates its tolerance to drought stress.

The drought tolerance of rocket seeds during germination might be related to the small seeds size, which provides greater total surface contact with the soil and would require less water for germination. Pereira et al. (2013) reported this finding when studying cameline. Rocket seeds like cameline possess another seed-specific property that explains its excellent performance during germination and early seeding growth in dry environments: the presence of mucilage (Čanak et al. 2020). Mucilage consists of polysaccharides, accumulated in the cell wall, which have a high binding water capacity.Mucilage consists of polysaccharides, accumulated in the cell wall, which has a high binding water capacity because they broaden during the imbibition phase, fragmenting the outer cell wall, and surrounding the seed with viscous gel. By maintaining seed hydration when water is deficient, the gel enables the process of seed germination and seedling formation during abiotic stress (Tsai et al. 2021).

Salinity also plays a critical role during seeds germination. It is a limiting factor for many plant species worldwide, especially in the early stages of growth (Vahabinia et al. 2019). Salt stress can affect seed germination in two ways: osmotically and Ionically. When affected osmotically, seed water uptake is reduced, which is common in halophyte cultures (Chen et al. 2021). When affected ionically, sodium and chloride accumulation increases, leading to unbalanced nutrient uptake and toxic effects on seed metabolic processes (i.e. DNA repair and protein synthesis). This is commonly observed in non-halophyte cultures (Bewley et al. 2013; Seal et al. 2018). Salinity can also hamper the use of seed reserves (Gupta et al. 2002) coming from hydrolysis thus depriving the embryo access to a vital source of energy (Misra and Dwivedi 2004). Additionally, salt stress can reduce the activity of enzymes related to seed germination and can terminate normal seedling growth and development (Parmoon et al. 2018)

According to Bajji et al. (2011), a plant is resistant to salt stress if it germinates in media that contain up to 3 $g.L^{-1}$ of salt. With a germination rate of 65.33% at 14.5$g.L^{-1}$ of salt, our results strongly indicate that arugula seeds are well suited to grow in environments that contain high levels of salt.

Rocket seeds that were exposed to salinity potentials ranging from -0.24 MPa to -0.96 MPa returned germination rates similar to no-salt conditions after a 96-hour recovery in distilled water. These results show that salt effects are osmotic at first when salinity is moderate, which in turn enabled a full germination recovery once the stress was removed (Diallo et al. 2013). At -1.2 and -1.4 MPa (medium salinity) germination recovery is under 51%. When exposed to higher concentrations of NaCl (-1.68 MPa and -1.92 MPa) seed germination is inhibited permanently, with no recovery occurring after the removal of the salty environment. Toxicity action has been shown to also occur due to the accumulation of Na^+ and Cl^- ion which in turn caused a decrease in the germination rate. High concentrations of sodium chloride may lead to the accumulation of toxic ions in the embryo, which will impair the metabolic process of seed

germination (Bajji et al. 2011). In contrast, when rocket seeds were exposed to PEG_{6000}, germination was reduced and recovery was total, this indicate that the exposure didn't result in a loss vigour, which is observed when submitted to salt stress. We can also conclude that the inhibition of seed germination under PEG_{6000} stress was due to the low water potential caused by osmosis.

There are few studies on the effects of osmotic and salt stress on phenolic compounds accumulation during seed germination. Plants naturally produce phenolic compounds during growth and development to defend against biotic and abiotic stresses (Nderitu et al. 2013). Several study findings support the idea that phenolic compounds levels increase with germination (Khang et al. 2016). Our study results show that osmotic and salt stress during germination caused an increase in phenolic compounds accumulation (total polyphenols, total flavonoids and total anthocyanins). Ungerminated seeds when subjected to a potential of -0.98 MPa in PEG_{6000} and -1.92 MPa in NaCl, had the highest levels of total polyphenols, total flavonoids, and total anthocyanins. It has been reported that stress stimulated by PEG_{6000} and NaCl will favour oxidative stress which could induce subsequently the release of phenolic compounds being stored in the seed. Phenolic compounds are antioxidants and their release will help seeds set up a defence system against reactive oxygen species produced by these two abiotic stresses. These results are well aligned with previous findings from Cevallos-Casals and Cisneros-Zevallos (2010), Pajak et al. (2014), Khang et al. (2016). Cevallos-Casals and Cisneros-Zevallos (2010) reported that when seeds break their latent state, a protective response takes place through the synthesis of phenolic compounds. They also reported accumulations of phenolic compounds in mung bean seeds (increase of 841% compared to ungerminated seeds), sunflower (232%), radish (206%) and broccoli (68%). Yuan et al. (2010) also showed that salt stress has a significant effect on the accumulation of phenolic compounds and plays an important physiological role in seeds' tolerance to oxidative stress. This accumulation was also shown to reach a peak after 4 days of germination. The approach, presented in this study, targeted specific class of phenolic compounds: phenols, flavonoids and anthocyanin. While all three classes increased, anthocyanins saw the greater accumulation (10x increase for PEG and NaCl) compared to phenols (7x for PEG, 3x for NaCl) and flavonoids (4x for PEG, 3x for NaCl). These findings corroborate proven published facts that anthocyanin is an important antioxidant and, based on our results, may play an important role in the protective response that takes place during germination. The physiological mechanisms and molecular pathways involved in the accumulation of these phenolic compounds in the germinating seed should be further investigated to fully understand seeds responses to oxidative stress.

Eruca vesicaria ssp. sativa seeds are resilient to outside stress factors such as salt and drought. Evaluation of the seeds tolerance to osmotic stress showed a resistance to moderate stress and a high germination recovery when transferred to normal germination conditions. *Eruca vesicaria ssp. sativa* is well adapted to dry environments and our study results are in line with this known fact and confirm its ability to germinate under osmotic stress. When rocket seeds were submitted to salt stress, good resistance to harsh conditions was observed. However, germination recovery was greatly affected when submitted to severe salt stress indicating the pronounced toxic effect salt accumulation has on the seed vigour. These findings can be explained at a biochemical level by an increase in accumulation of phenolic

compounds during seeds germination as suggested by measurements carried after seeds were subjected to osmotic and salt stresses. Accumulation of anthocyanins, flavonoids and phenols was shown to occur with anthocyanins recording the biggest increase. This finding highlights the preponderant role that anthocyanins have when fighting oxidative stress.

Methods

Plant Material

Rocket seeds were provided by a seed producer in Tamanrasset, Algeria. Seeds were stored in the dark at 5°C. Seed viability was evaluated under optimal conditions. A germination rate of over 98% was recorded.

Effect of osmotic and salt stress on germination

In this experiment, seed batches were used to study species responses to drought and salinity induced respectively by polyethylene glycol 6000 (PEG_{6000}) and sodium chloride (NaCl).

Distilled water potential was used as a control. Ten levels of water potential -0.04, -0.14, -0.29, -0.46, -0.70, -0.75, -0.80, -0.86, -0.92 and -0.98 MPa and eight levels salinity -0.24, -0.46, -0.72, - 0.96, - 1.20, -1.44, -1.68, -1.92 MPa were used.

Five replicates of 100 seeds were used in each treatment (osmotic and salt stress). Seeds were placed on two sheets of filter paper Whatman No. 1 and inserted in 8-cm Petri dishes for each replicate. To provide adequate moisture for the seeds during the experiment, 3 mL of test solutions (including distilled water) were used for each treatment. Petri dishes were incubated at 26°C in the dark for 216 hours. Seeds were considered to have germinated once the radicle has reached a length of at least 2 mm. The experiments were terminated when new germination was not observed for three consecutive days.

Measured parameters

Several parameters were studied to evaluate the effect of water and salt stress on germination:

Germination Kinetic: This parameter was assessed using the below mathematical model.

$$Y_{(t)} = Y_{max}(1 + e^{-k(t-t0)}$$

Where $Y_{(t)}$ represents the number of germinated seeds at time t, Y_{max} is the plateau reached by $Y_{(t)}$ and corresponds to the number of viable seeds, k is the time required for the germination of 50% of the viable seeds. (t_0) represents latency of germination (time during which the seeds acquire the aptitude to germinate).

The Final germination percentage (GP): This parameter was expressed as described in Come (1970). and as follows:

$$GP\ \% = N_i/N_t \times 100$$

Where N_i is the number of germinated seeds, and N_t is the number of total seeds per replicate.

Germination speed: This parameter relates to the germination energy required to exhaust all seeds reserves. The time necessary to attain 50% germination (T_{50}) was calculated in accordance with the method described in Come (1970)

$$T_{50}\,(\%) = T_1 + (0.5 - G_1 / G_2 - G_1)\,x\,(T_2 - T_1)$$

Where G1 = Cumulative percentage of germination whose value is closest to 50% by lower value and G2 = Cumulative percentage germination whose value is closest to 50% by highest value.

Mean germination time (MGT) was defined according to Kotowski (1926) as:

$$MGT = (1/Vc)\,x\,100$$

with Vc (velocity coefficient) = $N_1+N_2+...+N_i/100$ x $N_1T_1+...+N_iT_i$; where N is the number of seeds germinated every day and T is the number of hours from seeding corresponding to N.

Mean germination hours (MGH): corresponding to the final germination percentage on number of hours to final germination (Osborne et al., 1993).

Germination recovery

After 210 hours, ungerminated seeds from the PEG_{6000} and NaCl treatments were rinsed three times using distilled water, then transferred to new Petri dishes containing distilled water, and maintained in the control conditions for four days to study germination recovery. The recovery test was made from the osmotic potential of -0.75 MPa for osmotic stress and -0.96MPa for salt stress.

Phenolic compounds

Biochemical analyses were performed on the seeds that were submitted to different osmotic potentials induced by PEG_{6000} and NaCl.

Determination of total polyphenols. Total phenolic content (TPC) was assayed as described by Singleton and Rossi (1965), using Folin-Ciocalteu reagent with final reaction measurements carried out at λ

(nm)=760 nm. Gallic acid was used as a calibration standard, and the results were calculated as mg gallic acid equivalent (GAE) per gram of germinated seeds (mg GAE.g^{-1} seeds).

Determination of total flavonoids content. The aluminium chloride colorimetric method was used for the determination of total flavonoids as described in Lamaison and Carnet (1990). Quercetin was used as a calibration standard, and the results were calculated as quercetin equivalent (QE) per gram of germinated seeds (mg QE.g^{-1} seeds).

Determination of total anthocyanins. Total anthocyanin content was measured according to the procedure described by Rabino and Mancinelli (1986).

The formula Absorbance (A) = (A_{530} - 0.25 A_{657}) was employed to compensate for the contribution of chlorophyll and its by-products at 530 nm. The anthocyanin content is expressed as mg cyanidin-3-glucoside equivalent per gram of germinated seeds (mg Cyd-3-glu.g^{-1} seeds).

$$\text{Total Anthocyanin} = \frac{\text{Absorance (A)} \times 448.2 \times \text{Dilutionfactor} \times \text{Volume}}{29600 \times \text{Sample weight}}$$

Statistical Analysis

Data was analysed using one way Analysis of Variance (ANOVA) provided by Statistica 10.0 software (Statsoft. Inc.). Significant differences between the means of five (05) repetitions and standard deviations were evaluated using the Duncan post-hoc test. For our study, $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered statistically significant.

Declarations

Author contributions: S. Barris designed the experiment and wrote the manuscript**. M. Toumi** presented some experimental ideas and contributed to the writing of the manuscript. **A.Belkebir** revised the manuscript.

Conflict of interest: There is no conflict exists among all the authors, and the contribution of the authors is clear and unquestionable. All of them declare that they have no conflict of interest whether financial, commercial, contractual, or patent related. Therefore, all authors are allowed to publish the article

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Figures

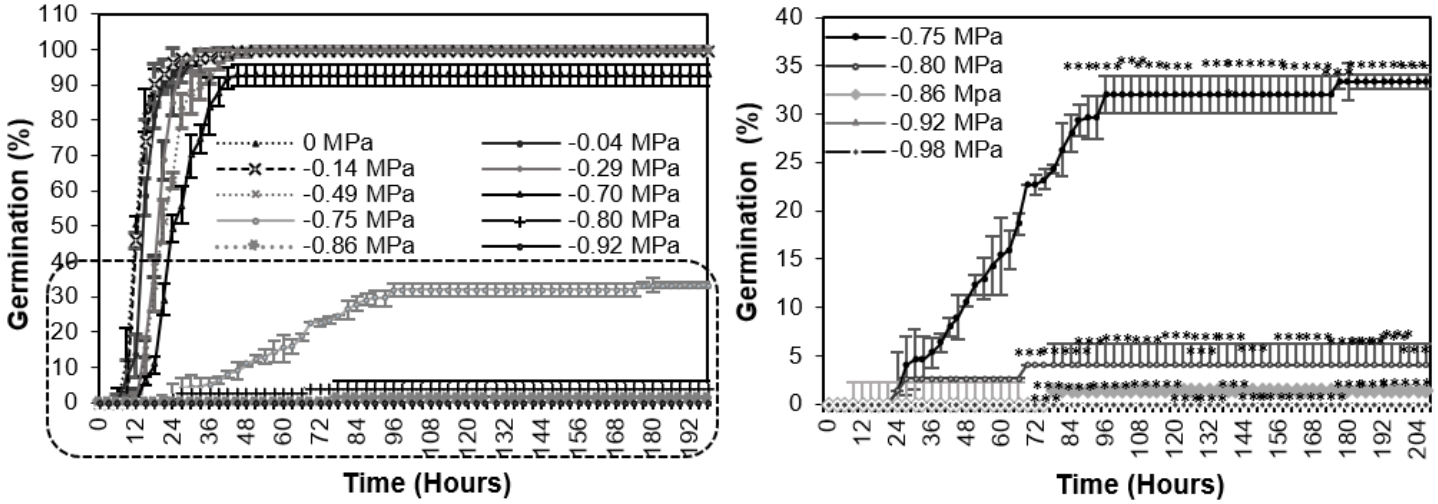

Figure 1

Cumulative germination time courses of *Eruca vesicaria ssp. sativa* L. under different levels of osmotic stress induced with PEG_{6000}

Bars represent standard error of mean (n = 6). Asterisks indicated significant difference with control at * P≤ 0.05,

** P≤ 0.01, *** P≤ 0.001 by Duncan test

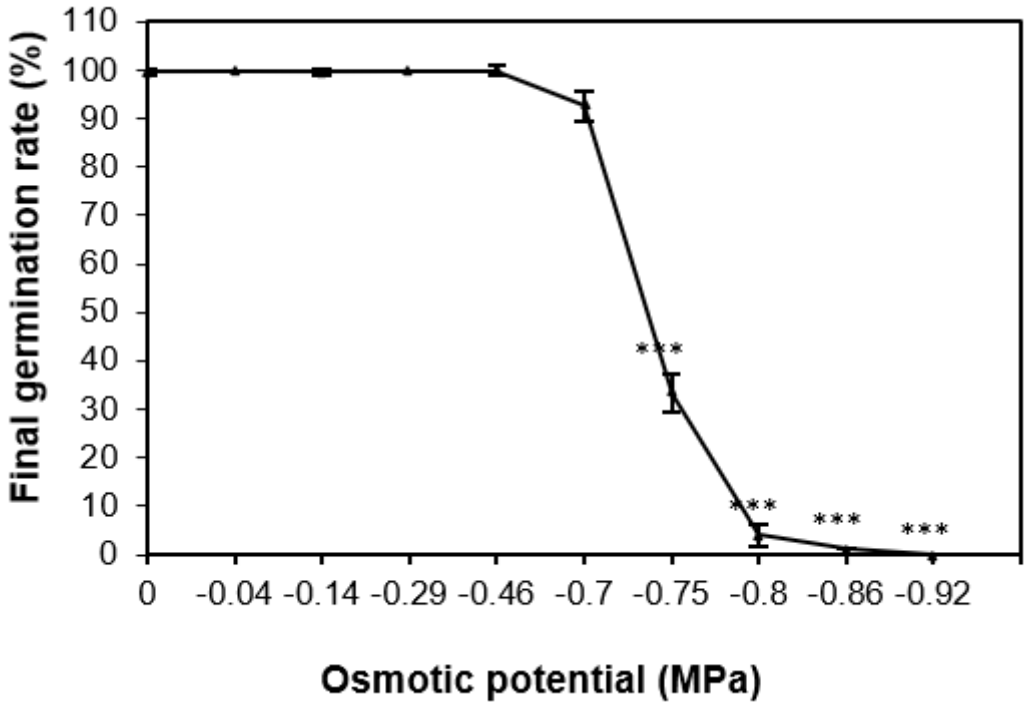

Figure 2

Evolution of germination rate of *Eruca vesicaria ssp sativa* under different levels of osmotic stress induced with PEG_{6000}

Bars represent standard error of mean (n = 6). Asterisks indicated significant difference with control at* P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001 by Duncan test

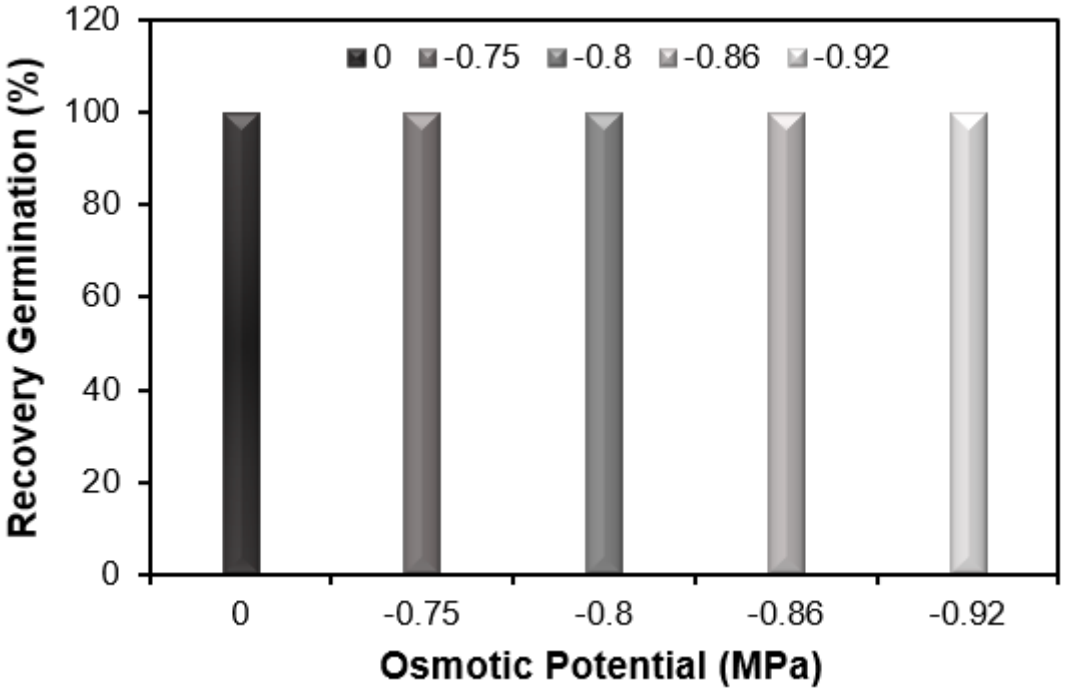

Figure 3

Recovery germination of *Eruca vesicaria ssp sativa* seeds after PEG_{6000} pretreatment

Bars represent standard error of mean (n = 6). Asterisks indicated significant difference with control at * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001 by Duncan test

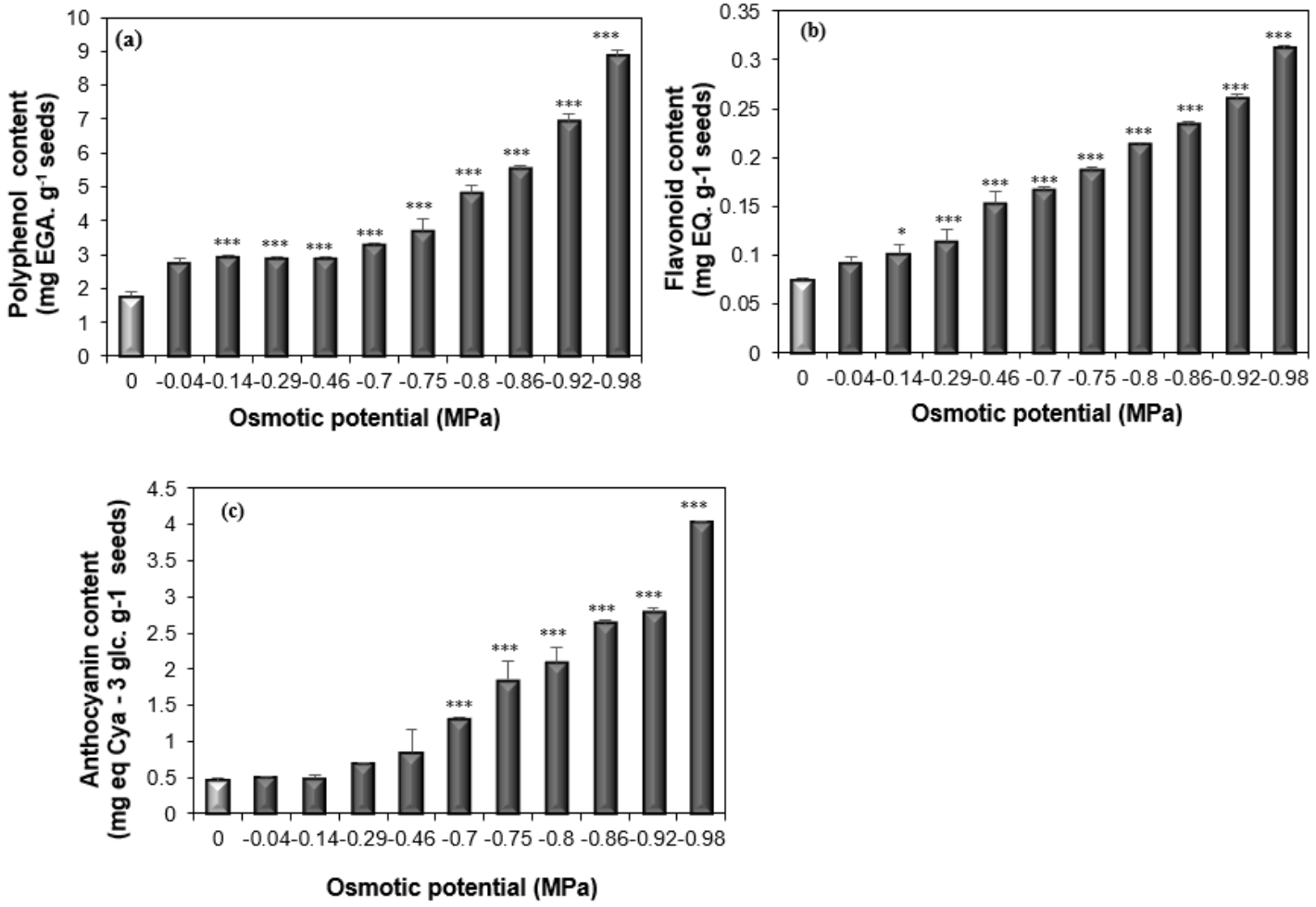

Figure 4

Effect of different levels of osmotic stress induced with PEG $_{6000}$ on polyphenol content (a), flavonoid contentment (b) and anthocyanin content (c) of *Eruca vesicaria ssp sativa* germination

Bars represent standard error of mean (n = 6). Asterisks indicated significant difference with control at * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001 by Duncan test

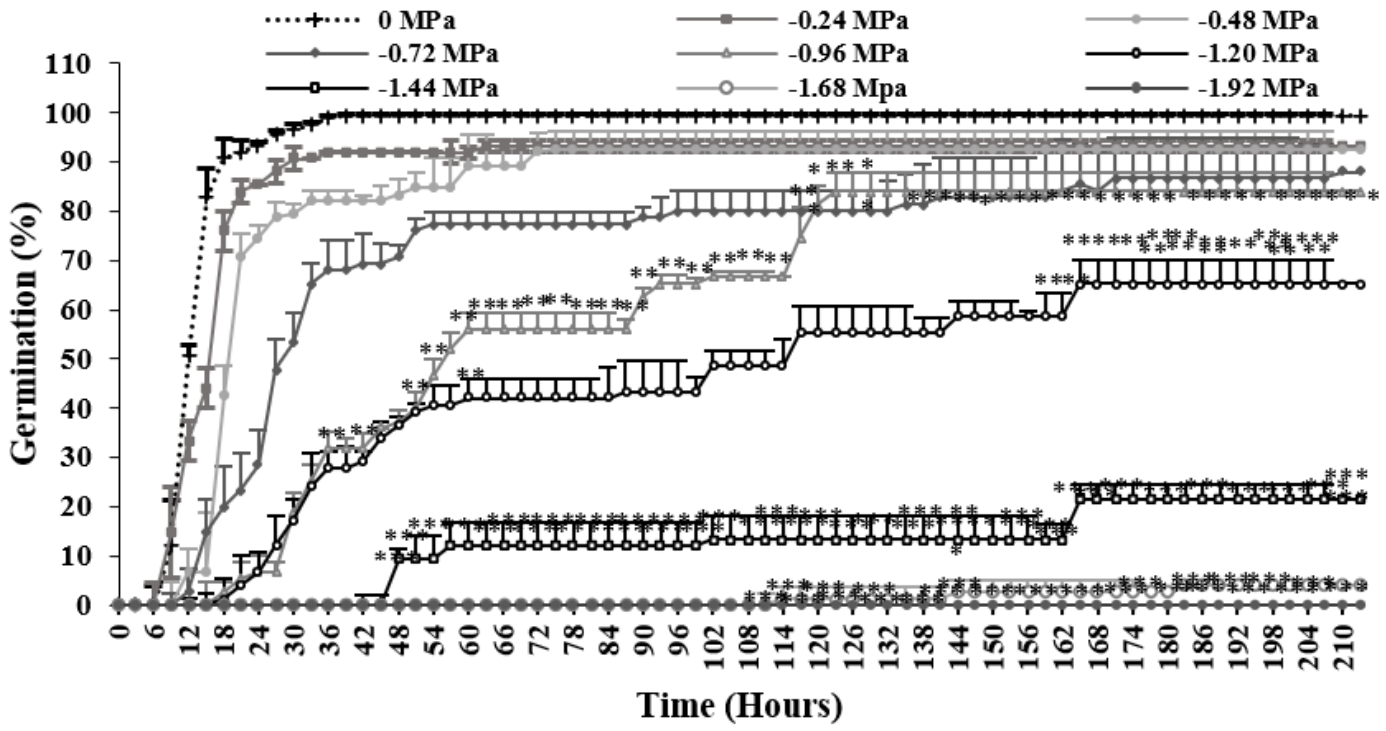

Figure 5

Cumulative germination time courses of *Eruca vesicaria ssp. sativa* L. under different levels of salt stress induced with NaCl

Bars represent standard error of mean (n = 6). Asterisks indicated significant difference with control at * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001 by Duncan test

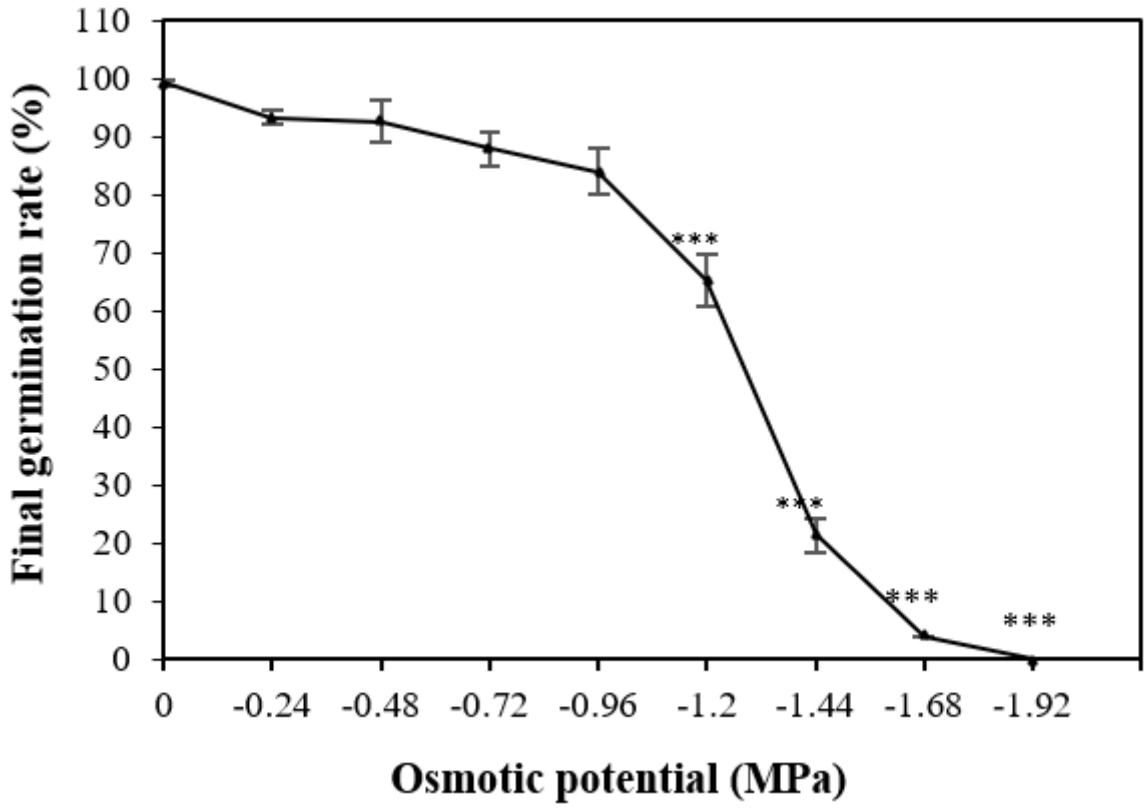

Figure 6

Evolution of germination rate of *Eruca vesicaria ssp sativa* under different levels of salt stress induced with NaCl

Bars represent standard error of mean (n = 6). Asterisks indicated significant difference with control at * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001 by Duncan test

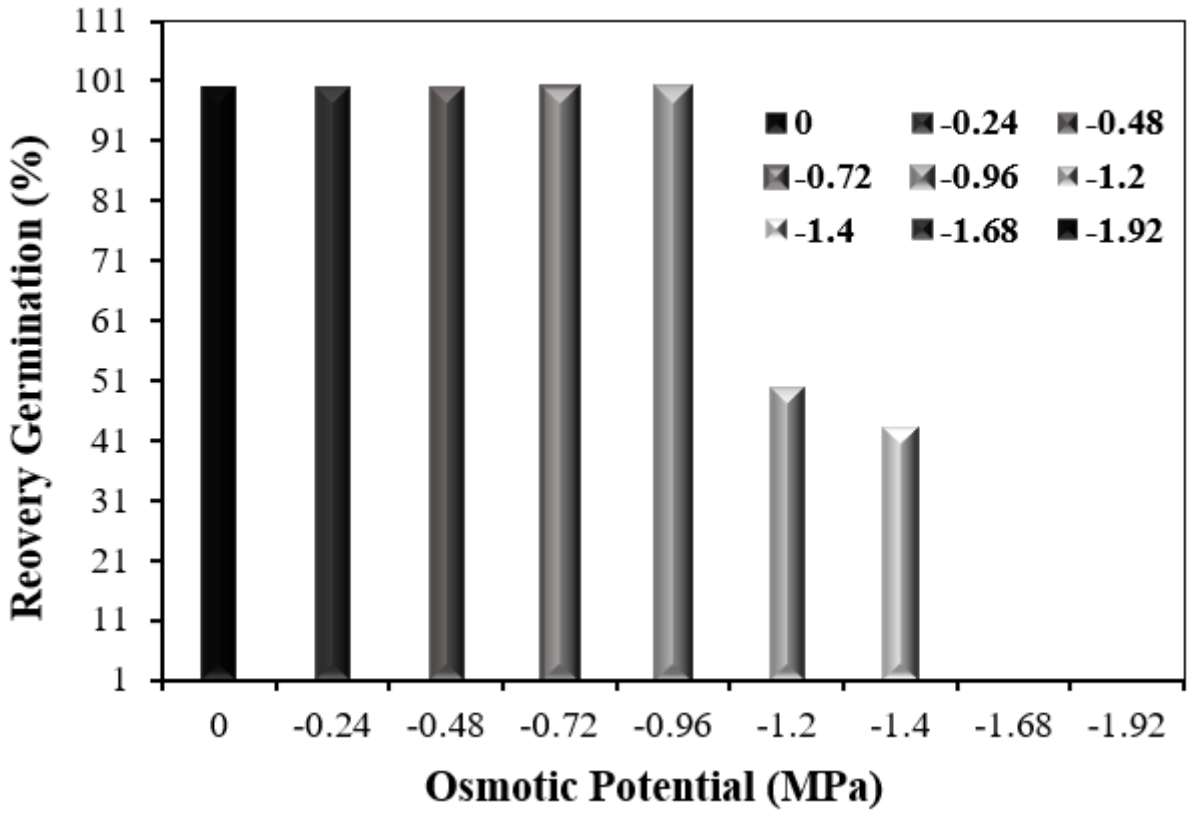

Figure 7

Recovery germination of *Eruca vesicaria ssp sativa* seeds after NaCl pretreatment

Bars represent standard error of mean (n = 6). Asterisks indicated significant difference with control at * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001 by Duncan test

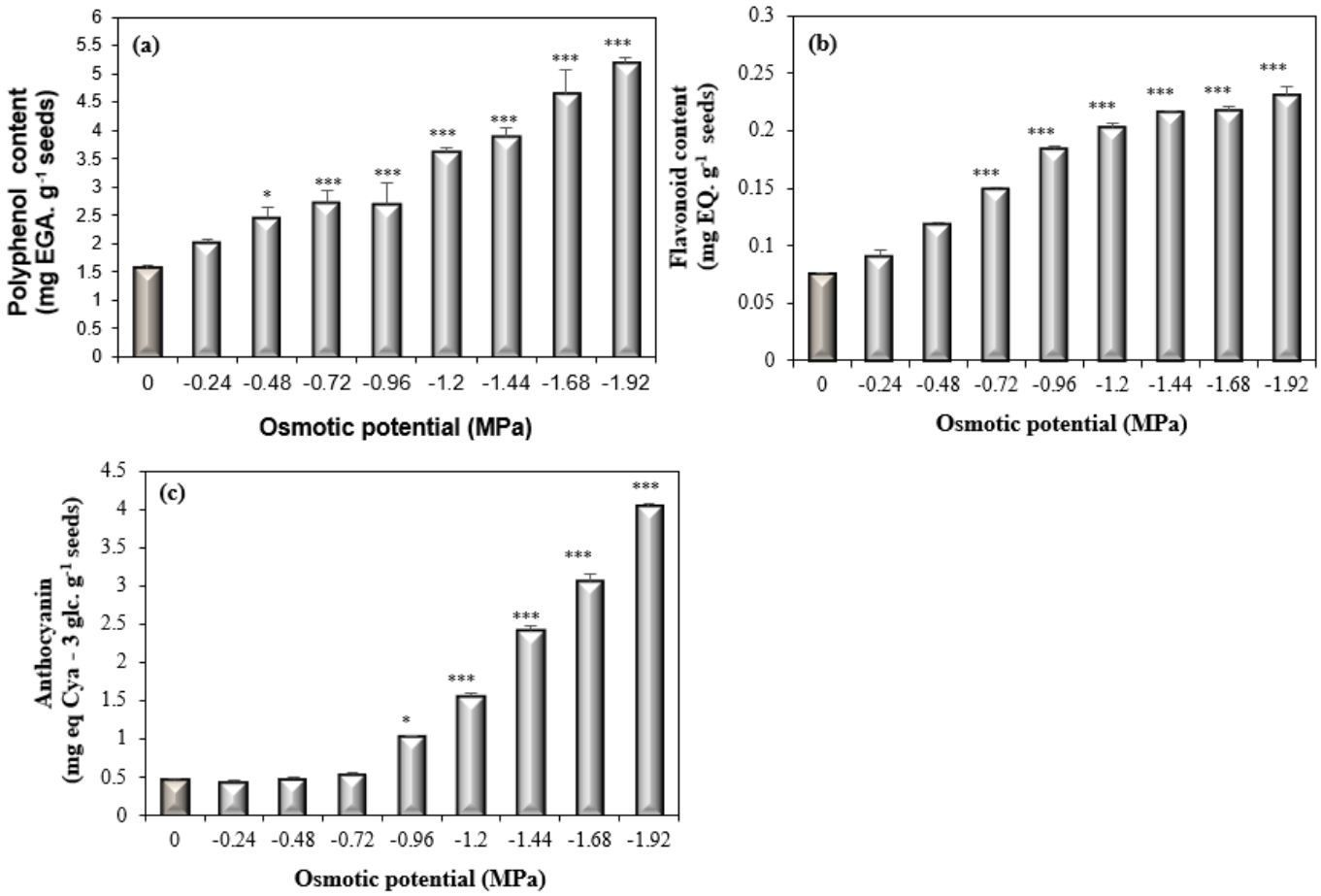

Figure 8

Effect of different levels of osmotic stress induced with NaCl on polyphenol content (a), flavonoid contentment (b) and anthocyanin content (c) of *Eruca vesicaria ssp sativa* germination

Bars represent standard error of mean (n = 6). Asterisks indicated significant difference with control at * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001 by Duncan test