INTERACTIONS IN GERMINATION RESPONSES TO SALINITY, TEMPERATURE AND LIGHT IN THE DESERT SHRUB AFRICAN RUE (*PEGANUM HARMALA* L.)

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Abstract. The African rue (*Peganum harmala* L.) is an intriguing perennial herbaceous plant with medicinal benefits that grows in semi aridic soil in areas of steppe and sandy soils. In the present study, we collected the African rue plant from Harimla, Saudi Arabia, and analyzed the relationships among salinity lethal dose (LD), temperature and photoperiod, germination rate, changes in mean daily germination (MDG) and germination speed (T_{50}). Therefore, the purpose of this study is to establish a high knowledge of the *Peganum* responses to the temperature, salinity, and photoperiod stresses and the interaction between factor affecting germination of African rue seeds. Seeds of *Peganum* were germinated for 28 d by soaking in distilled water or 100 mM, 200 mM, 300 mM and 400 mM of NaCl in variable dark/light conditions and temperature regimes (10-20, 15-25, 20-30 and 25-35 °C). At light germination, DL_{50} was attained at 100 mM NaCl for T (10-20 °C), 200 mM for T (15-25 °C), 300 mM for T (20-30 °C) and 400 mM for T (25-35 °C). DL_{100} was attained only at 300 mM for T (10-20 °C) and 400 mM for T (15-25 °C). At a high temperature (more than 25), DL_{100} was not reached. At dark germination, DL_{50} was attained at 0 mM NaCl for T (10-20 °C and 15-25 °C) and 100 mM for T (20-30 °C and 25-35 °C). DL_{100} was attained only at 100 mM for T (10-20 °C) and 300 mM for T (15-25 °C, 20-30 °C and 25-35 °C.
Keywords: *environmental factors, germination, Nitrariaceae, seeds, tolerance, viability*

Introduction

The African rue is a native species of the family Nitrariaceae. It grows in degraded and metalliferous lands of Mediterranean countries as well as in some areas of China and Saudi Arabia (Suleiman et al., 2011; Nedjimi et al., 2019). This species is capable of reproducing by seed and vegetatively by lateral roots (Michelmore, 1997), and young seedlings are capable of resuming growth following extreme water deficits (Abbott and Sterling, 2006). The African rue showed resistance to external factors due to the influence of various environmental elements (Seilkhan and Kulmanov, 2018). African rue commonly occurs in disturbed areas such as roadsides, right-of-ways, and near livestock watering facilities (Abbott and Sterling, 2006). Numerous reports have shown that stresses dramatically affect the seed germination and initial seedling development of many plant species such as *Peganum* (Karakas, 2020).

Germination is an important process to produce a new generation. It begins by water absorption, reserve mobilization and elongation of the embryonic axis and the emergence of the root through the surrounding seed (Smiri and Missaoui, 2014). Intense metabolic activities, gene expression and enzymes synthesis were induced to hydrolyze reserves for seedling development (Smiri et al., 2015). This phase required a decrease in the mechanical strength of seed coats and an increase in water absorption. It is the most sensitive plant growth period for abiotic stress (Smiri et al., 2010). The characteristics of seeds germination present several highly interesting problems. Careful study of the factors controlling the seed germination and the complex interactions which may occur between these factors reveals many important bearings and could clarify many ecological

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aspects. External factors such as drought and salinity as well as endogenous factors such as phytohormones, proteins, transcription and other substances can affect germination (Ahmed and Khan, 2010).

Many studies reported that salinity affects seed germination and early seedling growth (Ahmed et al., 2020). The germination success of halophytes was attributed to their tolerance to various combinations of ions, differences in ion transport and their physiological role at the cellular level. Sodium chloride is the most common salt found in the saline soils of both inland and coastal salt marshes and deserts; however, other chlorides and sulfate salts are also present in saline habitats. Total dissolved salts (monovalent or divalent) influenced seed germination (Khan et al., 2015). Temperature affects seed germination by affecting ion uptake (Ruiz-Agudo et al., 2011), enzyme activity, membrane permeability, and seed coat hardness, which would help determine the appropriate time for seed germination in a natural environment (Singh et al., 2021). Light influenced seed germination by controlling ion movement, altering permeability and membrane potentials (Lazar et al., 2003) and pumps and channels regulation (Kendrick and Kronenberg, 2006). Ahmed et al. (2020) reported the best seed germination conditions for Playa *Peganum harmala* L. crops are in freshwater, 20/30 °C thermoperiod and 12 h photoperiod. In addition, seed maintains their viability under extreme harsh conditions like high salinity, complete darkness and sub-optimal temperature regimes.

Drastic reduction of biomass production and nutritional quality have been often observed in crops grown in contaminated environments. Temperature, light and salt can affect one or more vital physiological processes in plants. Seed germination is an important stage of plant life, which is highly sensitive to surrounding medium changes since the germinating seed is the first interface of material exchange between the plant cycle and the environment. One of the underlying metabolic activities following the imbibition of seed is the renewal of metabolism, so an attempt has been made in the present study to indicate how temperature, light and salt stresses might impair seed germination in the African rue from desert region. We looked at the effects of temperature, light and salt using seed germination assay of *Peganum harmala* L. as model crops in the laboratory conditions.

The present study was designed to test the following hypotheses (1) seeds of the African rue from desert region germinate optimally under non-saline conditions, (2) *P. hermala* germinate quickly under a non-saline medium, (3) its maximum seed germination occurs at 20/30 °C, (4) seed germination decreases under (24 h) dark, (5) the combination of unfavorable environmental conditions (salinity, temperature and light) synergistically inhibits seed germination in comparison to factors affecting independently and (6) seeds of the African rue maintain their viability under harsh environmental conditions. We hypothesised that *P. harmala* would have evolved seed germination characteristics that enable it to exploit episodes of rainfall in an extremely arid environment.

To define the link between hypotheses, the dataset used for testing and statistical test selected for proof, the effect of different concentrations of sodium chloride salt on seed germination of Saudi Arabia African rue under various temperature regimes and two photoperiods were analyzed. Two mechanisms can explain the effect of salt on the germination process. The first is that the negative effect of salt on germination success varies synergistically with temperature. The second is that the negative effect of salt on germination success varies synergistically with light. The findings add new knowledge to

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understanding the response of wild crops to different abiotic stresses in relation to different geographic and climatic conditions.

The arid climate of Saudi Arabia supports many medicinally important species, such as *P. harmala* (Osman and El-Hameid Abdein, 2019). The soil of Hrimla is dominated by sandy loamy soils with very little clay and organic matter, high calcium carbon content and has the size of sand and silt particulates. Chemical analysis showed low salinity and slight alkalinity (Al-Dosary, 2022). It represents a typical extreme arid ecosystem, with a very low precipitation less than 73 mm per annual (Almadini et al., 2021; Al-Saeedi, 2022). Germination behaviour is crucial to their establishment in the face of low rainfall and high summer temperatures that produce high evapotranspiration and salt accumulation in the surface soil. Understanding these adaptive characteristics will assist the development of effective strategies for the conservation of medicinally important species in arid environments.

Materials and Methods

Plant material and growth conditions

We looked at the effect of salinity, temperature and light on the seed germination of African rue. Plants are among the most important medicinal crops naturally grow anywhere and everywhere (Abd-Elgawad and Alotaibi, 2019). They are easily cultured and maintained in laboratory conditions.

Study site

Seeds of the African rue were collected from the Harimla, Saudi Arabia Latitude: 25.18 North Longitude: 46.31 East Altitude: 685.00 m/2247.38 ft. Rainfall is regular in pattern (Riyadh region Meteorological station, 2014 – 2019) being predominantly in winter and spring: January (10.0 mm), February (12.0 mm), March (23.0 mm), April (29.0 mm). The highest temperature recorded was 43.5℃, 44.2℃ and 45.1℃ for June, July and August respectively and minimum temperatures ranging from 11.3℃ for December, 9.2℃, for January and 11.2℃ in February. The pH of the soils in the habitats: 8.45. Electrical conductivity (EC) measurements was 0.51 mmhos cm^{-1}. Corresponding concentration of sodium and chloride ions was (0.82 mM $Na^{+}100\ g^{-1}$, 1.95 mM $Cl^{-}100\ g^{-1}$).

Seed collection and germination protocol

Mature seeds of the African rue were collected from 40-70 plants, randomly selected from natural populations in Harimla on 15 Mai 2018. Seeds were collected from individuals of the entire population to get an adequate representation of genetic diversity. Inflorescences were air dried, seeds were separated from spikes and cleaned before surface sterilization with 0.8% sodium hypochlorite for 1 min and after thorough washing and subsequent drying stored in plastic jars at 4 ℃.

Seeds were disinfected with 2% sodium hypochlorite for 10 min, rinsed thoroughly to remove disinfectant and soaked in distilled water at 4 ℃ for 30 min to obtain the initial stage. Seeds were placed in incubators under controlled conditions, in 9 cm diameter Petri dishes on three layers of Whatman No. 1 filter paper that had been moistened with 5 ml of distilled water or 100 mM, 200 mM, 300 mM and 400 mM of NaCl solution. Petri dishes were sealed with plastic film to prevent evaporation. Seeds were incubated at five

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alternating temperatures (10/20, 15/25, 20/30 and 25/35°C), with 12 h darkness and 12 h light each day or at 24 h-darkness. In every treatment, five replicates of 20 seeds were used. Seed germination was counted daily for 28 days.

Treatments: 4 alternating temperatures x 5 salinities x 2 light regimes x 5 replicate dishes = 200 Petri dishes (each containing 20 seeds).

The effect of fluctuating temperature, light, and salinity was studied in a standardized experiment for two reasons: an asynchronous experiment would be impractical because other factors could be involved. In addition, it will not be possible to know their combined effects unless they are simultaneous.

Final total germination was recorded as well as the time is taken to reach 50% of the final germination percentage, across all the replicates (T_{50}). Germination in the light was monitored each day, at which time germinated seeds were counted and removed from the Petri dishes, for 28 days. For continuous darkness, the dishes were wrapped in an aluminum foil to prevent any exposure to light and seed germination was counted only after 28 days at the end of the experiment.

Estimation of germination rate, mean daily germination and germination speed

The germination rate was calculated as a percentage of the control and germinating seed sampled for assays. Mean daily germination (MDG) was defined as the number of seeds germinating per day relative to the maximum number of germinated seeds. Germination speed was estimated by the average time to germination of 50% of the seeds. Each experiment was carried out at least twice.

Statistical analysis

Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance (Ahmed and Khan, 2010). We present three distinct experiments, each designed to address a different aspect of germination (the effects of alternating temperature, salinity and light). Each treatment consisted of five replicates, and each experiment was carried out at least twice. The significant effects ($P < 0.05$) of temperature, salinity, light and their interactions on seed germination and rate of germination were determined by analysis of variance (ANOVA) using SPSS version 23 for Windows (SPSS Inc., 2015). If the ANOVA indicated a significant effect, then post hoc Bonferroni tests were performed to compare treatment means (SPSS Inc., 2015).

Results

Interaction between light, temperature and salinity during germination of the African rue was done in *Table 1*.

Table 1. *Results of temperature (T), salinity (S) and their interactions on rate of seed germination of P. harmala in 12 h photoperiod condition for 28 days under 0-400 mM NaCl at 4 different temperature regimes*

Independent variables	T	S	T x S	Error
df	4	5	20	30
P. hermala	24.75***	38.00***	136.48***	MS :18.00

Superscript *** denotes significant difference at $P < 0.05$, Data represent F values

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Salinity significantly affected seed germination of *P. harmala* compared with the control (0 mM NaCl) (F=38:00). In the same way, an increase in the temperature regime significantly increased the germination rate (F=24.75). The combined effect of salinity and temperature was significantly different compared with the single effect of temperature or salinity with a factor F=136.48. When we increased the temperature, the germination rate increased and reached 100% without NaCl. When added NaCl, the germination rate of *P. harmala* seeds didn't reached 100% and the highest rate was obtained with the high temperature regime (25-35 °C) and with the smallest dose of NaCl (100%). We suggested antagonist effect between temperature and salinity on light seed germination of *P. harmala*. It appeared from results in *Table 2* the influence of temperature (F=32.19), salinity (F=78.00) and photoperiod (L=351.00) on seed germination of *P. harmala*.

Table 2. *Results of temperature (T), salinity (S), light (L), and their interactions on seed germination parameters; germination in 12 h-photoperiod condition for 28 days under 0-400mM NaCl at 4 different temperature regimes and germination in 24 h-dark condition for 28 days under 0-400 mM NaCl at 4 different temperature regimes of P. harmala*

Independent variables	**T**	**S**	**L**	**T x S**	**T x L**	**S x L**	**T x S x L**	**Error**
df	4	5	1	20	4	5	20	60
P. hermala	32.19*	78.00*	351.00*	208.58*	249.19*	7.6*	443.16*	MS : 37*

Superscript *** denotes significant difference at P < 0.0001, Data represent F values

Combined effects significantly changed when we modified conditions and significantly influenced seed germination rate with a factor F=208.58 (T x S), F=249.19 (T x L), F=7.6 (S x L) and F=443.16 (T x S x L). Light significantly induced seed germination and we reached 100% at T (25-35) and S (0 mM NaCl). At dark germination rate didn't reach 100% and it's decreased in present of low temperature, and high salinity. We suggested a synergic effect between temperature and light on seed germination of the African rue, and an antagonist effect between light and salinity. At the light, more seeds germinated in non-saline control and the inclusion of NaCl in the medium substantially inhibited seed germination and few seeds germinated at 400 mM NaCl (*Figure 1*).

At similar salinity concentrations, seed germination was significantly reduced in dark in comparison to light. No seeds germinated in the dark at a salinity concentration of 300 mM and above (*Figure 2*). The median lethal dose (LD_{50}) presented the smaller dose of NaCl that caused 50% of ungerminated seeds and lethal dose 100 (LD_{100}) was the smaller dose of NaCl that caused 100% of ungerminated seeds (*Table 3*). At light germination, DL_{50} was attained at 100 mM NaCl for T (10-20 °C), 200 mM for T (15-25 °C), 300 mM for T (20-30 °C) and 400 mM for T (25-35 °C). We observed from those results that DL_{50} values were proportional to temperature. DL_{100} was attained only at 300 mM for T (10-20 °C) and 400 mM for T (15-25 °C). At a high temperature (more than 25), DL_{100} was not reached. We suggested from the results that temperature and salinity had antagonist effect on seed germination rate. At dark germination, DL_{50} was attained at 0 mM NaCl for T (10-20 °C and 15-25 °C) and 100 mM for T (20-30 °C and 25-35 °C). DL_{100} was attained only at 100 mM for T (10-20 °C) and 300 mM for T (15-25 °C, 20-30 °C and 25-35 °C). It appeared that darkness had synergic effect with salinity on seed germination and antagonist effect with temperature.

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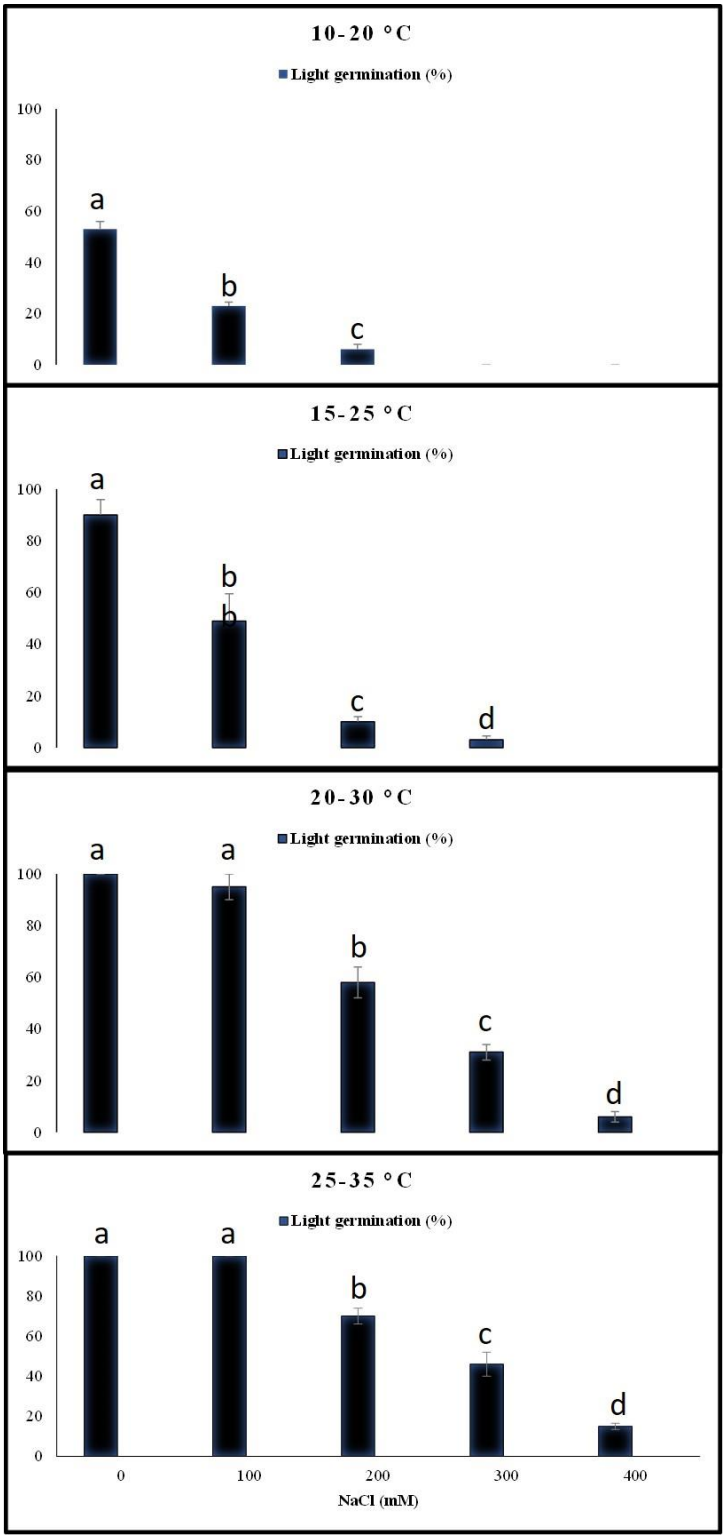

Figure 1. *Seed germination (%) of P. hermala. Data are the mean of two independent experiments (±SE). Each experiment was carried out with 2000 germinating seeds. Treatment: 4 alternating temperatures x 5 salinities x 1 light regime x 5 replicate dishes = 100 Petri dishes (each containing 20 seeds). Different letters indicate significant differences (P < 0.05) at 12 h-photoperiod (within one salinity and temperature level), and among different temperature regimes of non-saline control*

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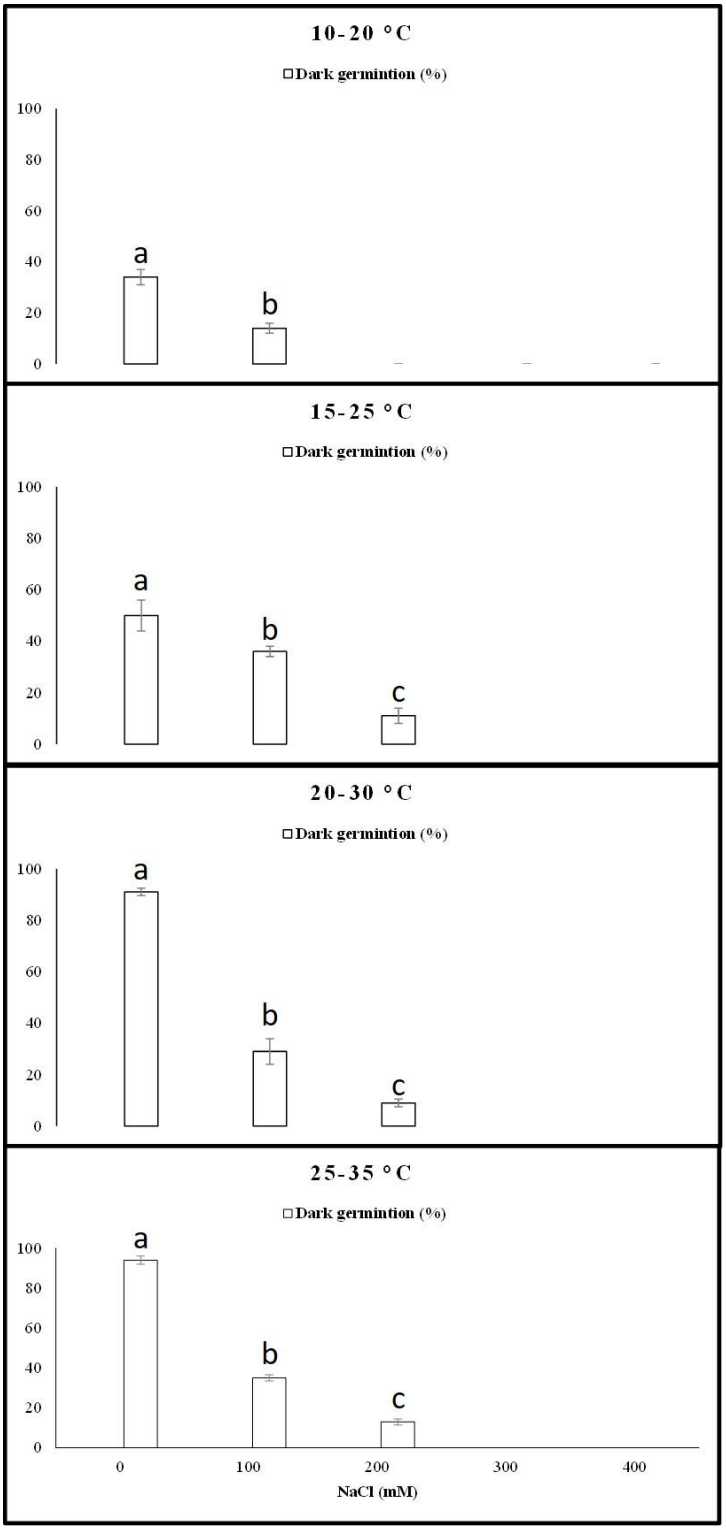

Figure 2. *Seed germination (%) of P. hermala. Data are the mean of two independent experiments (±SE). Each experiment was carried out with 2000 germinating seeds. Treatment: 4 alternating temperatures x 5 salinities x 1 light regime x 5 replicate dishes = 100 Petri dishes (each containing 20 seeds). Different letters indicate significant differences (P < 0.05) at 24 h-darkness (within one salinity and temperature level), and among different temperature regimes of non-saline control*

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Table 3. *Summary list of germination tests in experimental sequence of P. hermala with the salinity median lethal dose (LD_{50}) and lethal dose 100 (LD_{100})*

Treatments	Temperature °C	Salinity (mM)	Photoperiod	Germination rate (day 28)	DL_{50} (mM)	DL_{100} (mM)
1	10-20 °C	0	12-12h	53±3		
2	10-20 °C	100	12-12h	14±2	100 mM	
3	10-20 °C	200	12-12h	6±2		
4	10-20 °C	300	12-12h	0		300 mM
5	10-20 °C	400	12-12h	0		
6	15-25 °C	0	12-12h	90±6		
7	15-25 °C	100	12-12h	49±10.5		
8	15-25 °C	200	12-12h	10±2	200 mM	
9	15-25 °C	300	12-12h	3±1.5		
10	15-25 °C	400	12-12h	0		400 mM
11	20-30 °C	0	12-12h	100±0		
12	20-30 °C	100	12-12h	95±5		
13	20-30 °C	200	12-12h	58±6		
14	20-30 °C	300	12-12h	31±3	300 mM	ND
15	20-30 °C	400	12-12h	6±2		
16	25-35 °C	0	12-12h	100±0		
17	25-35 °C	100	12-12h	100±0		
18	25-35 °C	200	12-12h	70±4		
19	25-35 °C	300	12-12h	46±6		
20	25-35 °C	400	12-12h	15±1.5	400 mM	ND
21	10-20 °C	0	0-24h	34±3	0 mM	
22	10-20 °C	100	0-24h	14±2		
23	10-20 °C	200	0-24h	0		200 mM
24	10-20 °C	300	0-24h	0		
25	10-20 °C	400	0-24h	0		
26	15-25 °C	0	0-24h	50±6	0 mM	
27	15-25 °C	100	0-24h	36±2		
28	15-25 °C	200	0-24h	11±3		
29	15-25 °C	300	0-24h	0		300 mM
30	15-25 °C	400	0-24h	0		
31	20-30 °C	0	0-24h	91±1.5		
32	20-30 °C	100	0-24h	29±5	100 mM	
33	20-30 °C	200	0-24h	9±1.5		
34	20-30 °C	300	0-24h	0		300 mM
35	20-30 °C	400	0-24h	0		
36	25-35 °C	0	0-24h	94±2		
37	25-35 °C	100	0-24h	35±1.5	100 mM	
38	25-35 °C	200	0-24h	13±1.5		
39	25-35 °C	300	0-24h	0		300 mM
40	25-35 °C	400	0-24h	0		

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Rates of light seed germination (LSG), mean daily germination (MDG) and germination speed (T50) of *P. harmala* in response to variable salinity (0–400 mM NaCl) and temperature regimes (10-20, 15-25, 20-30 and 25-35 °C) were presented in *Table 4.*

Table 4. *Rates of light seed germination, mean daily germination (MDG) and germination speed (T_{50}) of P. hermala in response to variable salinity (0–400 mM NaCl) and temperature regimes (10-20, 15-25, 20-30 and 25-35 °C)*

Temperature	10-20			15-25			20-30			25-35		
	Germination rate	MDG	T_{50}	Germination rate	MDG	T_{50}	Germination rate	MDG	T_{50}	Germination rate	MDG	T_{50}
NaCl												
0	53^{a}	0.47	26	90^{e}	0.80	5	100^{h}	0.89	3	100^{h}	0.89	3
100	23^{b}	0.20	ND	49^{a}	0.43	26	95^{i}	0.84	4	100^{h}	0.89	3
200	6^{c}	0.05	ND	10^{f}	0.08	ND	58^{j}	0.51	11	70^{l}	0.62	15
300	0^{d}	0	ND	3^{g}	0.02	ND	31^{k}	0.27	ND	46^{a}	0.41	ND
400	0^{d}	0	ND	0^{d}	0	ND	6^{c}	0.05	ND	15^{m}	0.13	ND

Different letters via Bonferroni test indicate significant differences ($P < 0.05$) between salinity treatments (within one temperature treatment). ND, undefined

At 0 mM NaCl, MDG were about 0.47; 0.8; 0.89 and 0.89 seed/day and T_{50} were obtained after 26, 5, 3 and 3 days for temperature regimes (10-20, 15-25, 20-30 and 25-35 °C, respectively). After treatment with 100 mM NaCl, we showed a regression in MDG (0.20; 0.43; 0.84 and 0.89 seed/day for T: 10-20, 15-25, 20-30 and 25-35 °C, respectively) and T_{50} were obtained after 26, 4 and 3 days only for T: 15-25, 20-30 and 25-35 °C, respectively. 200 mM-treated seeds have MDG about 0.05; 0.08; 0.51 and 0.62 seed/day for T: 10-20, 15-25, 20-30 and 25-35 °C, respectively and T_{50} were obtained after 11 and 15 days only for T: 20-30 and 25-35 °C, respectively. For 300 mM-treated seeds, MDG were about 0; 0.02; 0.27; 0.41 seed/day and finally for 400 mM-treated seeds, MDG reached 0; 0; 0.05 and 0.13 seed/day but T_{50} were undefined (ND). It appeared from results presented in *Table 2* that salinity affected light germination process of *P. harmala* in negative correlation with temperature. With high temperature, germination speed, mean daily germination and germination rate increased and salinity effect regressed. To study the correlation between darkness, salinity and temperature in relation with dark seed germination (DSG), mean daily germination (MDG) and germination speed (T50) of *P. hermala* in response to variable salinity (0–400 mM NaCl) and temperature regimes (10-20, 15-25, 20-30 and 25-35 °C) were analyzed. Result presented in *Table 5* showed that MDG were about 0.30; 0.44; 0.81 and 0.83 seed/day and T_{50} were obtained after 15, 8, 5 for temperature regimes (15-25, 20-30 and 25-35 °C, respectively) when seeds grown without NaCl. Addition of 100 mM salt caused decrease in MDG to 0.12; 0.32; 0.25 and 0.31 seed/day for T: 10-20, 15-25, 20-30 and 25-35 °C, respectively) and T_{50} were undefined (ND). 200-mM treated seeds have MDG about 0; 0.09; 0.08 and 0.11 seed/day for T: 15-25, 20-30 and 25-35 °C, respectively. After treatment with 300 or 400 mM NaCl, in darkness, no germination for all seeds for different temperature regimes. Seed germination in complete darkness was substantially inhibited in comparison with 12h photoperiod. Light seemed to be a dominant factor in improving germination after salinity stress.

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Table 5. *Rates of dark seed germination, mean daily germination (MDG) and germination speed (T_{50}) of P. hermala in response to variable salinity (0–400 mM NaCl) and temperature regimes (10-20, 15-25, 20-30 and 25-35 °C)*

Temperature	10-20			15-25			20-30			25-35		
	Germination rate	MDG	T_{50}	Germination rate	MDG	T_{50}	Germination rate	MDG	T_{50}	Germination rate	MDG	T_{50}
NaCl												
0	34^{a}	0.30	ND	50^{d}	0.44	15	91^{e}	0.81	8	94^{e}	0.83	5
100	14^{b}	0.12	ND	36^{a}	0.32	ND	29^{a}	0.25	ND	35^{a}	0.31	ND
200	0^{c}	0	ND	11^{b}	0.09	ND	9^{b}	0.08	ND	13^{b}	0.11	ND
300	0^{c}	0	ND	0^{c}	0	ND	0^{c}	0	ND	0^{c}	0	ND
400	0^{c}	0	ND	0^{c}	0	ND	0^{c}	0	ND	0^{c}	0	ND

Different letters via Bonferroni test indicate significant differences ($P < 0.05$) between salinity treatments (within one temperature treatment). ND, undefined

Discussion

The African rue from the desert region presents a good germination success (20%) until a high dose of NaCl (400 mM), under light condition (24 h) and at high-temperature regime (25-35 C). Under the same conditions, *P. harmala* from the Playa region did not germinate (0%) (Ahmed and Khan, 2010). Seeds of *P. hermala* from desert regions germinate optimally under non-saline conditions (100%), quickly (T50 = 3 days), its maximum seed germination occurs at 20-30 °C (100%), seed germination decreases under (24 h) dark (96%), the combination of unfavorable environmental conditions (salinity, temperature and light) synergistically inhibits seed germination in comparison to factors affecting independently (0% germination with 400 mM NaCl, at 10-20 °C and at dark) and maintain their viability under harsh environmental conditions (400 mM of NaCl, at light condition (24 h) and at high-temperature regime (25-35 °C). The effect of external factors on the germination process of *Peganum* has been clarified. Effects vary with different factors and their severity. In previous studies by Al-Turki et al. (2022), the effects of factors on seed germination characteristics of some medicinally important desert plants from the Arabian Peninsula were studied in a separate way or in different circumstances than what was addressed in this study. Our results were sometimes close to previously published and sometimes different. The difference is sometimes due to the type of plant and in many cases to the external factor and its severity. The most important feature of this study is to clarify that the percentage of germination of *Peganum* is reduced at low temperature, high salinity and in the absence of lighting. Seed germination of *P. harmala* of the Saudi Arabia region was inhibited more in dark in comparison with light conditions. High salinity and less optimal temperatures have a synergistic effect in inhibiting seed germination. We observed from the results that light caused significant effects on the seed germination rate of *P. harmala* at different tested temperatures and only for 0, 100 and 200 mM NaCl. We suggested a positive effect of light on seed germination of *P. harmala* in conditions of salinity and temperature stress. Salinity and darkness reduced germination of *P. hermala*, however, temperature seemed to be a dominant factor in improving germination after the removal of salinity stress. With light, germination speed, mean daily germination and germination rate increased and salinity effect regressed. It appeared from results presented in Table 5 that darkness retarded seed

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germination of *P. harmala*. In dark seed germination, high temperature partially improved germination for low concentration of NaCl (0-300 mM). When compared with germination percentages obtained at different temperatures regime, the germination at 25-35 °C was considered optimum germination temperature by comparison with all other temperature's regimes at light and dark conditions. This weed is adapted to arid regions. Presence of salinity and absence of light reduced germination (Ahmed and Khan, 2010). Authors showed optimal responses for seed germination of *P. harmala* at light with temperature regime of 20-30 °C. Elimination of salinity stress while providing light and optimal temperature resulted in almost complete recovery of *Hemianthus glomeratus* seed germination and only partial for *Lepidium latifolium* and *P. hermala*. Halophytes species are quite sensitive to light variation during seed germination (Baskin and Baskin, 1998; Khan and Qaiser, 2006), and this is true for *P. hermala*. Ahmed and Khan (2010) reported no seed germination in dark both under saline and non-saline conditions for *L. latifolium*, while germination of *P. harmala* and *H. glomeratus* was substantially inhibited. Baskin and Baskin (1998) reported that 22 out of 27 species respond similarly to both light and dark condition. Light-requiring seeds tend to germinate at a time when other types of stresses are relatively low (Zheng et al., 2005). Ability of seeds to endure high salinity stress under both light and dark conditions was analyzed for *P. harmala*. Tolerance and recovery from salinity stress is species specific (Song et al., 2006), 500 mM NaCl for *Limonium stocksii* (Zia and Khan, 2004) and *Medicago ruthenica* and *Salsola affinis* (Wei et al., 2008; Guan et al., 2009) and *Halostachys capsica* (Zheng et al., 2005). However, seeds of other halophytes did not recover or showed little recovery response when subjected to high salinity stress (Khan and Gul, 2006). Ahmed and Khan (2010) showed that rate of germination of *P. hermala* was significantly decreased with an increase in salinity and also at temperature regimes higher or lower than optimal (20/30 °C), *L. latifolium* and *P. harmala* are moderately salt tolerant (300 mM NaCl) (Khan and Ungar, 1997). Other species like *H. glomeratus* have only few seeds germinated at 500 mM-800 mM NaCl (Khan et al., 2002). Some Halophytes are reported to be very highly tolerant to NaCl, like *Salicornia europaea* and *Allenrolfea occidentalis* (800 mM NaCl) (Guan et al., 2009), *Salicornia rubra*, *Kochia scoparia*, *Sarcobatus vermiculatus* and *Suaeda moquinii* (1000 mM NaCl) (Khan et al., 2002) and *Kochia americana* (1700 mM NaCl) (Clarke and West, 1969). Song et al. (2006) reported that seed germination depends on the range of salt tolerance and temperature. Previous studies reported that salinity affected seed germination and early seedling growth, especially on the thermoperiod and photoperiod (Ahmed and Khan, 2010; Rasheed et al., 2019). The effect of light on seed germination was previously documented, especially with desertic plant species, demonstrated that light positively affected germination in *A. cruentus*, *B. rapa* subsp. *Chinensis*, *C. olitorius*, *C. lanatus* and *S. retroflexum* (Motsa et al., 2015), *Halogeton glomeratus, Lepidium latifolium,* and *Peganum harmala* (Ahmed et al., 2020).

Genetic response of *P. harmala* was examined previously and authors proposed up-regulation and down-regulation of genes subsequent to salinity to explain response of seedlings for two salinity levels (150 and 200 mM NaCl) (Karam et al., 2016). Temperature influenced seed germination rate of three salt playa halophytes, *Halogeton glomeratus*, *Lepidium latifolium*, and *Peganum harmala* (Ahmed et al., 2014). Transition between seed dormancy and germination of halophytes is highly regulated by endogenous chemicals, which maximizes the chances of seedling establishment in variable and stressful environmental conditions, particularly in the absence of light (Gul et al., 2000). Seeds of *P. harmala* were non-dormant, while the presence of salinity and/or the absence

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of light caused enforced dormancy (Ahmed and Khan, 2010). Hussain and Nasrin (1985) reported that optimum light germination temperature of *P. harmala* seeds is 25–30 °C. Bozoğlu (1999) has determined that minimum light germination temperature of African rue seeds is 10-15 °C, optimum light germination temperature is 25–35 °C and maximum germination temperature is above 35 °C. Solak et al. (2015) showed that light germination speed and duration of *P. harmala* seeds change depending on the temperature. On the other hand, light seed germination of *P. harmala* decreased an increase of salinity concentrations (Amartuvshin, 2013). In previous study (Basahi, 2018), the *Anabasis setifera* seeds completed germination at 15/5, 20/10, and 20 °C; a higher percentage of seeds completed germination in light than in the dark at 20/10 and 25/15 °C and decreased as salinity increased from 0 to 700 mmol/L NaCl. Ahmed et al. (2020) and Rasheed et al. (2019) reported that *P. harmala* seed germination and recruitment must be precisely adapted to a specific time when soil moisture, salinity, temperature, and light are appropriate and successful recruitment could be achieved.

Conclusions

In conclusion, seed germination of the African rue depended on the light, salt concentration and temperature. *P. harmala* preferred light condition to make germination of seeds. The optimum temperature for all treatment tests was from regime 25-35 °C. Presence of NaCl in medium culture inhibited seed germination of tested plant for all temperature's regime in dark and light conditions. In addition, we analyzed the successful of seed germination and reported the effects of different environmental stresses on mean daily germination (MDG) and germination speed (T_{50}). All results proved significantly effects (increase of seed germination when temperature increased, and under light condition, but decreased when NaCl concentration increased). In darkness, we showed decrease in MDG and delay in seed germination (in major tests, T_{50} was not defined). We suggested synergic effect between light and temperature caused increase in seed germination when they are increased, and antagonist effect with salinity that caused decrease when NaCl concentration increased. Understanding the African rue adaptive characteristics will assist the development of effective strategies for the conservation of medicinally important species in arid environments.

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