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EFFECT OF DISINFECTANTS IN IMPROVING SEED GERMINATION OF *SUAEDA FRUTICOSA* UNDER SALINE CONDITIONS

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Abstract

This research examines the possible role of two commonly used disinfectants viz., hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl) in improving seed germination and rate of germination of a subtropical cash crop halophyte, *Suaeda fruticosa* under saline conditions. Brief seed pretreatment with H_2O_2 (0.34%) and NaOCl (2%) had the following effects in various salinity (0, 100, 200, 300 and 400 mM NaCl) and temperature (10/20, 15/25, 20/30 and 25/35^oC) regimes. H_2O_2 ameliorated seed germination and rate of germination at moderate (15/25 and 20/30^oC) temperatures while NaOCl was effective at all temperature regimes up to 200 mM NaCl. Seed pretreatment with H_2O_2 and NaOCl prior to seeding could increase emergence, which may help in mass propagation of *S. fruticosa*.

Introduction

Seed germination of halophytes distributed in arid environments is influenced by osmotic and ionic stresses caused due to major variations in water, salinity and temperature. Recruitment from seeds is therefore restricted to a period of brief monsoon rains when soil salinity and temperature are reduced allowing seed hydration and germination. The use of various growth regulators did not ameliorate seed germination of *Suaeda fruticosa* except for ascorbic acid (Khan & Gul, 2006; Khan *et al*., 2006; Khan, unpublished data). Hydrogen peroxide and Sodium hypochlorite disinfect seeds by oxidizing proteins and/or nucleic acids of microorganisms inhabiting seed surfaces (Bewley & Black, 1994; Miché & Balandreau, 2001; Commander *et al*., 2009). They may also improve seed germination, which is species-specific and dose-dependent (Chein & Lin, 1994; Fontaine *et al*., 1994; Ogawa & Iwabuchi, 2001; Matus- Ca´Diz & Hucl, 2005). Application of Sodium hypochlorite and hydrogen peroxide before seeding could increase the chances of emergence of *S. fruticosa* seedlings under saline conditions.

Suaeda fruticosa (L.) Forssk., or Lani is a perennial halophyte of inland and coastal salt marshes and salt deserts of Pakistan. It is used locally as fodder for livestock and is a source of washing soda or sajji / sajismati (Freitag *et al*., 2001). Leaves are used as poultice for treating ophthalmia while aqueous decoctions as an emetic (Chopra *et al*., 1986). Aqueous extracts of *S. fruticosa* are reported to have hypoglycemic (Benwahhoud *et al*., 2001) and hypolipidaemic effects in rats (Bennani-Kabachi *et al*., 1999). Seeds contain 25% fat which may be used as edible oil (Weber *et al*., 2007). Recently, Khan *et al*., (2009) demonstrated the utility of this leaf succulent species in maintaining soil salinity levels by removing excess salts from soil. The present study examined the possible role of H_2O_2 and NaOCl in alleviating salinity effects on seed germination and rate of germination of *S. fruticosa* under various temperature regimes.

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Materials and Methods

Mature inflorescence of *Suaeda fruticosa* were harvested from populations located at Hawks Bay, Karachi and transported to the laboratory in plastic bags. Seeds were cleaned, air dried and stored at room temperature. Four replicates of 25 seeds each were immersed in 5 ml test solutions in 50 mm dia. tight fitting Petri-plates, placed in a programmed incubator (Percival, USA) at 12:12 h Dark: Light photoperiod coinciding with 10/20, 15/25, 20/30 and 25/35^{o}C thermoperiods. Light source in the incubator were Philips cool white fluorescent lamps (25 μmol m^{-2} s^{-1}, 400-700 nm).

Seeds were pretreated with 3 dilutions of H_2O_2 (0, 0.17, 0.34 and 0.52%) and 4 of NaOCl (0, 2, 4, 6 and 8%) for 5 min., each. This was followed by air-drying of seeds before immersing in NaCl (0, 100, 200, 300, and 400 mM) solutions. Data for 0.34% H_2O_2 and 2% NaOCl are given here, as low concentrations had no effect and seed died at higher levels. Germination (radical emergence) was recorded every 48 h for 20-d. Rate of germination was calculated with the modified Timson's index (Khan & Ungar, 1984). All data were arcsine transformed to test for homogeneity of variance. General Linear Model (GLM) was used to compare treatment effects with three-way analysis of variance (ANOVA) to test for significance of main effects (temperature, salinity, disinfectant treatment) and their interactions on seed germination and rate of germination. A Bonferroni post-hoc test was used (p<0.05) to indicate significant differences both within and between salinity and temperature treatments on final germination percentage (FG) and rate of germination (RG) in response to disinfectant pretreatment. SPSS for Windows release 11 (SPSS, 2001) was used for statistical analyses.

Results and Discussion

There were significant (p<0.0001) differences in final germination percentage (FG) and rate of germination (RG) among temperature (F_{FG} = 30.92; F_{RG} = 49.67), salinity (F_{FG} = 589.80; F_{RG} = 964.17), disinfectant (F_{FG} = 52.98; F_{RG} = 80.63) and the interactions between temperature x salinity (F_{FG} = 2.63; F_{RG} = 5.19) as well as those of salinity x disinfectant (F_{FG} = 39.67; F_{RG} = 14.21). However, interactions between temperature x disinfectant (F_{FG} = 1.52, p=0.27) on FG and those among all three factors combined (F_{FG} = 1.41; F_{RG} = 2.13, p=0.28) were non-significant. Seeds of halophytes germinate optimally in the absence of salinity and do not require low water potential to germinate (Ungar, 1991; Khan & Gul, 2006). Similar response was observed in case of *S. fruticosa* where FG and RG were optimal under non-saline conditions at moderate temperatures of 20/30^{o}C (Figs. 1 and 2).

Various techniques have been used to improve seed germination under saline conditions such as application of various chemicals (Matus-Ca´diz & Hucl, 2005; Khan & Gul, 2006; Shahba *et al*., 2008), seed priming (Wahid *et al*., 2006) and brief seed pretreatment with various chemicals (Ashraf & Foolad, 2005). Pretreatment of *S. fruticosa* seeds both with H_2O_2 and NaOCl significantly enhanced germination probably due to seed coat scarification leading to increased seed hydration and aeration (Chein & Lin, 1994; Yildiz & Er, 2002). These chemicals could oxidize germination inhibiting substances in the seed coat (Ogawa & Iwabuchi, 2001). H_2O_2 pretreatment promoted germination in *Zinnia elegans* (Ogawa & Iwabuchi, 2001) while a pretreatment NaOCl promoted seed germination of *Limonium stocksii* and *Linum usitatissimum* (Yildiz & Er, 2002; Khan & Zia, 2007).

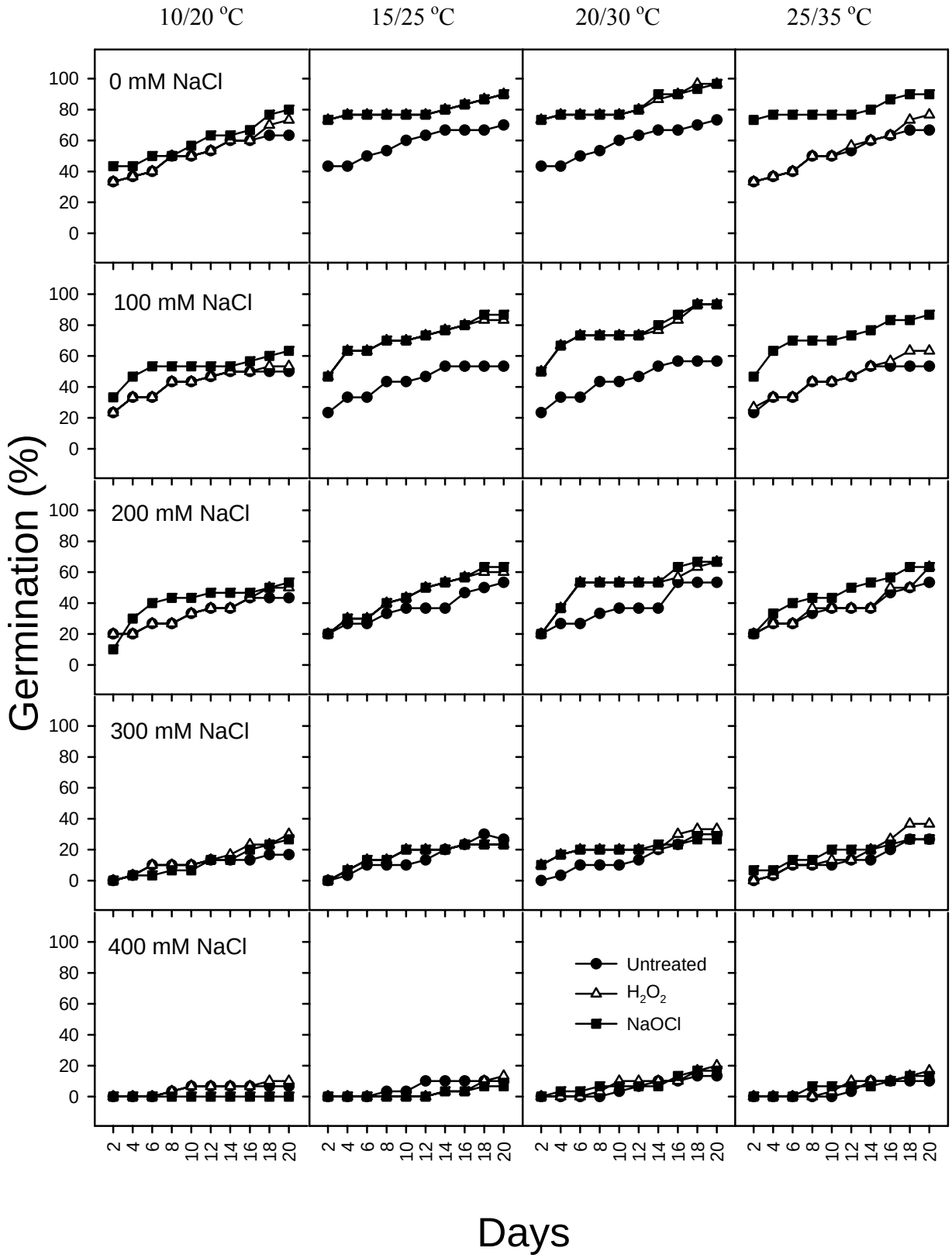

Fig. 1. Day-wise germination percentages of untreated, H_2O_2 pretreated and NaOCl pretreated seeds of *Suaeda fruticosa* under different salinity (0, 100, 200, 300 and 400 mM NaCl) and temperature (10/20, 15/25, 20/30 and 25/35°C) regimes.

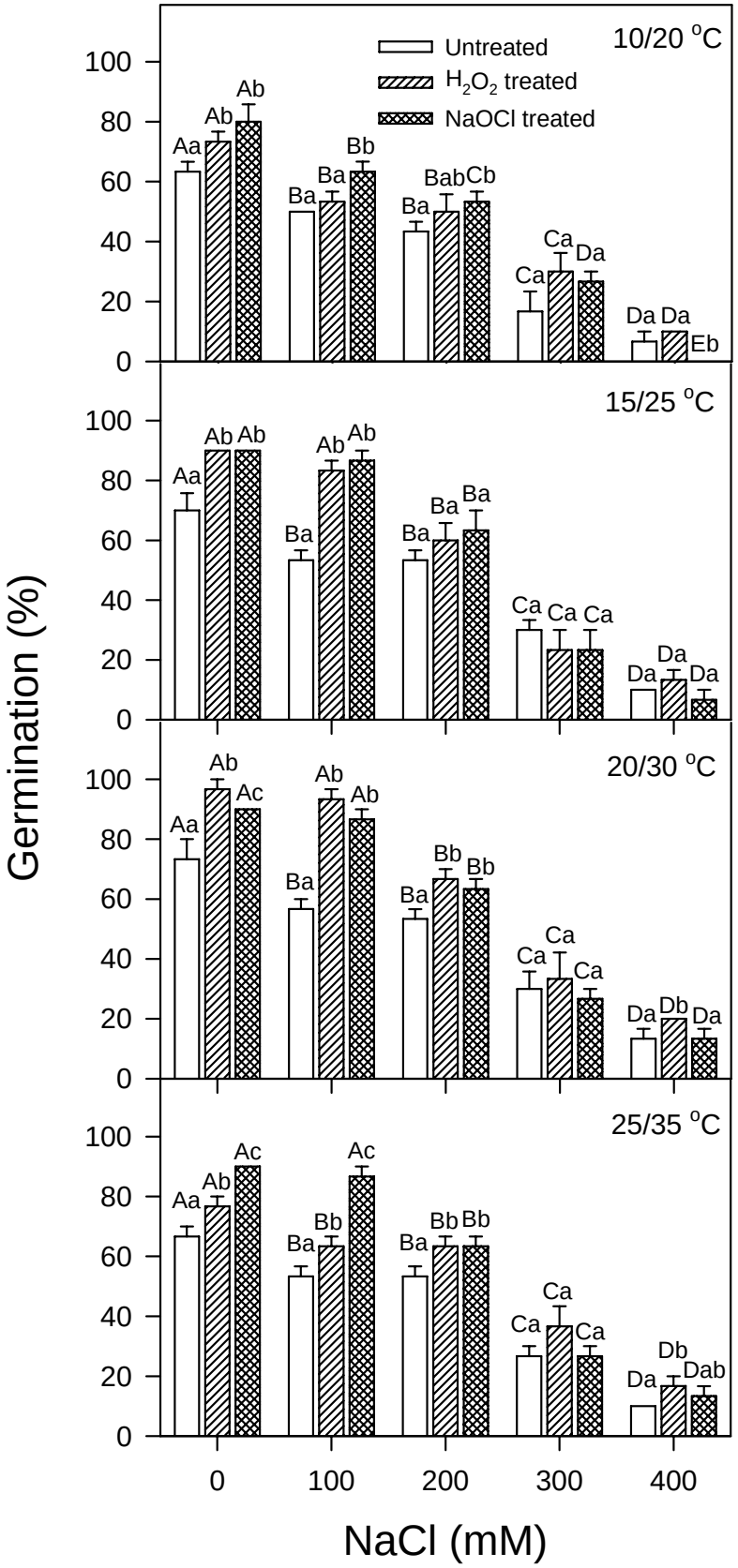

Fig. 2. Final germination percentages of untreated, H_2O_2 pretreated and NaOCl pretreated seeds of *Suaeda fruticosa* under different salinity (0, 100, 200, 300 and 400 mM NaCl) and temperature (10/20, 15/25, 20/30 and 25/35°C) regimes. Bars represent means (±S.E.). Similar capital letters among salinity treatments and small letters within each salinity are not significantly (p<0.05) different from each other, Bonferroni test.

Table 1. Rate of germination (RG) of untreated, H_2O_2 pretreated and NaOCl pretreated seeds of *Suaeda fruticosa* under different salinity (0, 100, 200, 300 and 400 mM NaCl) and temperature (10/20, 15/25, 20/30 and 25/35°C) regimes.

Salinity (mM)	Treatment	Temperature °C			
		10/20	15/25	20/30	25/35
0	Untreated	25.50±0.5Aa	29.17±1.9Ba	29.50±2.1Ba	26.00±0.5Aa
	H_2O_2	26.33±0.4Aa	39.83±1.0Bb	41.50±1.2Bb	27.00±0.3Aa
	NaOCl	29.67±1.6Ab	39.83±1.0Bb	41.50±1.2Bb	40.17±1.4Bb
100	Untreated	21.17±0.8Aa	21.83±1.4Aa	22.33±1.3Aa	21.83±1.4Aa
	H_2O_2	21.50±0.5Aa	35.50±1.5Bb	37.83±2.2Bb	23.17±1.2Aa
	NaOCl	26.33±1.6Ab	35.83±1.7Bb	38.17±2.2Bb	36.17±1.9Bb
200	Untreated	16.50±1.5Aa	18.33±0.9Aa	18.83±0.7Aa	18.33±0.9Aa
	H_2O_2	17.17±1.5Aa	22.17±1.4Bb	25.50±1.5Bb	19.17±1.4Aa
	NaOCl	20.50±1.4Ab	22.50±1.2Ab	26.00±1.3Bb	23.33±1.5Ab
300	Untreated	5.33±2.1Aa	7.33±2.0Aa	7.50±2.2Aa	6.67±1.6Aa
	H_2O_2	7.00±2.7Aa	8.17±2.2Aa	11.17±3.0Bb	8.50±1.2Aa
	NaOCl	5.83±1.0Aa	8.17±2.2ABa	10.33±2.5Bb	8.83±2.0ABa
400	Untreated	2.17±1.0Aa	2.83±0.3Aa	2.83±0.6Aa	2.17±0.2Aa
	H_2O_2	2.50±0.8Aa	1.50±0.5Ab	4.00±0.3Ba	3.17±0.2Ab
	NaOCl	0.00±0.0Ab	1.00±0.6ABb	4.00±1.3Ba	3.17±0.9Bb

Numbers represent mean (±S.E.). Similar capital letters within rows and small letters in columns are not significantly (p<0.05) different from each other, *Bonferroni test*.

In *Avena fatua* both H_2O_2 and NaOCl promoted seed germination (Hsiao & Quick, 1984). The two disinfectants used in this study significantly ameliorated seed germination in up to 200 mM NaCl, however H_2O_2 was more effective at moderate temperatures while NaOCl did equally well at all temperature regimes tested. Seed pretreatments enhanced RG more in comparison to FG (Table 1; Fig. 1). Khan & Zia (2007) also reported that NaOCl pretreatment ameliorated seed germination of *Limonium stocksii* in up to 300 mM NaCl at cooler temperature regime of 10/20°C.

Suaeda fruticosa is a leaf succulent halophyte, which dominates sandy saline soils with wide ecological amplitude from inland salt flats to coastal dunes and intertidal salt marshes. Ash content approaching 50% of dry weight indicates its potential in saline soil reclamation. In addition, presence of high quality edible oil in seeds and medicinal value of leaves makes it an important non-conventional crop for arid saline wastelands. Cultivating *S. fruticosa* successfully would require higher emergence. Our results suggest that pretreatment with H_2O_2 (0.34%) and NaOCl (2%) may increase seed germination under both non-saline and moderately saline conditions contributing in efforts to exploit the potential of *S. fruticosa* as a cash-crop.

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