Pak. J. Bot., 49(6): 2079-2088, 2017.

EFFECT OF HUMIC ACID ON SEED GERMINATION OF SUB-TROPICAL HALOPHYTES UNDER SALT STRESS

SONIA BANO ABBASI[1], BILQUEES GUL[1*], NASIRUDDIN KHAN[2], SALMAN GULZAR[1], AND MUHAMMAD ZAHEER AHMED[1]

[1]*Institute of Sustainable Halophyte Utilization, University of Karachi, Karachi-75270, Pakistan*
[2]*Department of Chemistry, University of Karachi, Karachi-75270, Pakistan*
[*]*Corresponding author's email: bilqueesgul@uok.edu.pk; Phone: (9221) 34820253; Fax: (9221) 34820258*

Abstract

This study was carried out to determine the effects of exogenous applications of three humic acid types (HA1, HA2, HA3) and their concentrations (250 and 500 mg L^{-1}) on seed germination of ten halophytes. The species included *Aeluropus lagopoides, Arthrocnemum macrostachyum, Cyperus conglomeratus, Desmostachya bipinnata, Halocnemum strobilaceum, Halopeplis perfoliata, Halopyrum mucronatum, Phragmites karka, Sporobolus ioclados* and *Urochondra setulosa* whereas, treatments used were (1) thermoperiod (10/20 and 20/30°C); (2) photoperiod (12/12h, light/dark; and 24h dark) under non-saline conditions. Effects of humic acids on alleviation of seed germination were also studied in response to 1) the individual and combined effects of NaCl treatments (reported to cause 50% germination inhibition in species tested) and 2) photoperiod (12/12h, light/dark; and 24h dark). In non-saline conditions, humic acid did not alleviate seed germination under the sub-optimal thermoperiod (10/20°C) in most halophyte seeds studied except for that of HA1 in *A. lagopoides* (34%), *A. macrostachyum* (15%), *D. bipinnata* (12%) and *H. perfoliata* (20%). Humic acid did not improve seed germination in either photoperiod under non-saline conditions at the optimal thermoperiod (20/30°C). In the presence of the light (12/12h; light/dark photoperiod), humic acid alleviated seed germination of *U. setulosa* under saline conditions but not in any of the other test species. Under saline condition and complete darkness, the lower concentration treatment (250 mg L^{-1}) of all three humic acid types significantly improved seed germination of *A. lagopoides*, *C. conglomeratus*, *D. bipinnata*, *H. perfoliata*, *P. karka* and *U. setulosa.* Under saline conditions and absence of light, higher concentration (at 500 mg L^{-1}) of all humic acid types alleviated seed germination in *S. ioclados* however, no effect of either humic acid type was observed on seed germination of *H. strobilaceum*. In conclusion, HA could partially substitute the light requirement for seed germination of test species under saline conditions.

Key words: Halophytes, Humic acid, Light, Seed germination, Temperature.

Introduction

About 7% of the world's total land area is affected by salinity. In Pakistan, more than 6.3 million ha of agricultural land has become saline (Yensen, 1995; Flowers & Colmer, 2008). This has led to decrease in crop yield especially in arid and semi-arid regions (Yensen, 1995; Tester & Davenport, 2003; Ashraf & Foolad, 2007; Flowers & Colmer, 2008). Halophytes are naturally resistant to high soil salinity and appear to have an obligate salt requirement for optimal growth however, most are sensitive to saline environment sat seed germination (Bewley & Black, 1982). Most halophytes can resist considerably higher levels of salinity compared to salt sensitive crops at the germination level. Osmotic and/or ionic effects of salinity on seed germination have been studied at physiological, biochemical and molecular levels (Mahajan & Tuteja, 2005; Munns & Tester, 2008). Halophyte seed germination is usually higher in the absence of salinity (Khan *et al*., 2001; Bu *et al*., 2008; Guan *et al*., 2009). Considerable variation in salt resistance at germination has been reported for some halophytes from low (*Halopeplis perfoliata*, Mahmoud *et al*., 1983), to substantially high levels (*Salicornia herbacea*,1700 mM NaCl).Seed germination of *Sarcocornia fruticosa*, *Arthrocnemum macrostachyum* and *Halocnemum strobilaceum* was inhibited by osmotic rather than ionic effects under saline conditions (Pujol *et al*., 2000). Whereas, inhibition of seed germination in *Aristida adscensionis*, *Artemesia ordosica* (Tobe *et al*., 1999) and *Prosopis strombulifera* (Sosa *et al*., 2005) was caused by ion toxicity. Inhibition of seed germination could occur due to disturbances at various stages such as imbibition, cell-wall loosening, mobilization of food reserves and radicle elongation (Khajeh-Hosseini *et al*., 2003; Kaya *et al*., 2006).

Other environmental extremes affecting seed germination include light and temperature (Zehra *et al*., 2013). Phytochromes are reported to be involved in light responses of photoblastic seed during germination (Fenner & Thompson, 2005). Halophytes vary in their optimal light and temperature requirement for seed germination (Gul *et al*., 2013).Sub-optimal temperatures also inhibited seed germination possibly by altering ion flux and enzyme kinetics (Tlig *et al*., 2008). Most sub-tropical halophytes germinate best at moderate (20/30°C; night/day) thermoperiods while cooler (10/20°C) temperature regimes are inhibitory for seed germination (Khan & Gul, 2002; Gul *et al*., 2013).

Exogenous application of chemicals could alleviate seed germination of halophytes under sub-optimal photoperiod and thermoperiod and salinity regimes (Gulzar & Khan 2001; Masciandaro *et al*., 2002; Bartels & Sunkar, 2005; Yamaguchi & Blumwald, 2005). Application of GA3, kinetin, thiourea, nitrate, ethephone, fusicoccin, proline, and betaine alleviated seed germination of halophytes (Gulzar & Khan, 2001; Ahmed *et al*., 2014). Humic acid could also be used to improve seed germination and improve our understanding of underlying mechanisms of salt resistance of halophytes at this critical stage of life cycle (Khalesro *et al.,* 2015). Humic acid is the main organic constituent of municipal waste compost (Senesi *et al*., 1996).Higher seed germination due to humic acid under saline conditions is attributed to improved soil quality(higher microorganism population, nutrient status, water holding capacity, organic content (Mcdonnell *et al.,* 2001). Humic substances are previously reported with similar effect to plant hormones (Nardi *et al*., 2002; Pizzeghello *et al*., 2013). Applications of humic acid increased seed germination of parsley, celery and leek at various temperature regimes (Walker & Bernal, 2004) and of barley, cowpea, wheat, bean, watermelon, geranium, cucumber and marigold seeds under saline and non-saline conditions (Hartwigsen & Evans, 2000; El-Hefny, 2010; Szczepanek & Wilczewski, 2011; SilvaMatos *et al*., 2012). Literatu research between "1966-2016" using the ISI web of knowledge and using the key words "*humic acid"AND "halophyte"* revealed only 11 research articles.

The present study investigated: 1) the effects of three humic acids on seed germination of ten sub-tropical halophytes in response to various thermoperiod and photoperiod regimes with and without NaCl treatments.

Materials and Methods

Seed collection and storage: Seeds of ten halophytic species (*Aeluropus lagopoides*, *Arthrocnemum macrostachyum*, *Cyperus conglomeratus*, *Desmostachya bipinnata*, *Halocnemum strobilaceum*, *Halopeplis perfoliata*, *Halopyrum mucronatum*, *Phragmites karka*, *Sporobolus ioclados* and *Urochondra setulosa*), were collected from their populations. The list of test species and experimental conditions used in this study are given in Table 1. Seeds were detached from inflorescence, surface sterilized with 0.85% Clorox (commercial NaOCl), air dried and stored in a plastic bottle at 4ºC.

Extraction of humic acid (HA) from coal: Coal samples were thoroughly washed, oven dried for 24 h at 105°C and ground to mesh size #100. Humic acid was extracted from coal (10g) added to 50 ml of 0.25 M NaOH and allowed to mix on an orbital shaker for 24 h. The samples were centrifuged at 1000 r.p.m. and filtered thrice to separate from residue. The filtrate was acidified to pH 1 with the help 6 M HCl and precipitates allowed to settle overnight before centrifugation to recover the humic acid crystals. The crystals of humic acid were washed with 20 ml of 0.5% hydrochloric-hydrofluoric acids (HCL-HF) solution and then repeatedly rinsed with distilled water to eliminate inorganic impurities. After drying the purified HA for 24h at 105 °C the yield of HA was calculated.

Seed germination tests: All germination experiments were carried out in 50 × 9 mm, air-tight, plastic Petri plates. Seeds were treated with three humic acid types; HA1 and HA2 (derived from mined coal) and HA3 a commercially available (Sigma Aldrich-53680-10G) form of humic acid. Four replicates of twenty-five seeds each filled with 5 ml of respective test solution were used in this study.

Experiment-1

Effect of humic acid and thermoperiod on seed germination of halophytes under non-saline conditions: Seeds of each of the ten above-mentioned halophyte species, were placed in programmed incubator sat 10/20 and 20/30°C (12/12h; night/day) thermoperiod and allowed to germinate in distilled water with and without HA1, HA2 and HA3 at 250 and 500 mg L^{-1}. Seed germination was recorded every alternate day for 20 d.

Experiment-2

Effect of humic acid and photoperiod on seed germination of halophytes under non-saline conditions: The treatments consisted of non-saline with three concentration (0, 250 and 500 mg L^{-1}) of different types of HA (HA1, HA2, HA3). The seeds were placed in the Petri plates and immersed with test 5ml solutions under programmed incubators (Percival, Boone, USA) with optimal regime (20/30°C - night/day), photoperiod (12/12h, light/dark; and 24h dark) (25μmol photons m^{-2} s^{-1}; 400–700 nm Sylvania cool white lamp).

Table 1. List of selected ten halophytes with the detail of experimental conditions.

Species	SAL	PP	TP (ºC)	Reference
Aeluropus lagopoides	0, 500	L /D	10/20; 20/30	(Gulzar & Khan, 2001)
Arthrocnemum macrostachyum	0, 500	L	10/20; 20/30	(Khan, 1999)
Cyperus conglomeratus	0, 100	L /D	10/20; 20/30	(El-Keblawy *et al*., 2009)
Desmostachya bipinnata	0, 500	L /D	10/20; 20/30	(Gulzar *et al*., 2007)
Halocnemum strobilaceum	0, 500	L /D	10/20; 20/30	(Qu *et al*., 2007)
Halopeplis perfoliata	0, 500	L /D	10/20; 20/30	(Rasool *et al*., 2016)
Halopyrum mucronatum	0, 300	L	10/20; 20/30	(Noor & Khan, 1995)
Phragmites karka	0, 500	L/D	10/20; 20/30	(Zehra *et al*., 2013)
Sporobolus ioclados	0, 300	L/D	10/20; 20/30	(Gulzar & Khan, 2003)
Urochondra setulosa	0, 200	L /D	10/20; 20/30	(Gulzar *et al*., 2001)

Sal-salinity (mM NaCl); PP- photoperiod (L: 12 h /12 h light and dark, D: 24h dark); TP- thermoperiod (ºC) (based on mentioned references)

Table 2. Analysis of variance showing effect of humic acid types (HAT) and concentrations (HAC) on final seed germination of test species under 12 h photoperiod (dark/light) at different thermoperiods (TP) .

Species	HAT	HAC	TP	HAT*HAC	HAT*TP	HAC*TP	HAT*HAC*TP
A. lagopoides	382^{***}	154^{***}	989^{***}	69^{***}	86^{***}	36^{***}	29^{***}
A.macrostachyum	138^{***}	236^{***}	3^{ns}	102^{***}	67^{***}	245^{***}	56^{***}
C. conglomeratus	4764^{***}	2^{ns}	3724^{***}	2^{ns}	844^{***}	10^{***}	7^{***}
D. bipinnata	39^{***}	23^{***}	266^{***}	6^{**}	5^{ns}	17^{***}	5^{**}
H. strobilaceum	108^{***}	11^{***}	5^{ns}	5^{**}	50^{***}	19^{***}	5^{**}
H. perfoliata	2945^{***}	1407^{***}	1764^{***}	311^{***}	307^{***}	2^{ns}	51^{***}
H. mucronatum	15^{***}	0^{ns}	96^{***}	5^{**}	7^{***}	12^{***}	7^{***}
P. karka	16^{***}	1^{ns}	62^{***}	13^{***}	4^{ns}	0^{ns}	5^{**}
S. ioclados	7^{**}	285^{***}	651^{***}	8^{***}	36^{***}	341^{***}	11^{***}
U. setulosa	6^{**}	32^{***}	441^{***}	17^{***}	45^{***}	6^{ns}	1^{ns}

Experiment-3

Effect of humic acid and photoperiod on seed germination of halophytes under non-saline and saline conditions: The treatments consisted of two NaCl concentrations (non-saline and inhibitory, concentration as mentioned in Table 1) with three concentration (0, 250 and 500 mg L^{-1}) of HA (HA1, HA2, HA3). The seeds were placed in the petri plates and immersed with test solutions under programmed incubators with optimal temperature regime (20/30°C - night/day), photoperiod (12/12h, light/dark) (25µmol photons m^{-2} s^{-1}; 400–700 nm Sylvania cool white lamp). Seeds of test species (light sensitive seeds Table 2) were also germinated in complete darkness where germination of seeds kept under 24h darkness was noted only once after 20 days. Seeds were considered to be germinated with radical emergence (Bewley & Black, 1994).

Statistical analysis: Germination data were arcsine transformed before statistical analyses to test for homogeneity of variance. Three-way analysis of variance (ANOVA) were performed with the help of SPSS version 11.0 for Windows (SPSS Inc., 2006) to determine the individual and interactive effects of various parameters as follows:

1. HA types x HA concentrations x thermoperiods
2. HA types x HA concentrations x photoperiods

3a. HA types x HA concentrations x NaCl treatments (in 12/12h; dark/light photoperiod)

3b. HA types x HA concentrations x NaCl treatments (in 24 dark photoperiod)

The post-hoc Bonferroni test was also carried out to determine differences between individual treatment means. ANOVA and Bonferroni tests were conducted at $p<0.05$, on a maximum total of 100 values for seed germination (in 4 replicates with 25 sub-replicates each).

Results

Experiment-1

Effect of humic acid and thermoperiod on seed germination of halophytes under non-saline conditions: The three-way analysis of variance (ANOVA) indicated significant effects ($p<0.05$) of HA type and its interaction with HA concentration and thermoperiod, except for *U. setulosa* (Table 2) HA1 significantly ($p<0.0$) mitigated inhibitory effect of thermoperiod compared to un-treated controls at 10/20°C on seed germination of *A. lagopoides* (34%; 250 mg L^{-1} HA), *A. macrostachyum* (15%; 500 mg L^{-1}), *D. bipinnata* (12%; both 250 and 500 mg L^{-1}) and *H. perfoliata* (20%), respectively (Supplementary Table 1) However, HA inhibited seed germination of *C. conglomeratus* and *P. karka* at 10/20°C, while germination remained unaffected in case of *S. ioclados*, *H. strobilaceum*, *H. mucronatum* and *U. setulosa* (Supplementary Table 1).

Experiment-2

Effect of humic acid and photoperiod (12/12h; light/dark and 24h dark) under non-saline conditions at 20/30°C: The three-way analysis of variance (ANOVA) indicated significant effects of photoperiod (except for *A. lagopoides* and *P. karka*) among all test species while there were no interactive effects of all three parameters taken together (Table 3). Two interactions of HA type with HA concentration and of HA type with photoperiod (except for *C. conglomeratus*) were also non-significant. In general, humic acids showed no alleviation of seed dormancy in response to photoperiod under non-saline conditions and except for *C. conglomeratus* (in 12/12h; light/dark photoperiod) compared to un-treated controls (Supplementary Table 2).

Experiment-3

a. Effect of humic acid and salinity treatments on seed germination in light (12/12h; dark/light) at 20/30°C: Three-way ANOVA of HA type, HA concentration, NaCl treatments and their interactions showed significant ($p<0.05$) individual and combined effects on seed germination of *A. lgopoides, C. conglomeratus, D. bipinnata, H. perfoliata ,S. ioclados* and *U. setulosa* (Table 4). Salinity effects were highly significant ($p<0.001$) among all test species as in case of HA type except for *H. mucronatum*, which remained generally unaffected by HA type, HA concentrations and their interactions (Table 4). However, HA concentration did not affect seed germination of *A. macrostachyum*, *H. mucronatum* (Supplementary Table 2), *P. karka* and *U. setulosa.* Among all species tested, the substantial alleviatory effect of HA (particularly 500 mg L^{-1}) under saline conditions, was observed only in *U. setulosa* with either an inhibitory effect or no effect in the other species (Figs. 1-8).

Table 3. Analysis of variance showing effect of humic acid types (HAT) and concentration (HAC) on final seed germination of test species under 12 h photoperiod (dark/light) and 24 hours darkness.

Species	HAT	HAC	PP	HAT*HAC	HAT*PP	HAC*PP	HAT*HAC*PP
A. lagopoides	1.6^{ns}	8.5***	1.5^{ns}	0.3^{ns}	0.4^{ns}	5.6**	0.3^{ns}
C. conglomeratus	15.4***	0.5^{ns}	40.6***	0.7^{ns}	4.1*	0.3ns	0.4^{ns}
D. bipinnata	1.6^{ns}	2.6*	13.6***	0.9^{ns}	0.2^{ns}	2.6*	0.4^{ns}
H. strobilaceum	1.0^{ns}	7.9**	891***	0.6^{ns}	2.2^{ns}	1.6^{ns}	0.2^{ns}
H. perfoliata	1.6^{ns}	11.3***	342.5***	0.6^{ns}	0.1^{ns}	0.8^{ns}	0.5^{ns}
P. karka	2.8*	0.3^{ns}	22.2***	1.7^{ns}	1.3^{ns}	0.5^{ns}	2.1^{ns}
S. ioclados	0.3^{ns}	12.1***	8.8**	0.3^{ns}	0.5^{ns}	5.5**	0.3^{ns}
U. setulosa	0.2^{ns}	1.5^{ns}	3.5*	0.7^{ns}	0.4^{ns}	0.7^{ns}	1.2^{ns}

Table 4. Analysis of variance showing effect of humic acid types (HAT) and concentrations (HAC) on final seed germination of test species under 12 h photoperiod (dark/light) under salinity.

Species	HAT	HAC	SAL	HAT*HAC	HAT*SAL	HAC*SAL	HAT*HAC*SAL
A. lagopoides	151***	146***	5596***	39***	118***	48***	34***
A. macrostachyum	7.0**	2^{NS}	98***	6***	6***	9***	3^{ns}
C. conglomeratus	158***	36***	2172***	38***	64***	8***	6**
D. bipinnata	10***	54***	1360***	7***	13***	7**	5**
H. strobilaceum	10***	6***	191***	1^{ns}	4^{ns}	1^{ns}	2^{ns}
H. perfoliata	61***	404***	3472***	19***	101***	71***	26***
H. mucronatum	1 ^{ns}	1 ^{ns}	47***	2^{ns}	0^{ns}	1^{ns}	1^{ns}
P. karka	23***	1 ^{ns}	639***	19***	6*	4^{ns}	4^{ns}
S. ioclados	69***	1671***	5828***	61***	33***	888***	19***
U. setulosa	15***	3 ^{ns}	362***	7***	33***	258***	29***

Table 5. Analysis of variance showing effect of humic acid types (HAT) and concentrations (HAC) on final seed germination of test species under 24 h darkness under salinity.

Species	HAT	HAC	SAL	HAT*HAC	HAT*SAL	HAC*SAL	HAT*HAC*SAL
A. lagopoides	36***	871***	471***	11***	7**	585***	5**
C. conglomeratus	1535***	12***	2249***	44***	507***	62***	35***
D. bipinnata	10***	18***	28***	4^{ns}	2^{ns}	11***	3^{ns}
H. strobilaceum	2^{ns}	111***	55***	5**	1^{ns}	37***	0^{ns}
H. perfoliata	26***	199***	290***	31***	1***	163***	5***
P. karka	52***	15***	517***	49***	22***	59***	25***
S. ioclados	24***	296***	113***	0^{ns}	17***	1059***	1^{ns}
U. setulosa	0^{ns}	18***	240***	14***	28***	22***	4^{ns}

Experiment-3

b. Effect of humic acid and salinity treatments on seed germination in dark (24h complete darkness) at 20/30ºC: Three way ANOVA indicated significant effects of HA concentration and salinity treatments and their interaction on seed germination of all halophyte test species under complete darkness (except for *D. bipinnata*, *S. ioclados* and *U. setulosa*) (Table 5). In general, all HA types (250 mg L^{-1}) enhanced seed germination of *A. lagopoides* (60%) (Fig. 1), *C. conglomeratus* (20%) (Fig. 2), *D. bipinnata* (20%) (Fig. 3), *P. karka* (60%) (Fig. 6), *H. perfoliata* (10%) (Fig. 5) and *U. setulosa* (60%) (Fig. 8), except of *S. ioclados* (20%) (Figure 7) where high concentration of HA (500 mg L^{-1}) was more effective in comparison with untreated seeds for each species in complete dark along with salinity. However, HA showed no effect on seed germination of *H. strobilaceum* (Fig. 5).

Discussion

Humic acid was reported to improve seed germination of many cultivated species (Sera and Novak, 2011; Turkmen *et al*., 2004; Antosova *et al*., 2007; Namdaran-Gooran *et al*., 2014; Akinci, 2009; Patil & Wadje, 2010; Masciandaro *et al*., 2007; Ebrahimi & Miri, 2016). However, inhibitory effects of HA were also reported for germination of barley seeds (Cavusoglu *et al*., 2015), with no effect in lettuce and tomato (Piccolo *et al*., 1993; Turkmen *et al*., 2004; Antosova *et al*., 2007).

Table 6. Summary of the effects of humic acid (HA1) on seed germination responses of ten halophytic species under sub-optimal salinity (50% inhibition), photoperiod (24h dark) and temperature (10/20ºC).

Species	Seed germination		
	High salinity	Complete dark and saline	Sub-optimal temperature
Aeluropus lagopoides	↓	↑	↑
Arthrocnemum macrostachyum	↓	ND	↑
Cyperus conglomeratus	—	↑	—
Desmostachya bipinnata	↓	↑	↑
Halocnemum strobilaceum	—	—	—
Halopeplis perfoliata	↓	↑	↑
Halopyrum mucronatum	—	ND	—
Phragmites karka	↓	↑	↓
Sporobolus ioclados	↓	↑	—
Urochondra setulosa	↑	↑	↓

Arrow indicate the significant increase (upward) or decrease (downward compared to their respective controls. Horizontal lines indicate no change while ND indicates species not use in that particular experiment

Experiment-1

Humic acid and theromoperiod effects: The influence of HA during seed germination at sub-optimal temperature and photoperiod was also determined. HA1 partially alleviated the effects of the lower temperature regime (10/20ºC) on seed germination of *A. lagopoides* and *A. macrostachyum* whereas, HA2 and HA3 mitigated this effect in case of *U. setulosa* and *P. karka.* No effect of HA was found on seed germination of *A. macrostachyum, D. bipinnata, H. strobilaceum, H. mucronatum* and *S. ioclados* at 10/20ºC. Humic acid is reported to increased seed germination of parsley, leek, celery, tomato, lettuce, basil, radish and garden cress vegetable species in a wide range of temperatures (Yildirim *et al*., 2002). Sub-optimal temperatures either delay seed germination or induce seed dormancy by altering hormonal balance. The kinetin-like hormonal property of HA appears to overcome damaging effects of sub-optimal temperatures on seed germination as in case of sugar beet (Akeson *et al*., 1980). Seed germination inhibition at low temperature was associated with problems in binding of nitrate with and activation of phytochromes involved in GA production (Kendrick & Kronenberg, 2012). HA possibly increases the nitrogen contents of seeds there by skipping the role of phytochrome receptors (Keuskamp *et al*., 2010; Bouwmeester & Karssen, 1989) as also reported in *Spergula arvensis* (Karssen *et al*., 1988).

Experiment 2 - Effects of humic acid and photoperiod: Humic acid appeared to inhibit seed germination of all test species under both thermoperiods tested possibly due to the presence of phenolic compounds (Pellisier, 1993; Halley & Pellisier, 1997) as in case of some conifers and leguminous species (Muscolo *et al*., 2013). Another possible reason could be the decrease in water potential as a result of high water binding ability of humic acid (Pettit *et al*., 2004).

Experiment-3a

Effects of humic acid, photoperiod and salinity in response to light (12/12h; light/dark): Application of HA improved seed germination of garden cress, wheat, parsley, celery and leek was under NaCl treatments (Yildirim *et al*., 2013; Masciandaro *et al*., 2002; Al-Erwy *et al*., 2016), but not in case of onion (Yildirim *et al*., 2014). On the contrary, inhibitory effects of HA were recorded in *Dracocephalum moldavica* and *Satureja hortenssis* (Khalesro *et al*., 2015). In the present study, humic acid improved the final germination of *U. setulosa* by 2 folds under saline conditions particularly in the higher HA concentration (500 mg L^{-1}) tested. Effects of HA appeared to be related to ROS signaling (Bailly *et al*., 2008) for radicle emergence during seed germination (Cordeiro *et al*., 2011). In addition, alleviatory effects of HA on processes such as respiration, cell division, minerals uptake on seed germination under saline conditions, indicate hormone-like activity (Pettit, 2004). HA enhanced the ethylene production under saline conditions via the *ETHYLENE RESPONSE FACTOR1* (Mora *et al*., 2012).

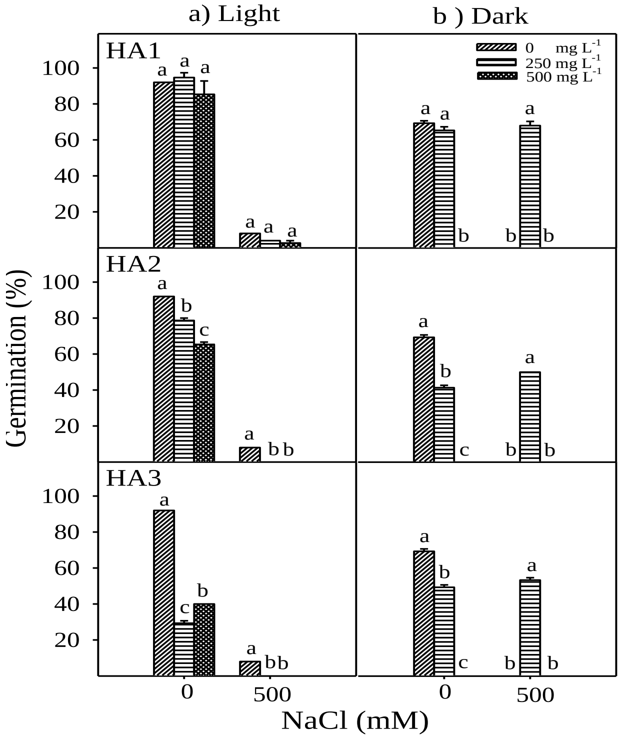

Fig. 1. Effect of humic acid types (HA1, HA2, HA3) and HA concentrations (0, 250, 500 mg L^{-1}) on seed germination of *Aeluropus lagopoides* under non-saline and saline conditions (affecting 50% germination inhibition, reported for each species) at photoperiod **a)**12/12h; light/dark, **b)** 24h dark; 20/30ºC. Bars indicate means (± SE; n = 4 replicates with 25 seeds each) while different letters over bars indicate significant (p<0.05) differences among humic acid concentrations (Bonferroni test).

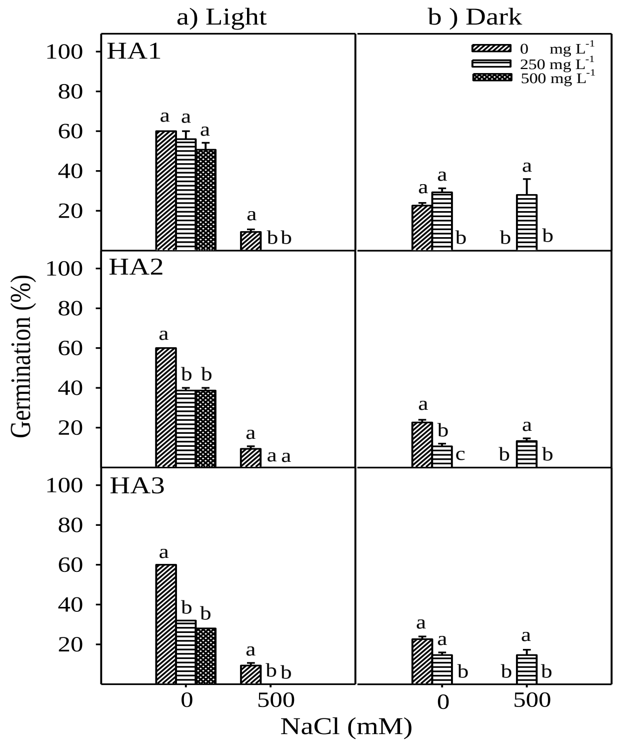

Fig. 3. Effect of humic acid types (HA1, HA2, HA3) and HA concentrations (0, 250, 500 mg L^{-1}) on seed germination of *Desmostachya bipinnata* under non-saline and saline conditions (affecting 50% germination inhibition, reported for each species) at photoperiod **a)**12/12h; light/dark, **b)** 24h dark; 20/30 ºC. Bars indicate means (± SE; n = 4 replicates with 25 seeds each) while different letters over bars indicate significant (p<0.05) differences among humic acid concentrations (Bonferroni test).

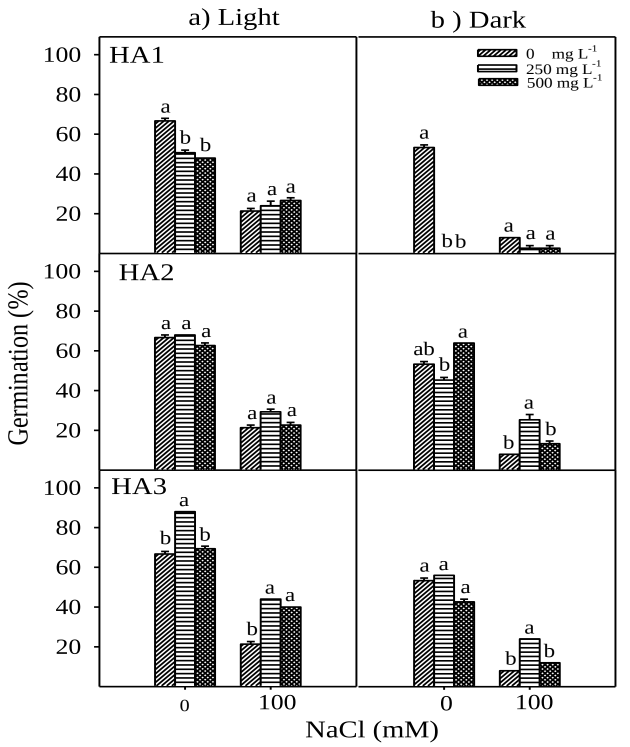

Fig. 2. Effect of humic acid types (HA1, HA2, HA3) and HA concentrations (0, 250, 500 mg L^{-1}) on seed germination of *Cyperus conglomeratus* under non-saline and saline conditions (affecting 50% germination inhibition, reported for each species) at photoperiod **a)**12/12h; light/dark, **b)** 24h dark; 20/30ºC. Bars indicate means (± SE; n = 4 replicates with 25 seeds each) while different letters over bars indicate significant (p<0.05) differences among humic acid concentrations (Bonferroni test).

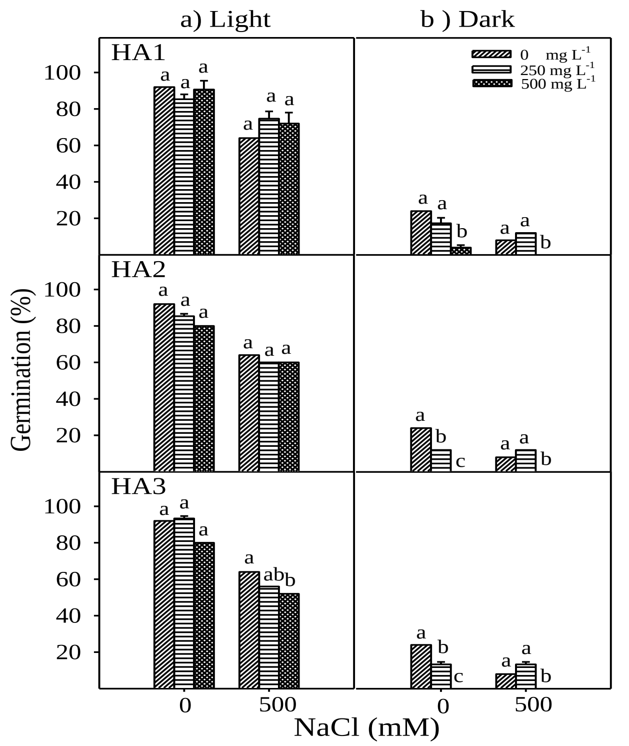

Fig. 4. Effect of humic acid types (HA1, HA2, HA3) and HA concentrations (0, 250, 500 mg L^{-1}) on seed germination of *Halocnemum strobilaceum* under non-saline and saline conditions (affecting 50% germination inhibition, reported for each species) at photoperiod **a)**12/12h; light/dark, **b)** 24h dark; 20/30 ºC. Bars indicate means (±SE; n = 4 replicates with 25 seeds each) while different letters over bars indicate significant (p<0.05) differences among humic acid concentrations (Bonferroni test).

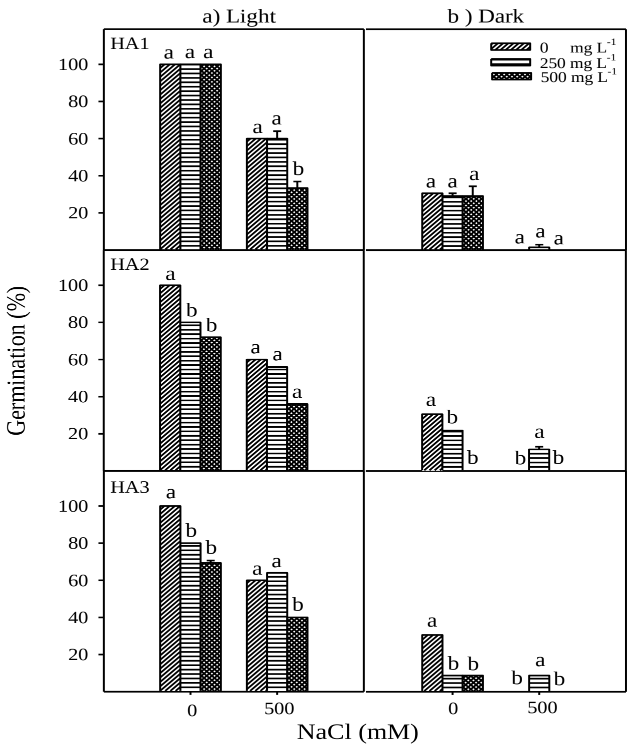

Fig. 5. Effect of humic acid types (HA1, HA2, HA3) and HA concentrations (0, 250, 500 mg L^{-1}) on seed germination of *Halopeplis perfoliata* under non-saline and saline conditions (affecting 50% germination inhibition, reported for each species) at photoperiod **a)**12/12h; light/dark, **b)** 24h dark; 20/30 ºC. Bars indicate means (± SE; n = 4 replicates with 25 seeds each) while different letters over bars indicate significant (p<0.05) differences among humic acid concentrations (Bonferroni test).

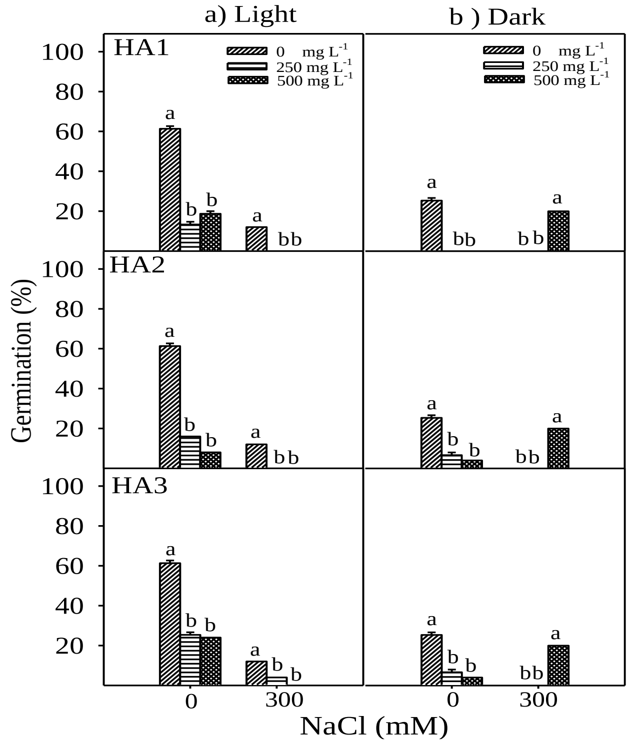

Fig. 7. Effect of humic acid types (HA1, HA2, HA3) and HA concentrations (0, 250, 500 mg L^{-1}) on seed germination of *Sporobolus iocladosunder* non-saline and saline conditions (affecting 50% germination inhibition, reported for each species) at photoperiod **a)**12/12h; light/dark, **b)** 24h dark; 20/30ºC. Bars indicate means (± SE; n = 4 replicates with 25 seeds each) while different letters over bars indicate significant p<0.05) differences among humic acid concentrations (Bonferroni test)

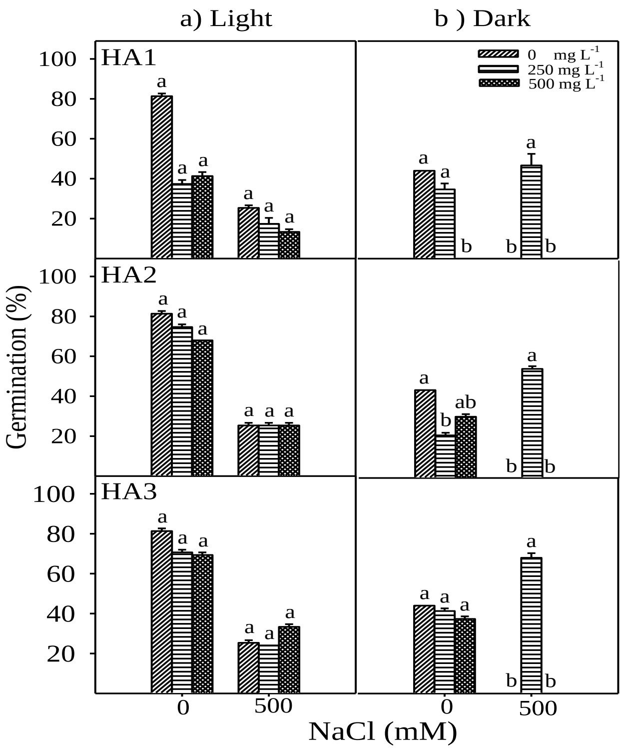

Fig. 6. Effect of humic acid types (HA1, HA2, HA3) and HA concentrations (0, 250, 500 mg L^{-1}) on seed germination of *Phragmites karka* under non-saline and saline conditions (affecting 50% germination inhibition, reported for each species) at photoperiod **a)**12/12h; light/dark, **b)** 24h dark; 20/30 ºC. Bars indicate means (±SE; n = 4 replicates with 25 seeds each) while different letters over bars indicate significant (p<0.05) differences among humic acid concentrations (Bonferroni test

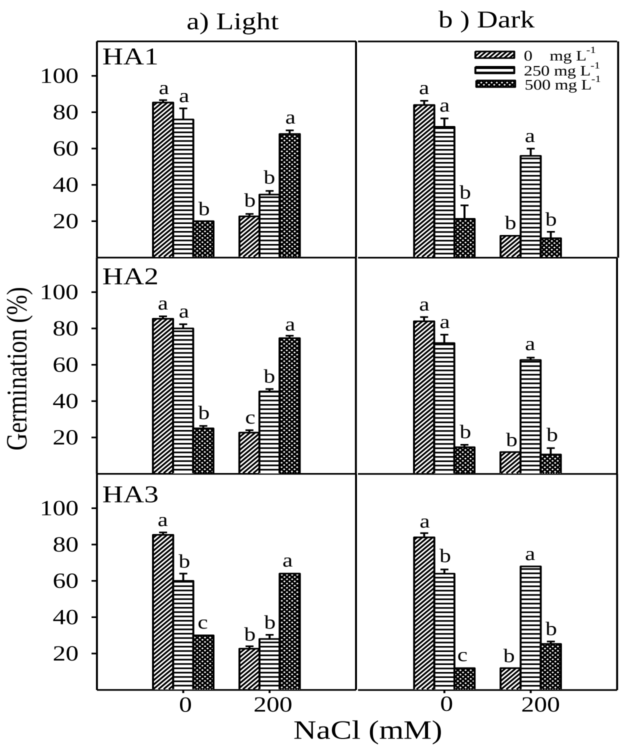

Fig. 8. Effect of humic acid types (HA1, HA2, HA3) and HA concentrations (0, 250, 500 mg L^{-1}) on seed germination of *Urochondra setulosa* under non-saline and saline conditions (affecting 50% germination inhibition, reported for each species) at photoperiod **a)** 12/12h; light/dark, **b)** 24h dark; 20/30ºC. Bars indicate means (± SE; n = 4 replicates with 25 seeds each) while different letters over bars indicate significant (p<0.05) differences among humic acid concentrations (Bonferroni test).

Supplementary Table 1. Effects of humic acid type (HA1, HA2, HA3) and HA concentration (0, 250, 500 mg L^{-1}) on seed germination of ten halophytic species at different thermoperiod (TP; 10/20 and 20/30ºC).

Species	TP (ºC)	C	HA1		HA2		HA3	
		0	250	500	250	500	250	500
A. lagopoides	10/20	43 ± 1 bc	77 ± 1 a	52 ± 0 b	27 ± 1 d	20 ± 0 d	25 ± 1 d	40 ± 0 c
	20/30	92 ± 1 a	94 ± 0 a	85 ± 4 ab	79 ± 1 bc	65 ± 1 c	29 ± 0 e	40 ± 0 d
A. macrostachyum	10/20	52 ± 0 ab	53 ± 4 ab	64 ± 2 a	53 ± 1 ab	48 ± 0 b	52 ± 0 ab	48 ± 0 b
	20/30	71 ± 1 a	45 ± 1 c	60 ± 0 b	51 ± 1 b	20 ± 0 d	53 ± 1 d	52 ± 0 b
C. conglomeratus	10/20	0 ± 0 c	0 ± 0 c	3 ± 0 b	0 ± 0 c	0 ± 0 c	0 ± 0 c	15 ± 0 c
	20/30	67 ± 1 a	51 ± 1 bc	48 ± 0 c	68 ± 0 a	63 ± 0 ab	68 ± 0 a	69 ± 1 a
D. bipinnata	10/20	21 ± 1 ab	33 ± 4 a	35 ± 3 a	13 ± 1 b	20 ± 2 ab	25 ± 3 ab	20 ± 2 ab
	20/30	60 ± 0 a	56 ± 4 a	51 ± 4 a	39 ± 1 b	39 ± 1 b	32 ± 0 b	28 ± 0 b
H. strobilaceum	10/20	80 ± 0 a	80 ± 0 a	80 ± 0 a	81 ± 0 a	81 ± 0 a	80 ± 0 a	80 ± 0 a
	20/30	92 ± 0 a	85 ± 3 a	91 ± 5 a	85 ± 1 a	80 ± 0 a	93 ± 1 a	80 ± 0 a
H. perfoliata	10/20	80 ± 0 b	100 ± 0 a	93 ± 1 a	55 ± 1 d	60 ± 0 cd	69 ± 1 bc	52 ± 0 d
	20/30	100 ± 0 a	100 ± 0 a	100 ± 0 a	80 ± 0 b	72 ± 0 b	80 ± 0 b	69 ± 1 b
H. mucronatum	10/20	88 ± 0 a	88 ± 2 a	96 ± 2 a	89 ± 1 a	84 ± 4 a	100 ± 0 a	95 ± 1 a
	20/30	100 ± 0 a	100 ± 0 a	100 ± 0 a	92 ± 0 a	96 ± 2 a	95 ± 1 a	97 ± 1 a
P. karka	10/20	47 ± 1 b	36 ± 0 b	39 ± 4 b	49 ± 1 ab	41 ± 0 b	52 ± 0 a	52 ± 0 a
	20/30	81 ± 1 a	37 ± 2 b	41 ± 2 b	75 ± 1 a	68 ± 0 a	71 ± 1 a	69 ± 0 a
S. ioclados	10/20	13 ± 1 ab	8 ± 4 b	11 ± 3 a	20 ± 0 a	16 ± 0 a	12 ± 0 ab	9 ± 1 b
	20/30	61 ±1 a	13 ± 1 b	19 ± 1 b	16 ± 0 b	8 ± 0 c	25 ± 1 b	24 ± 0 b
U. setulosa	10/20	59 ± 1 a	60 ± 2 a	17 ± 1 b	11 ± 1 b	8 ± 0 c	8 ± 0 c	8 ± 0 c
	20/30	85 ± 1 a	76 ± 6 a	20 ± 0 b	80 ± 2 a	25 ± 1 b	60 ± 4 a	30 ± 0 b

Supplementary Table 2. Effect of humic acid type (HA1, HA2, and HA3), HA concentration (0, 250, 500 mg L^{-1}) and photoperiod (12/12h; light/dark and 24h dark) on seed germination of ten halophytic species at 20/30ºC.

Species	pp	C	HA1		HA2		HA3	
		0	250	500	250	500	250	500
A. lagopoides	L	92 ± 0 a	94 ± 2 a	85 ± 7 ab	78 ± 1 a	65 ± 1 c	29 ± 1 e	40 ± 0 d
	D	69 ± 1 a	65 ± 2 a	0 ± 0 c	41 ± 1 b	0 ± 0 c	49 ± 1 b	0 ± 0 c
C. conglomeratus	L	67 ± 1 a	51 ± 1 bc	48 ± 0 c	68 ± 0 a	63 ± 1 ab	68 ± 0 a	68 ± 0 a
	D	53 ± 1 ab	0 ± 0 c	0 ± 0 c	45 ± 1 b	64 ± 0 a	56 ± 0 ab	43 ± 0 b
D. bipinnata	L	60 ± 0 a	56 ± 4 ab	51 ± 4 ab	39 ± 1 bc	39 ± 0 bc	32 ± 0 c	28 ± 0 c
	D	9 ± 1 a	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
H. strobilaceum	L	92 ± 0 a	85 ± 3 ab	91 ± 5 ab	85 ± 1 ab	80 ± 0 b	93 ± 1 a	80 ± 0 b
	D	24 ± 0 a	17 ± 3 ab	4 ± 1 c	12 ± 0 b	0 ± 0 d	13 ± 1 b	0 ± 0 d
H. perfoliata	L	100 ± 0 a	100 ± 0 a	100 ± 0 a	80 ± 0 b	72 ± 0 bc	80 ± 0 bc	69 ± 1 c
	D	28 ± 0 a	27 ± 1 a	27 ± 1 a	20 ± 0 a	0 ± 0 c	8 ± 0 b	8 ± 0 b
P. karka	L	81 ± 1 a	37 ± 2 c	41 ± 2 c	75 ± 1 ab	68 ± 0 b	71 ± 1 ab	69 ± 0 b
	D	44 ± 0 a	35 ± 3 a	0 ± 0 c	21 ± 1 b	31 ± 1 a	41 ± 1 a	37 ± 1 a
S. ioclados	L	61 ± 1 a	13 ± 1 bc	19 ± 1 b	16 ± 0 b	8 ± 0 c	25 ± 1 b	24 ± 0 b
	D	25 ± 1 a	0 ± 0 c	0 ± 0 c	7 ± 1 b	4 ± 0 b	7 ± 1 b	4 ± 0 b
U. setulosa	L	85 ± 1 a	76 ± 6 ab	20 ± 0 c	80 ± 2 a	25 ± 1 c	60 ± 4 b	30 ± 0 c
	D	82 ± 2 a	72 ± 5 ab	21 ± 2 b	72 ± 1 a	15 ± 1 c	68 ± 0 b	25 ± 1 b

Values are means ± SE

Supplementary Table 3. Effect of humic acid types (HA1, HA2, HA3) and concentrations (0, 250, 500 mg L^{1}) on seed germination % *A. macrostachyum* of and *H. mucronatum* in non-saline (NS) and saline (S) condition at 12 h photoperiod with 20/30ºC.

Species	ST	C	HA1		HA2		HA3	
		0	250	500	250	500	250	500
A. macrostachyum	NS	71 ± 1	45 ± 1	60 ± 0	51 ± 1	20 ± 0	53 ± 1	52 ± 0
	S	56 ± 0	59 ± 1	45 ± 1	31 ± 3	49 ± 1	52 ± 0	36 ± 0
H. mucronatum	NS	100 ± 0	100 ± 0	100 ± 0	92 ± 0	96 ± 2	95 ± 1	97 ± 1
	S	29 ± 3	28 ± 3	32 ± 0	37 ± 3	25 ± 1	24 ± 0	24 ± 0

Values are means ± SE

Experiment-3b

Effects of humic acid, photoperiod and salinity in response to darkness (24h dark): Under saline conditions, lower dose (250 mg L^{-1}) of humic acid had a positive effect on seed germination of photoblastic seeds except for *H. strobilacium*, while higher doses (500 mg L^{-1}) of humic acid had negative effects on seed germination. This dose-specific response of HA was also reported for some other species (Masciandaro *et al.*, 2002; Ebrahimi & Miri, 2016). The alleviation of photoblastic seed germination by humic acid from complete darkness could be a hormone-like activity (Pizzeghello *et al*., 2001; Sutcliffe & Whitehead, 1995). Photoblastic seeds had higher ROS in the presence of light (Leymarie *et al*., 2012).

Humic acid could alleviate salt induced seed dormancy in the absence of light for most of the halophytic species tested. Biochemical and molecular studies could further elucidate the underlying mechanisms of these responses. Results obtained in this study could have practical applications for growing cash crop halophytes using direct seeding method (Table 6). However, field trials need to be conducted to evaluate the feasibility of using humic acid on a larger scale.

References

Ahmed, M.Z., S. Gulzar and M.A. Khan. 2014. Role of dormancy regulating chemicals in alleviating the seed germination of three playa halophytes. *Ekoloji.*, 23: 1-7.

Akeson, W.R., M.A. Henson, A.H. Freytag and D.G. Westfall, 1980. Sugar beet fruit germination and Emergence under moisture and temperature stress. *Crop Sci*., 20: 735-739.

Akinci, S., T. Bueyuekkeskin Buyukeskin, A. Eroglu and B.E. Erdogan. 2009. The effect of humic acid on nutrient composition in broad bean (*Vicia faba* L.) roots. *Notulae Scientia Biologicae.*, 1: 81-87.

Antosovak, J., J. Kozler, J. Kubicek and I. Kimmerova. 2007. Methodic for testing biological activities of humic substances in higher plants, In: (Ed.): Barroso, M.I. *Reactive and Functional Polymers Research Advances*. New York, Nova Science Publisher, pp: 191-203.

Ashraf, M. and M. Foolad. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.*, 59: 206-216.

Bailly, C. 2004. Active oxygen species and antioxidants in seed biology. *Seed Sci Res.*, 14: 93-107.

Bartels, D. and R. Sunkar. 2005. Drought and salt tolerance in plants. *Crit. Rev. Plant Sci*., 24: 23-58.

Bewley, J.D. and M. Black. 1982. Viability and longevity Physiology and Biochemistry of Seeds in relation to germination. Springer.

Bewley, J.D. and M. Black. 1994. Seeds. In seeds. Springer US.

Bouwmeester, H.J. and C.M. Karssen. 1989. Environmental factors influencing the expression of dormancy patterns in weed seeds. *Ann. Bot.*, 63: 113-120.

Bu, H., G. Du, X. Chen, X. Xu, K. Liu and S. Wen. 2008. Community-wide germination strategies in an alpine meadow on the eastern Qinghai-Tibet plateau: phylogenetic and life-history correlates. *Plant Ecol.*, 195: 87-98.

Çavuşoglu, K. and H. G. Ergin. 2015. Effects of humic acid pretreatment on some physiological and anatomical parameters of barley (*Hordeum vulgare* L.) exposed to salt stress. *Bangladesh J. Bot*., 44: 591-598.

Cordeiro, F.C., C. Santa-Catarina, V. Silveira and S.R. de Souza. 2011. Humic acid effect on catalase activity and the generation of reactive oxygen species in corn (*Zea mays*). *Biosci. Biotechnol. Biochem.*, 75: 70-74.

Ebrahimi, M. and E. Miri. 2016. Effect of humic acid on seed germination and seedling growth of *Borago officinalis* and *Cichorium intybus*. *Ecopersia.*, 4: 1239-1249.

El-Hefny, E.M. 2010. Effect of saline irrigation water and humic acid application on growth and productivity of two cultivars of cowpea (*Vigna unguiculata* L. Walp). *Aust. J. Basic* & *Appl. Sci*., 4: 6154-6168.

El-Keblawy, A., M.V. Rao., A. Al-Marzouqi Arzouqi and S.S. AL-Neyadi. 2009. Germination ecology of indigenous *Cyperus conglomeratus* seeds: a sand dune binder. In: Proceedings of the Tenth Annual Conference for Research Funded by UAE University. pp. 111–120. Al-Ain, United Arab Emirates: UAE University.

Fenner, M. and K. Thompson. 2005. The ecology of seeds. Cambridge University Press.

Finch-Savage, W.E. and G. Leubner-Metzger. 2006. Seed dormancy and the control of germination. *New Phytol*., 171: 501-523.

Flowers, T.J. and T.D. Colmer. 2008. Salinity tolerance in halophytes. *New Phytol*., 179: 945-963.

Guan, B., D. Zhou, H. Zhang, Y. Tian, W. Japhet and P. Wang. 2009. Germination responses of *Medicago ruthenica* seeds to salinity, alkalinity, and temperature. *J. Arid Environ*., 73: 135-138.

Gul, B., R. Ansari, T.J. Flowers and M.A. Khan. 2013. Germination strategies of halophyte seeds under salinity. *Environ Exp Bot.*, 92: 4-18.

Gulzar, S. and M.A. Khan, 2001. Seed germination of a halophytic grass *Aeluropus lagopoides*. *Ann. Bot.*, 87: 319-324.

Gulzar, S., M.A. Khan and X. Liu. 2007. Seed germination strategies of *Desmostachya bipinnata:* a fodder crop for saline soils. *Rangeland Ecol. Manag.*, 60: 401-407.

Halley, C. and F. Pellissier. 1997. Phenolic compounds in natural solutions of a coniferous forest. *J. Chem. Ecol*, 23: 2401-2412.

Hartwigsen, J.A. and M.R. Evans. 2000. Humic acid seed and substrate treatments promote seedling root development. *Hort. Sci*., 35: 1231-1233.

Karssen, C.M., P.M. Derkx and B.J. Post. 1988. Study of seasonal variation in dormancy of *Spergula arvensis* L. seeds in a condensed annual temperature cycle. *Weed Res*., 28: 449-457.

Kaya, M.D., G. Okçu, M. Atak, Y. Cikili and O. Kolsarıcı. 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *Eur. J. Agron.*, 24: 291-295.

Kendrick, R.E. and G.H. Kronenberg. 2012. *Photomorphogenesis in plants*. (Eds.). Springer Science and Business Media.

Keuskamp, D.H., S. Pollmann, L.A. Voesenek, A.J. Peeters and R. Pierik, 2010. Auxin transport through PIN-FORMED 3 (PIN3) controls shade avoidance and fitness during competition. *Proc. Natl. Acad. Sci*., 107: 22740-22744.

Khajeh-Hosseini, M., A.A. Powell and I.J. Bingham. 2003. The interaction between salinity stress and seed vigour during germination of soya bean seeds. *Seed Sci and Technol*., 31: 715-725.

Khalesro, S., M. Salehi and B. Mahdavi. 2015. Effect of humic acid and salinity stress on germination characteristic of savory (*Satureja hortensis* L.) and dragonhead (*Dracocephalum moldavica* L.). *Biol. Forum*., 7: 554-561.

Khan, M.A. 1999. Comparative influence of salinity and temperature on the germination of subtropical halophytes. Halophyte uses in different climates. I: *Ecol. Ecophysiol. Studies*., 13: 77-88.

Khan, M.A. and B. Gul. 2002. *Arthrocnemum macrostachyum*: A potential case for agriculture using above seawater salinity. In: (Eds.): Ahmed, R. and Malik, K.A. *Prospects for Saline Agriculture*. Vol. 37, Springer. Netherlands, pp: 353-364.

Khan, M.A. and S. Gulzar. 2003. Germination responses of *Sporobolus ioclados*: a saline desert grass. *J. Arid. Environ.*, 53: 387-394.

Khan, M.A., B. Gul and D.J. Weber. 2001. Seed germination characteristics of *Halogeton glomeratus*. *Can. J. Bot*., 79: 1189-1194.

Leymarie, J., G. Vitkauskaite, H.H. Hoang, E. Gendreau, V. Chazoule, P. Meimoun and C. Bailly. 2012. Role of reactive oxygen species in the regulation of *Arabidopsis* seed dormancy. *Plant and Cell Physiol.*, 53: 96-106.

Mahajan, S. and N. Tuteja. 2005. Cold, salinity and drought stresses: an overview. *Arch. Biochem. Biophys.,* 444: 139-158.

Mahmoud, A., A.M. El-Sheikh and S. Abdul Baset. 1983. Germination of two halophytes: *Halopeplis perfoliata* and *Limonium aillare* from Saudi Arabia. *J. Arid Environ.,* 6: 87-98.

Masciandaro, G., B. Ceccanti, V. Ronchi, S. Benedicto and L. Howard. 2002. Humic substances to reduce salt effect on plant germination and growth. *Commun. Soil Sci. Plant Anal*., 33: 365-378.

McDonnell, R., N.M. Holden, S.M. Ward, J.F. Collins, E.P. Farrell and M.H.B. Hayes. 2001. Characteristics of humic substances in heathland and forested peat soils of the Wicklow Mountains. In: *Biology and Environment*. Proceedings of the Royal Irish Academy, 187-197.

Mora, V., R. Baigorri, E. Bacaicoa, A.M. Zamarreno and J.M. Garcia-Mina. 2012. The humic acid-induced changes in the root concentration of nitric oxide, IAA and ethylene do not explain the changes in root architecture caused by humic acid in cucumber. *Environ. Exper. Bot*., 76: 24-32.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.,* 59: 651-681.

Muscolo, A., M. Sidari and J.A.T. da Silva. 2013. Biological effects of water-soluble soil phenol and soil humic extracts on plant systems. *Acta. Physiol. Plant.*, 35: 309-320.

Namdaran-Gooran, M., R. Haghparast, S. Jalali-Honarmand and R. Rjabi. 2014. The effects of the seed treatment with two commercial combinations containing fulvic acid and humic acid on wheat germination stress index. *Int. J. Biosci*., 5: 250-255.

Nardi, S., D. Pizzeghello, A. Muscolo and A. Vianello. 2002. Physiological effects of humic substances on higher plants. *Soil Biol. Biochem.*, 34: 1527-1536.

Noor, M.E.H.E.R. and M.A. Khan. 1995. Factors affecting germination of summer and winter seeds of *Halopyrum mucronatum* under salt stress. In: *Biology of Salt Tolerant Plants* (Eds.): Khan, M.A. and I.A. Ungar, pp. 51-58, Department of Botany, University of Karachi, Karachi.

Patil, R.B., S.S. Mokle and S.S. Wadje. 2010. Effect of potassium humate on seed germination, seedling growth and vegetative characters of *Triticum aestivum* (L.) Cv. Lokvan. *Int. J. Pharm. Biosci.,* 1: 1-4.

Pellissier, F. 1993. Allelopathic effect of phenolic acids from humic solutions on two spruce mycorrhizal fungi: *Cenococcum graniforme* and *Laccaria laccata*. *J. Chem. Ecol.*, 19: 2105-2114.

Pettit, R.E. 2004. Organic matter, humus, humate, humic acid, fulvic acid and humin: Their importance in soil fertility and plant health. *CTI Research*.

Piccolo, A., G. Celano and G. Pietramellara. 1993. Effects of fractions of coal-derived humic substances on seed germination and growth of seedlings (*Lactuga sativa* and *Lycopersicum esculentum*). *Biol. Fertil. Soil*, 16: 11-15.

Pizzeghello, D., G. Nicolini and S. Nardi. 2001. Hormone-like activity of humic substances in *Fagus sylvaticae* forests. *New Phytol*., 151: 647-657.

Pizzeghello, D., O. Francioso, A. Ertani, A. Muscolo and S. Nardi. 2013. Isopentenyladenosine and cytokinin-like activity of different humic substances. *J. Geochem. Explor.*, 129: 70-75.

Pujol, J.A., J.F. Calvo and L. Ramirez-Diaz. 2000. Recovery of germination from different osmotic conditions by four halophytes from southeastern Spain. *Ann. Bot.*, 85: 279-286.

Qu, X.X., Z.Y. Huang, J.M. Baskin and C.C. Baskin. 2007. Effect of temperature, light and salinity on seed germination and radicle growth of the geographically widespread halophyte shrub *Halocnemum strobilaceum*. *Ann. Bot.*, 101: 293-299.

Rasool, S.G., A. Hameed, M.Z. Ahmed, M.A. Khan and B. Gul. 2016. Comparison of seed germination and recovery responses of a salt marsh halophyte *Halopeplis perfoliata* to osmotic and ionic treatments. *Pak. J. Bot.,* 48: 1335-1343.

Senesi, N. and G. Brunetti. 1996. Chemical and physico-chemical parameters for quality evaluation of humic substances produced during composting. In: The science of composting. Springer Netherlands, pp: 195-212.

Sera, B. and F. Novak. 2011. The effect of humic substances on germination and early growth of Lamb's Quarters (*Chenopodium album agg*.). *Biologia.*, 66: 470-476.

Silva-Matos, R.R.S., I.H. Cavalcante, G.B.S. Junior, F.G. Albano, M.S. Cunha and M.Z. Beckmann-Cavalcante. 2012. Foliar spray of humic substances on seedling production of watermelon cv. crimson sweet. *J. Agron*., 11: 60.

Sosa, L., A. Llanes, H. Reinoso, M. Reginato and V. Luna 2005. Osmotic and specific ion effects on the germination of *Prosopis strombulifera*. *Ann. Bot*., 96: 261-267.

Szczepanek, M. and E. Wilczewski. 2011. Effect of humic substances on germination of wheat and barley under laboratory conditions. *Acta Sci. Pol., Agric*., 10.

Tester, M. and R. Davenport. 2003. Na+ tolerance and Na+ transport in higher plants. *Ann. Bot*., 91: 503-527.

Tlig, T., M. Gorai and M. Neffati. 2008. Germination responses of *Diplotaxis harra* to temperature and salinity. *Flora*, 203: 421-428.

Tobe, K., L. Zhang and K. Omasa. 1999. Effects of NaCl on seed germination of five nonhalophytic species from a Chinese desert environment. *Seed Sci. Technol.*, 27: 851-863.

Turkmen, O., A. Dursun, M. Turan and C. Erdinç. 2004. Calcium and humic acid affect seed germination, growth, and nutrient content of tomato (*Lycopersicon esculentum* L.) seedlings under saline soil conditions. *Acta Agric. Scand. B*., 54: 168-174.

Walker, D.J. and M.P. Bernal. 2004. The effects of copper and lead on growth and zinc accumulation of *Thlaspi caerulescens* J. and C. Presl: implications for phytoremediation of contaminated soils. *Water Air Soil Pollut*., 151: 361-372.

Yamaguchi, T., and E. Blumwald, 2005. Developing salt-tolerant crop plants: Challenges and opportunities. *Trend Plant Sci.*, 10: 615-620.

Yensen, N.P. 1995. International Symposium on High Salinity Tolerant Plants Summary of Papers Presented. In: (Eds.): Khan, M.A. and I.A. Ungar. *Biology of Salt Tolerant Plants*, Ohio University, Athens, 1-12.

Yildirim, E., A. Dursum, I. Guvenc and A.M. Kumlay. 2002. The effects of different salt, biostimulant and temperature levels on seed germination of some vegetable species. *Acta Hort. Cult.*, 579: 249-253.

Yildirim, N., G. Agar, M.S. Taspinar, M. Turan, M. Aydin and E. Arslan. 2014. Protective role of humic acids against dicamba-induced genotoxicity and DNA methylation in *Phaseolus vulgaris* L. *Acta Agric. Scand. B*., 64: 141-148.

Zehra, A., B. Gul, R. Ansari, A. Alatar, Hegazy and M.A. Khan. 2013. Action of plant growth regulators in alleviating salinity and temperature effects on the germination of *Phragmites karka*. *Pak. J. Bot.*, 45: 1919-1924.

(Received for publication ________)