

Seed dormancy and germination in *Dodonaea viscosa* (Sapindaceae) from south-western Saudi Arabia

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Accepted by the *Saudi Journal of Biological Sciences* on 19 May 2020.

Abstract

Dodonaea viscosa (Sapindaceae) is widespread in the mountainous highlands of the southwestern part of the Kingdom of Saudi Arabia, where it is a medicinally important species for the people of Saudi Arabia. Seeds of this species were collected from Mount Atharb in the Al-Baha region, at an altitude of 2100 m. The aims of this study were to determine if the seeds of *D. viscosa* have physical dormancy (i.e. a water-impermeable seed coat) and, if so, what treatments would break dormancy, and what conditions promote germination after dormancy has been broken. The dormancy-breaking treatments included: soaking of seeds in concentrated sulfuric acid (H₂SO₄) for 10 minutes, immersion in boiling water for 10 minutes and exposure to 50 °C for 1 minute. After seeds had been pre-treated with H₂SO₄, to break dormancy, they were incubated at constant temperatures from 5 to 35°C, under 12-h photoperiods or in continuous darkness, and germination recorded. Salinity tolerance was investigated by incubating acid-scarified seeds in 0, 100, 200 and 300 mM NaCl in the light at 25°C.

Untreated seeds had low final germination (30%). Seeds that had been acid-scarified, immersed in boiling water or exposed to 50 °C all achieved 91% subsequently when incubated at 25°C. Thus, seeds of this species in Saudi Arabia have physical dormancy, which can be broken by all three treatments designed to increase the permeability of the testa. After pre-treatment, there was a broad optimum constant temperature for germination that ranged between 5-25°C but germination was inhibited by higher temperatures (30 and 35°C). Light had little effect on this germination response. Scarified seeds were also sensitive to salinity, with the highest germination in distilled water and complete inhibition in 400 mM NaCl. Seeds that failed to germinate in saline treatments were mostly able to germinate on transfer to distilled water, suggesting osmotic inhibition.

Keywords: *Dodonaea viscosa*, physical dormancy, seed scarification, salinity (NaCl), seed dormancy, germination, temperature response, light response.

Introduction

Seed germination is one of the critical stages in the life cycle of the plant (e.g. Gutterman, 1993; Gutterman 2002; Song *et al.* 2005; Li *et al.* 2010). Seed dormancy may determine the timing of germination and various environmental factors, such as temperature, light and salinity may play a significant role in regulating germination behavior in the field (e.g. Al-Turki, 1992; Neo and Zedler 2000; Gutterman, 2002; Koornneef *et al.* 2002; Khan and Gul, 2006; Saeed *et al.*, 2011; Gulzar *et al.* 2013; Baskin and Baskin, 2014). One of the more important forms of seed dormancy is physical dormancy (PY), where the seed coat remains impermeable, as a result of a palisade layer of lignified malpighian cells (Baskin, 2003; Baskin *et al.*, 2000). Physical dormancy has been reported in more than 15 dicotyledonous and one monocotyledonous families (Baskin and Baskin, 2014; Baskin, 2003; Baskin *et al.*, 2000, 2006; Horn, 2004). Five genera of the family Sapindaceae, *Cardiospermum*, *Diplopeltis*, *Distichostemon*, *Dodonaea*, and *Koelreuteria*, have been reported to have PY (Rehman and Park, 2000; Turner *et al.* 2006; Baskin *et al.*, 2004; Johnston *et al.*, 1979; Harrington *et al.*, 2005; Cook *et al.*, 2008). Alternatively, some species have been found to have innate physiological dormancy of the embryo (Baskin and Baskin, 2014) and Naidu *et al.* (1999) have provided evidence that seeds of *Santalus trifoliatus* have physiological dormancy in addition to PY.

Physical dormancy can be broken by using various scarification methods to render the testa water-permeable: hot water (Baskin *et al.* 2004), dry heat (Mott *et al.* 1982), long-term dry storage (Morrison *et al.*, 1992), warm moist incubation (Jayasuriya *et al.* 2007), and soaking in concentrated sulfuric acid (Baskin and Baskin 2014). For instance, Cook *et al.* (2008) reported that PY in the seeds of *Dodonaea hackettiana* could be broken by exposing them to dry heat at 50°C for 30 seconds. Turner *et al.* (2009) have reported that PY in the field was broken in summer, when heavy rainfall events were followed within 24 h by soil temperatures > 50°C. Baskin *et al.* (2004) and Cook *et al.* (2008) reported that the PY in seeds of six species of *Dodonaea* could be removed by immersing seeds in hot water.

Seed germination behavior of *Dodonaea viscosa* has been investigated previously (Burrows, 1995; Phartyal *et al.* 2005; Naser *et al.*, 2013), but there are no previous reports on material from Saudi Arabia. The mature seeds of *D. viscosa* have been characterized as having a water impermeable seed coat (Demel, 1991; Negash, 1993; Phartyal *et al.*, 2005; Naser *et al.*, 2013). There is, however, geographical inconsistency in previous results. Seeds from Hawaii, New Zealand, Mexico, Australia and Brazil have shown physical dormancy (Baskin *et al.*, 2004),

whereas those from China, Pakistan (Qadir and Lodhi, 1971) and Botswana (Tietema *et al.*, 1992) were reported to be non-dormant. Details of the anatomy and morphology of fruits, seeds and seedlings of *D. viscosa* are provided by Khan and Ismail (2019).

The genus *Dodonaea* L. comprises about 60-70 species that grow in a great range of habitats, including woodland, forest and shrubland communities (West 1984; Reynolds, 1985; Shepherd *et al.* 2007). *D. viscosa* is very widely distributed, including in Australia, Africa, Mexico, New Zealand, India, South America. The center of origin of *D. viscosa* is believed to be Australia. The species is a dioecious or monoecious woody, perennial that grows up to 7m tall, as a multi-stemmed or single stemmed shrub (Rani *et al.*, 2009). In Saudi Arabia, *Dodonaea viscosa* subspecies *angustifolia* is found widely in high mountains in the south-west of the country, where it is an important component of the flora (Migahid, 1996; Chaudhary, 1999). In fact, it is the only species in the Sapindaceae in flora of Saudi Arabia (Migahid, 1996; Chaudhary, 1999; Collenette 1999). *D. viscosa* has been reported as a medicinal plant used for skin disease in cattle, and in humans as a cure for sore throats (Jansen, 1981). It is also used for lowering fever and rheumatism in folk medicine (Khurram *et al.*, 2009). In addition, different parts of this plant - stem, leaves, seeds, roots, bark – have been used as antibacterial, analgesics and antivirals (Rani *et al.*, 2009).

The aims of the present study were: (1) to determine whether the seed coat of *D. viscosa* from Saudi Arabia has physical dormancy; (2) to test the effects of mechanical scarification (immersion in boiling water and heating in a drying oven at 50°C) and chemical scarification (concentrated sulfuric acid); (3) to find the optimum temperature for germination after dormancy has been broken by investigating its responses to a wide range of constant temperatures (5 - 35°C), in alternating photoperiods (12h light: 12h dark) and continuous darkness; and (4) to examine the effects of sodium chloride (NaCl) concentration on seed germination of *D. viscosa*.

Materials and methods

Study area

Al-Bahah region is located in the south of Saudi Arabia, at an elevation above sea level ranging between 130 and 2450 m (Al-Aklabi, *et al.* 2016). The temperature ranges between 10 - 22°C in winter and 22 - 32°C in summer (Aref *et al.*, 2011). The mean monthly rainfall is variable. January, April and December have the highest average monthly rainfalls of 85 mm, 75 mm and 75 mm, respectively, whereas September, June, August and July have the lowest, with 7 mm, 10 mm, 15 mm and 20 mm respectively (Aref *et al.*, 2011).

Seed collection

Mature seeds of *Dodonaea viscosa* were collected from 20-30 plants, randomly selected from a natural population on 7 April 2017. The population sampled was at Jabal Athrab, at an elevation of 2130 m (latitude 19.694967 - 19.830936; longitude 41.596633 - 41. 759573). Seeds were air-dried, cleaned and examined immediately.

Experiment 1: Breaking dormancy

Seeds of *D. viscosa* were divided into four groups as follows (1) controls, without treatment, (2) treated with boiling water for 10 minutes, (3) treated with dry heat at 50°C for 1 minute, and (4) treated with concentrated sulfuric acid (H₂SO₄) for 10 minutes. All the seeds were then incubated at a constant temperature 25°C (12h: light: 12h: dark) in growth a chamber (LEEC, Nottingham, UK, Model PL33). Five replicate 9-cm Petri dishes with 20 seeds in each were used for each treatment. Seeds were placed on two layers of filter papers (Whatman No.1) moistened with 7 ml of distilled water. Seed germination was counted daily for 30 days. Petri dishes were randomly distributed in the temperature-controlled incubators and their positions were changed daily. Germination was defined as the first emergence of the radicle. Final germination percentage was recorded.

Experiment 2: Effects of temperature and light on germination.

Seeds of *D. viscosa* were pretreated by soaking in concentrated H₂SO₄ for 10 minutes and then rinsing three times in distilled water, before being incubated at a range of constant temperatures (5, 10, 15, 20, 25, 30 and 35°C), in 12h light: 12h dark or continuous darkness. Five replicates of 20 seeds were used, as previously described. Seed germination was counted daily for 30

days. For continuous darkness, the dishes were wrapped in aluminum foil to prevent any exposure to light and seed germination was counted only after 15 days at the end of experiment.

Experiment 3: Effects of salinity on seed germination and recovery

The effects of 0, 100, 200, 300 and 400 mM concentrations of NaCl on seed germination were tested at 25 °C (12 h light: 12h dark). Five replicate Petri dishes were used for each treatment, with 20 seeds per dish, as previously. Dishes were moistened with 7 ml of the appropriate solution and sealed with Nescofilm, to reduce evaporation, before being placed the incubator. The solutions were replaced at 7-day intervals. The number of seeds germinated was counted daily for 30 days and germinating seeds were removed from the Petri dishes. Seeds remaining ungerminated at the end of experiment were rinsed twice in distilled water and then transferred to dishes moistened with distilled water to assess whether salinity had inhibited germination; then they were incubated further for 15 days at 25 °C and germinations recorded daily. After this, seeds still ungerminated were tested for viability with a 1% aqueous solution of 2,3,5-triphenyl-tetrazolium chloride (TTC) (Mackay, 1972; Moore 1985). Seeds were soaked in this solution in Petri dishes covered with aluminum foil to exclude light, and were incubated for 24 h at 20/10 °C. Dehydrogenase enzymes within the seed tissues reduce the colorless tetrazolium chloride solution to form insoluble red formazan, so living cells appear red while dead cells appear colorless. Final germination was calculated as well as the time taken to reach 50% of the final germination percentages, across all the replicates (TG_{50}).

Statistical Analysis

Percentage germination data were arcsine transformed before statistical analysis, in order to meet assumptions of normality and homogeneity of variance, then and subjected to a one-way analysis of variance (ANOVA), with post hoc tests between treatment (Sokal and Rohlf, 1995).

Results

Experiment 1: Breaking dormancy

Treatment of the seeds of *D. viscosa* with sulfuric acid, boiling water and dry heat at 50°C all proved to be effective pretreatments, resulting in the highest germination (91%) at 25 °C, compared with only 30% in untreated seeds (Fig. 1). Significant differences ($P<0.0001$, $F=122$) for germination percentages were found between untreated seeds and the other treatments. However, there were no significant differences ($P>0.05$) in seed germination percentage observed between seed treatments using H₂SO₄, boiling water and dry heat at 50°C.

Experiment 2: Effects of temperature and light on germination.

The final germination percentage of *Dodonaea viscosa* seeds showed a broad, poorly defined optimum for constant temperature conditions with 12-hour photoperiods (Fig.2). Between 5°C and 25°C germination was high (80-90%) and did not differ statistically. However, at higher temperatures (30°C and 35°C) germination was significantly lower ($P<0.0001$, $F=28.56$) from that at the lower temperatures. In the dark, the germination response to temperature was extremely similar to that in the light (Fig.3). Germination at and 30°C and 35°C was again significantly lower than in the range 5°C - 25°C ($P<0.0001$, $F=11.68$).

Experiment 3: Effects of salinity on seed germination and recovery

In the salinity experiment, the highest germination percentage of *D. viscosa* seeds (91%) occurred in the distilled water control, and germination percentage decreased linearly with increasing salinity to 46% at 300 mM (Fig. 4). No seeds germinated at 400mM NaCl. Overall, mean germination percentage was significantly different between treatments ($P<0.0001$, $F=224.32$) Furthermore, all treatments were significantly different from each other, apart from the comparison of 200 and 300 mM ($P<0.05$) (Fig.4). Increasing salinity also delayed the time of which 50% germination was achieved: t_{50} increased from 2 days at 0 mM to 12 days at 300mM NaCl.

The germination of seeds after being transferred to distilled water from 100, 200. 300 and 400mM NaCl was 18%, 38%, 50%, and 93%, respectively.

Discussion

Dormancy is a way of creating a long-lived seed bank, which may be a mechanism for success in annual plants in unreliable habitats. The effects of innate dormancy and impermeable seed coats are significant mechanisms in delaying germination, especially among desert plant

species where rainfall is irregular, until the environment is favorable for the development of the seedlings (Toole *et al.* 1956). Delayed or inhibited germination resulting from an impermeable seed coat is a common phenomenon among desert plants (Koller *et al.* 1962; Koller 1969; Mahmoud 1977; 1985a, 1985b; Mahmoud and El-Sheikh 1978; Mahmoud *et al.* 1981a). It has been reported by Hudson *et al.* (2015) that 25% of the plant species have physically dormant seeds because of a hard water-impermeable testa.

The present study demonstrated clearly that the seeds of *D. viscosa* from Saudi Arabia have physical dormancy (i.e. water-impermeable seed coat) which was broken readily by three different methods: sulfuric acid, boiling water and dry heat at 50°C. The final germination percentage of untreated seeds was much lower than that of the seeds that had been scarified in any of the three ways. These results are in good agreement with the results of most other researchers (Hussain *et al.*, 1991; Bhagat and Singh, 1994; Baskin *et al.*, 2000; Baskin *et al.*, 2004; Phartyal *et al.*, 2005; Cook *et al.*, 2008, Naser *et al.*, 2013), who reported that seeds of various species of *Dodonaea* can germinate rapidly under optimal conditions once physical dormancy has been removed, reaching percentages >80% over a range of temperatures. Only the germination percentage of *D. petiolaris* has been reported as being much lower, at 36%, and neither did treatment of its seeds with warm or cold stratification, or 6 months of after-ripening at 30°C, improve germination (Turner *et al.* 2009).

In the case of *Dodonaea viscosa*, physical dormancy has been reported previously as present or absent in populations from different parts of the world. Our results agree with those for seeds collected from Hawaii, Australia, Brazil, Mexico and New Zealand (Baskin *et al.* 2004) and north-west India (Phartyal *et al.* 2005). However, our data for Saudi Arabia contrast with the reports that physical dormancy was absent, or nearly absent, in seeds collected from China, Pakistan (Baskin *et al.* 2004), Botswana (Tietema *et al.*, 1992) and Islamabad in Pakistan (Qadir and Lodhi 1971). Its absence in that latter collections is possibly related to them having less than fully mature embryos, as suggested by Baskin *et al.* (2004). Where dormancy exists, it can be broken by methods similar to ours. Bhagat and Singh (1994) used sulfuric acid on seeds of *D. viscosa* collected from north-west India to obtain a final germination of 90%. Similarly, Phartyal *et al.* (2005) reported that manual scarification of seeds from north-west India increased germination from 24%, to 84%, and boiling water increased it to 77%. Furthermore, Nasr *et al.* (2013) reported that untreated seeds of *Dodonaea viscosa* can germinate up to 40%, while the seeds treated with sulfuric acid for 45 min or boiling water can germinate up to 90.8% and 50%, respectively. In their natural habitats, species exhibiting

physical dormancy will have their dormancy weakened with time by daily temperature fluctuations in the soil (Baskin and Baskin, 2000) and also by exposure to heat shock during wildfires (Hodgkinson and Oxley, 1990; Hodgkinson, 1991). Also, some researchers (e.g. Gogue and Emimo, 1979; Morpeth and Hall, 2000) have suggested that the impermeable seed coat of some species can be weakened by fungal activity in the soil.

This present study showed that seeds of *Dodonaea viscosa* can germinate readily over a remarkably wide range of constant temperature, in light and darkness, once physical dormancy has been removed. The indifference of its germination to light is typical of plants with relatively large seeds (Fenner and Thompson, 2004). Final germination percentage is clearly inhibited at higher temperatures (30 and 35°C). This response could be attributed to adaptation to the environmental conditions in the habitat of this species in Saudi Arabia - cool, montane areas with predominantly winter rainfall. A similar result was reported by Al-Farraj *et al.*, (1988), who found that the seeds of *Verbesina enceliodes* (Cav) Benth. (Asteraceae) which had been collected some 35 km to the west of Riyadh, did not tolerate temperatures in excess of 14/28°C. Also, Al-Turki (1992) showed that seeds of *Suaeda aegyptiaca* from Al-Awashzia village (Al-Qassim region, 350 North-West of Riyadh city), attained high germination percentages (70% - 96%) between 15/5 and 30/25°C temperature regimes, though only 44% at 35/25°C. Similarly, the germination of *Suaeda monoica* was apparently inhibited by highest temperature it was tested at (35/25°C) (Al-Turki, 1992). Recently, Hadi *et al.* (2018) showed that the optimum temperatures for seed germination of *Salvadora persica* were in the range 10/20 to 15/25°C and its germination behavior in this respect is very similar to that of *Dodonaea viscosa*. The germination responses to temperature observed thus probably represent genetic adaptation to the habitat of this species. Also, it can be suggested from these results that germination of this species in Saudi Arabia would be more likely to occur in winter. However, seedlings have not been seen in the field in their natural habitats. Local adaptation species to the very different environmental conditions that prevail in Saudi Arabia has been considered previously by Abdulfatih and Bazzaz (1985) and Al-Turki (1992). Overall, our findings agree with previous studies (e.g. Baskin *et al.* 2004) that seeds of *Dodonaea viscosa* can germinate over a wide range of constant and alternating temperatures, and under various light regimes.

The germination response of *D. viscosa* indicated clearly that it is sensitive to salinity, since the final germination percentage dropped dramatically with increasing NaCl concentration, and it was completely unable to germinate at 400mM NaCl. Many previous workers (e.g. Song *et al.* 2005; Hadi *et al.* 2018) have suggested that inhibition of seed germination under saline

conditions could result from either osmotic or ionic effects of salinity. Our study showed seeds of this species exposed to 400 mM NaCl remained viable (93%), suggesting that germination was prevented by the osmotic effect of salinity. Similar results have been reported in some desert plants. For example, seeds of *Salvadora persica* failed to germinate at 400 mM NaCl (Hadi *et al.*, 2018), and most of ungerminated seeds of this species germinated readily when transferred to distilled water from 400 mM NaCl.

Conclusion

We conclude from this investigation that physical dormancy is present in the seeds of *Dodonaea viscosa* from Saudi Arabia, and that this dormancy can be broken equally by sulfuric acid (H₂SO₄), boiling water or dry heat 50°C treatments, all of which would disrupt the impermeability of the testa. Seeds from Saudi Arabia can germinate readily over a wide range of temperature in light or darkness, with an optimum temperature ranging 5°C – 25°C. The germination was clearly inhibited by higher temperatures. The seeds of this species are not salt tolerant but some germination can occur up to 300 mM NaCl.

Acknowledgments

This research was supported by King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia. We are very grateful to Prof Carol Baskin (University of Kentucky, USA) for her assistance in reviewing the manuscript.

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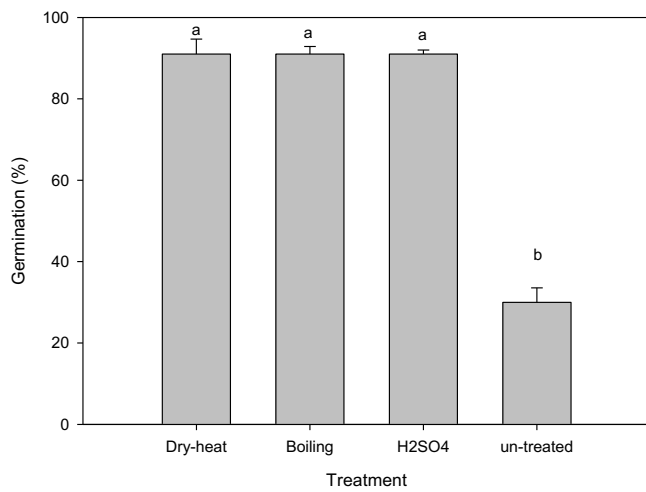


Fig. 1. The effect of sulfuric acid (H₂SO₄), boiling water and dry-heat on the final germination percentage (mean +se) of seeds of *Dodonaea viscosa* and seeds were exposed to 25°C (12 h light: 12 h dark), (Treatments that do not share a letter are significantly different).

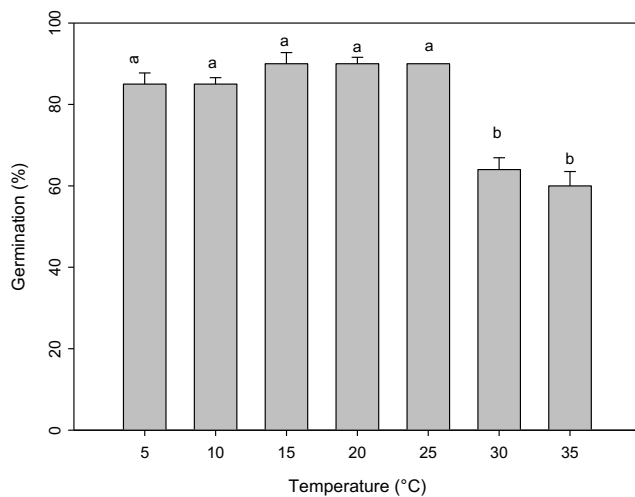


Fig. 2. The final germination percentage (mean +se) of seeds of *Dodonaea viscosa* at five constant temperatures (5, 10, 15, 20, 25, 30 and 35°C) (12 h light: 12 h dark). (Treatments that do not share a letter are significantly different).

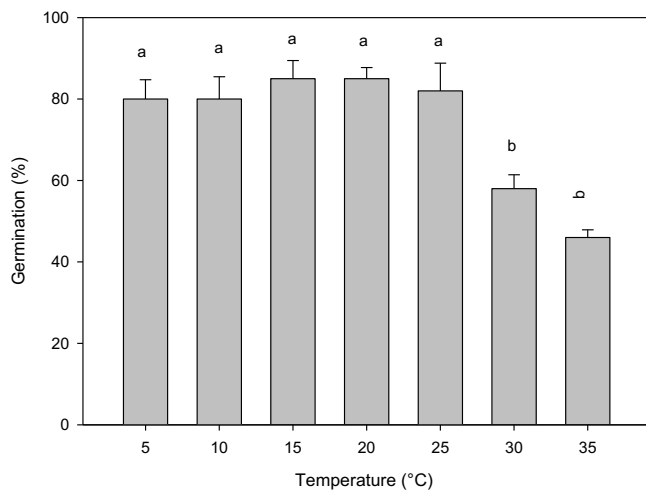


Fig. 3. The final germination percentage (mean \pm se) of seeds of *Dodonaea viscosa* at five constant temperatures (5, 10, 15, 20, 25, 30 and 35°C) (continuous darkness) (Treatments that do not share a letter are significantly different).

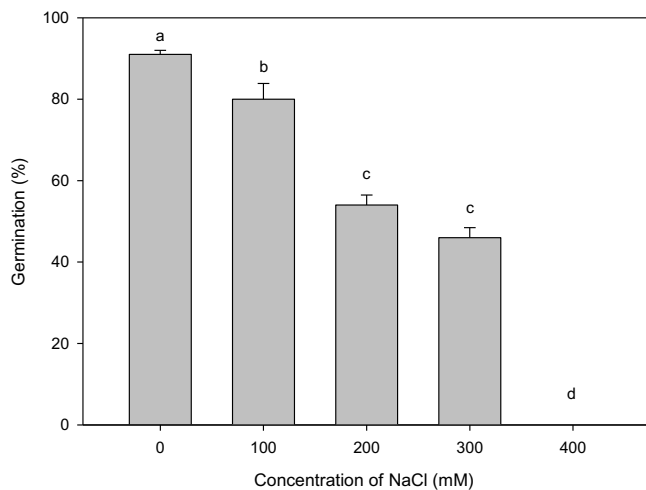


Fig.4. The effects of salinity (NaCl) on germination percentage (mean \pm se) of seeds of *Dodonaea viscosa* and seeds were exposed to 25°C., (12 h light: 12 h dark), (Treatments that do not share a letter are significantly different).