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ALLEVIATION OF SALINITY EFFECTS BY SODIUM HYPOCHLORITE ON SEED GERMINATION OF *LIMONIUM STOCKSII*

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Abstract

This research presents the role of Sodium hypochlorite (NaOCl) in alleviation of salinity effects on the germination of *Limonium stocksii* seeds. Seeds were either pre-treated with Sodium hypochlorite or it was added to a medium with and without salinity (0-400 mM NaCl) at various temperature regimes (10-20, 15-25, 20-30 and 25-35 °C). The application of Sodium hypochlorite was found to be beneficial to seed germination under saline conditions. Pretreatment for one minute in a 10 % Sodium hypochlorite solution appeared to be the most effective treatment in alleviating salinity-induced dormancy. Sodium hypochlorite alleviated salinity effects more under lower temperature regimes on the seed germination indicating the ability of Sodium chloride was progressively reduced with increase in temperature.

Introduction

Limonium stocksii (Boiss.) Kuntze, of the family Plumbaginaceae is a low branched perennial, salt secreting, halophytic shrub that unlike neighbouring halophytes, is primarily propagated through seed germination. *Limonium stocksii* could be used as a fodder crop and as an ornamental plant to beautify the beaches. Seeds of *L. stocksii* could germinate in up to 400 mM NaCl at moderate temperature regimes (20-30^{o}C) for Karachi (Zia & Khan, 2004). However, its level of salinity tolerance could be considered as moderate because other species viz., *Cressa cretica* and *Arthrocnemum macrostachyum* in the vicinity could germinate in 1000 mM NaCl (Khan & Gul, 2002). Exposure to high salinity and temperature do not have any effect on seed viability of *L. stocksii* (Zia & Khan, 2004). Previous studies on seed germination of *L. stocksii* have indicated that seeds washed in Sodium hypochlorite germinated better under high salinity conditions (unpublished data). These findings have encouraged us to look into the responses of *L. stocksii* seeds to Sodium hypochlorite application during germination under saline conditions.

A wide range of surface disinfectants such as ethanol, hydrogen peroxide, bromine water, mercuric chloride, silver nitrate, Sodium hypochlorite and antibiotics are used for seed surface sterilization (Bewley & Black, 1994). Sodium hypochlorite has been most commonly used as a disinfectant, which releases oxygen gas as a by-product and is highly effective against all kinds of bacteria, fungi and viruses, by oxidizing biological molecules such as proteins and nucleic acids (Bloomfield & Arthur, 1991). Higher concentrations of Sodium hypochlorite have shown negative effects both on the germination and growth (Hsiao, 1979; Hsiao & Hans, 1981; Hsiao & Quick, 1984; Ilahi & Hussain, 1988) in *Avena* and *Reptonia* species. The oxygen gas releasing property of Sodium hypochlorite enhances the oxidative respiration, which promotes seed germination (Bewely & Black, 1994; Ogawa & Iwabuchi, 2001; Vujanovic *et al.*, 2000).

Sodium hypochlorite is also known for its effect to overcome seed dormancy (Galleta *et al.*, 1989; Igbinnosa & Okonkowo, 1992; Bewely & Black, 1994) by decomposing germination inhibitors (Mackinnon & Alderton, 2000; Ogawa & Iwabuchi, 2001), bleaching of seed coat (Vujanovic *et al.*, 2000; Böhm, 2003), lowering of pH (Böhm, 2003), and promoting an enhancement of α-amylase activity (Kanecko & Morohashi, 2003). Böhm (2003) found that Sodium hypochlorite was able to release coat-imposed dormancy in orchid seeds by chemical bleaching of the additional coat present around the embryo**.** It was suggested that weak acids such as hypochlorite, overcome dormancy by lowering the pH and as a result promoting oxygen uptake. Clevering (1995) reported that the germination of *Scirpus lacustris* and *S. maritimus* was significantly improved when seeds were presoaked in Sodium hypochlorite.

Little information is available on the effect of Sodium hypochlorite in alleviating the salinity effects on seed germination. This situation justifies the main objective of the present study i.e., to test the hypothesis that application of Sodium hypochlorite alleviates salinity effects on seed germination of *L. stocksii*.

Materials and Methods

Inflorescences of *Limonium stocksii* were collected from a salt flat at the upper end of Manora Creek near Hawks Bay, Karachi (24ºN45'-25ºN and 66º45'-67ºE) and seeds were manually separated and stored at 4ºC. Germination was tested soon after using 5 cm diameter tight fitting plastic Petri dishes with 5 ml of test solution prepared by using distilled water, Sodium chloride and Sodium hypochlorite. Four replicates of 25 seeds each were used for each treatment. Seeds were considered germinated with emergence of the primary root.

Seeds were pre-soaked with 0, 10, 20, 30 and 40% Sodium hypochlorite for 1, 5, 10 and 20 minutes prior to germination and washed thoroughly with distilled water. After air-drying seeds were germinated in five concentrations (0, 100, 200, 300 and 400 mM) of NaCl at 20-30ºC in a 12 h light photoperiod. Seed germination was recorded on alternate days for 20 days.

Seeds were also germinated in mixtures of Sodium hypochlorite (2, 4, 6 and 8%) and NaCl solutions (0, 100, 200, 300 and 400 mM) under four alternating temperature regimes of 10-20, 15-25, 20-30 and 25-35ºC in incubators (Percival Scientific, USA). A 24 hr cycle was used where higher temperatures (20ºC, 25ºC, 30ºC, 35ºC) coincided with the 12 hr light period (Sylvania cool white fluorescent lamps, 25µM m^{-2} s^{-1}, 400-750 nM) and lower temperatures (10ºC, 15ºC, 20ºC, 25ºC) coincided with the 12 hr dark period. Percent germination was recorded on alternate days for 20 days.

Rate of germination was estimated by using modified Timson's index of germination velocity, $\Sigma G/t$, where G is percentage of seed germination at 2-d intervals, and t is total germination period (Khan & Ungar, 1997). The maximum value possible for our data using this index was 50 (i.e. 1000/20) & the higher value corresponded to a faster germination.

Germination data were transformed (arcsine) before statistical analysis. These data were analyzed using SPSS for windows release 11 (SPSS 2002). A three way ANOVA was also used to demonstrate the interaction between various factors in affecting germination. A Bonferonni test was used ($p < 0.05$) to determine significant differences between individual means.

Results

Three way ANOVA of percentage germination indicated a significant effect of salinity, Sodium hypochlorite, pretreatment time, and their interaction (Table 1). Results of pretreatment experiment indicate that there was little effect of Sodium hypochlorite in non-saline control and at low salinity treatments up to 100 mM NaCl (Fig. 1). However, alleviation of salinity effects on germination was recorded at NaCl concentrations of 200 mM or above. Best results were obtained when seeds were pretreated with 10% Sodium hypochlorite for one minute at 200 mM NaCl (Fig. 1). Rate of germination was also higher when seeds were pretreated for one minute using 10% Sodium hypochlorite solution (Table 2). The criterion for selecting the best hypochlorite treatments is the one which provides best germination under saline conditions.

Three way ANOVA of germination indicated a significant main effect of salinity, temperature, Sodium hypochlorite inclusion and their interaction (Table 3). The promoting effect of Sodium hypochlorite was obvious at all salinity treatments and the highest germinations were obtained when 6 and 8% Sodium hypochlorite was mixed with 200 mM NaCl at 10-20 ^{o}C. The salinity alleviating effect of Sodium hypochlorite progressively decreased (Fig. 2) with the increase in temperature. Inclusion of Sodium hypochlorite substantially improved the rate of germination and changed little with increase in Sodium hypochlorite concentrations (Fig. 2). Rate of germination increased with the increase in temperature peaking at 20-30 ^{o}C both in the absence and presence of Sodium hypochlorite (Table 4).

Discussion

Sodium hypochlorite is a potent disinfecting agent which is widely used for seed surface sterilization (Bewley & Black, 1994; Kaneko & Morohashi, 2003). It is also known to favour seed germination or to overcome seed dormancy of different species (Frank & Larson, 1970; Abdul-Baki, 1974; Major & Wright, 1974; Fieldhouse & Sasser 1975, Hsiao 1979, Hsiao *et al*., 1981, Drew & Brocklehurst 1984, Galletta *et al*., 1989; Igbinnosa & Okonkowo, 1992; Clevering, 1995; Fushing *et al*., 1998; Miyoshi & Mii, 1999; Vujanovic *et al*., 2000; Ervin & Wetzel, 2002). Yildiz & Celal (2002) reported that pre-treating *Linum usitatissimum* with Sodium hypochlorite for short period stimulated germination. They attributed this stimulation to the scarification of the seed coat that allows more water and oxygen absorption or perhaps due to enhanced oxidative respiration by an extra supply of oxygen from decomposition of Sodium hypochlorite.

Pretreatment of *L. stocksii* seeds with Sodium hypochlorite caused a substantial leaching of colored material but showed little effect on seed germination of *L. stocksii* at non-saline control and low saline conditions. However, pre-treatment of seeds for one and five minutes with 10-30% Sodium hypochlorite substantially alleviated salinity effects on seed germination (Fig. 1). Inclusion of Sodium hypochlorite (2%) in the medium has also significantly improved *L. stocksii* seed germination at 200 and 300 mM NaCl (Fig. 1) and this effect does not change with any further increase in Sodium hypochlorite concentrations. Leaching of the chemicals noticed during pretreatment could be instrumental in increased imbibitions of water from saline solution and resulted in improved germination. Germination promoting chemicals like GA_3, fusicoccin, ethephon, thiourea, proline, betaine and potassium nitrate failed to improve *L. stocksii* seed germination under saline conditions, except for kinetin (Zia & Khan, 2003). Improvement

Table 1. A three-way ANOVA of final germination of *Limonium stocksii* seeds due to salinity (SAL), Sodium hypochlorite (NaOCl), pretreatment time (TIM) and their interactions.

	Sum of square	df	F
Salinity	510379	4	1271***
NaOCl	4195	4	87***
Time	97	3	40**
SAL x NaOCl	5813	16	5***
SAL x TIM	1937	12	5*
NaOCl x TIM	4107	12	6***
SAL x NaOCl x TIM	5316	48	2*
SAL SAL x HYP x TIM			

*** = p< 0.001, ** = p< 0.01, * = p< 0.05.

Table 2. Rate of germination of *L. stocksii* using modified Timson's index in different salinities with various pretreatment times of different hypochlorite (NaOCl) concentrations.

NaOCl (%)	NaCl (mM)	Control	Time (minutes)			
			1	5	10	20
0	0	47.31±0.19	48.40±2.20	47.35±2.41	47.75±1.26	48.55±1.06
	100	38.35±2.35	33.10±0.33	37.13±2.07	37.05±1.07	36.30±1.40
	200	20.25±3.49	30.15±0.83	25.40±1.73	23.10±2.64	20.00±2.98
	300	05.35±2.35	06.80±1.33	07.15±2.86	06.25±1.57	06.80±3.60
	400	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
10	0	49.45±0.19	49.80±0.20	45.35±1.49	46.75±1.26	48.55±1.06
	100	45.35±2.35	47.10±0.33	44.25±1.04	47.05±1.07	46.30±1.40
	200	26.25±3.49	42.95±0.83	38.60±0.93	34.10±2.64	33.00±2.98
	300	08.35±2.35	21.80±1.33	10.15±1.97	09.25±2.57	13.80±3.60
	400	01.80±0.98	01.50±0.58	05.25±0.96	03.95±1.10	01.85±0.87
20	0	49.45±0.19	47.20±1.84	45.95±1.81	47.15±1.49	47.00±1.29
	100	45.35±2.35	43.60±2.27	46.80±1.24	41.85±1.65	45.80±2.21
	200	26.25±3.49	30.45±2.06	28.20±3.48	26.75±3.2	33.60±3.87
	300	08.35±2.35	08.20±0.84	10.05±1.89	11.15±0.73	13.65±1.37
	400	01.80±0.98	02.85±1.28	03.50±0.91	03.05±1.37	03.65±1.93
30	0	49.45±0.19	45.35±1.90	43.30±0.86	48.40±1.01	45.80±0.14
	100	45.35±2.35	45.45±2.18	41.40±2.84	44.20±1.54	43.85±2.66
	200	26.25±3.49	38.05±1.84	36.60±3.14	38.10±1.39	29.65±2.31
	300	08.35±2.35	18.90±2.34	15.70±1.85	22.65±1.06	20.05±5.28
	400	01.80±0.98	04.85±0.73	05.65±1.46	10.65±1.00	05.65±1.58
40	0	49.45±0.19	43.30±2.40	45.30±3.19	45.50±2.63	47.15±0.93
	100	45.35±2.35	42.30±1.09	45.65±2.11	44.65±1.22	41.90±1.99
	200	26.25±3.49	26.85±3.82	35.00±2.80	32.25±4.24	35.40±4.00
	300	08.35±2.35	07.25±2.82	12.05±2.48	16.70±0.79	11.75±4.03
	400	01.80±0.98	00.30±0.30	07.30±1.50	06.80±3.04	04.35±3.42

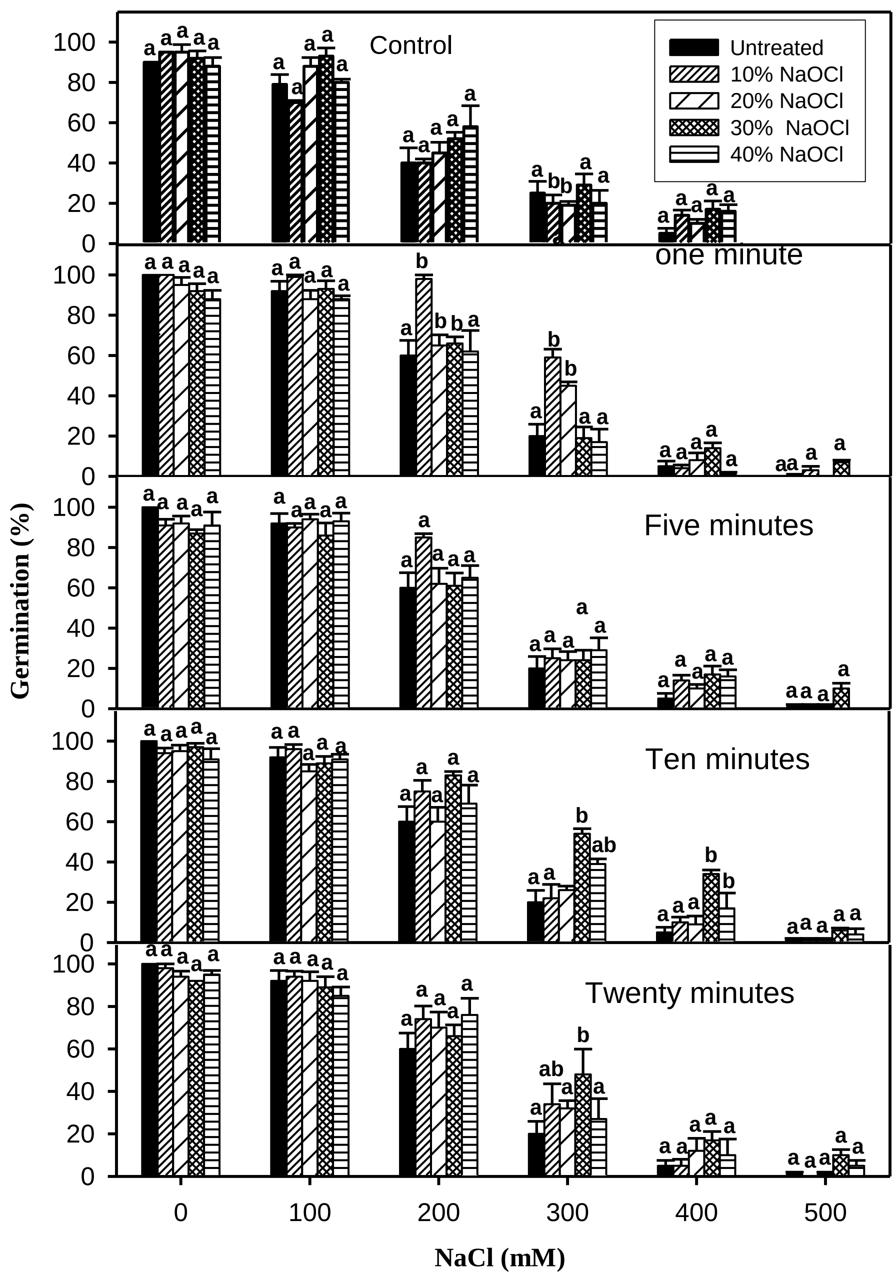

Fig. 1. Effect of different pretreatment timings and various concentrations of Na-hypochlorite on the germination percentage of *Limonium stocksii* in saline solutions. Letters indicate Bonferroni test within each salinity treatment.

Table 3. A three-way ANOVA of final germination of *Limonium stocksii* due to salinity (SAL), temperature (TEM), sodium hypochlorite (NaOCl), and their interactions.

	df	SS	F
Temperature	3	27337	55.9***
Salinity	4	329455	461***
NaOCl	4	6564	9***
TEM x SAL	12	14629	7***
TEM x NaOCl	12	34163	6***
SAL x NaOCl	16	20754	7***
TEM x SAL x NaOCl	48	3641	2*

*** = $p < 0.001$, * = $p < 0.05$

of seed germination by Sodium hypochlorite pretreatment could be due to increased availability of oxygen (Ogawa & Iwabuchi, 2001) increase in amylase activity (Kaneko & Morohashi, 2003), destruction of inhibitors (Makinnon & Alderton, 2000; Ogawa & Iwabuchi, 2001) and facilitation in leaching inhibitors (Zia & Khan, unpublished data).

Effect of Sodium hypochlorite in alleviating salinity effects on germination was optimal at cooler thermoperiod (10-20 ºC), however, rate of germination was higher at warmer thermoperiod (20-30 ºC). Promotion of seed germination at cooler temperatures could be ascribed to decreased penetration of Sodium hypochlorite into the seeds and simultaneous decomposition to provide oxygen (Drew & Brockelhurst, 1984; Yildiz & Celal, 2002) while decrease in seed germination at higher temperatures could be due to facilitation in penetration of Sodium hypochlorite through seed coat and increased accumulation in the seeds. High concentrations of Sodium hypochlorite may cause the oxidation of biological molecules such as proteins and nucleic acids, which could result in germination inhibition (Bloomfield & Arthur, 1991).

Washing seeds with Sodium hypochlorite appears to be quite useful in getting successful recruitment of species. This compound not only protects germinating seeds from all kinds of pathogens but also facilitates the leaching of toxic compounds and scarification of seed coat to improve the permeability for water and oxygen. These properties could contribute in increased germination which otherwise would have been difficult. This research first time reports that inhibiting effect of high salinity could also be alleviated both by pretreatment or inclusion of Sodium hypochlorite in the medium. The exact mechanism of this alleviation is not known, however, one hypothesis may be that it could be mediated through leaching and destruction of inhibitors produced by high salt stress and increased uptake of water from saline solution due to scarification of seeds. The exact mechanism through which Sodium hypochlorite alleviates salinity effects on germination needs to be explored further. However, application of Sodium hypochlorite would facilitate the recruitment of cash-crop halophytes under saline conditions and will therefore contribute significantly in utilizing the degraded saline lands. This research therefore validates the hypothesis that application of Sodium hypochlorite alleviates the salinity effects on seed germination of *Limonium stocksii*.

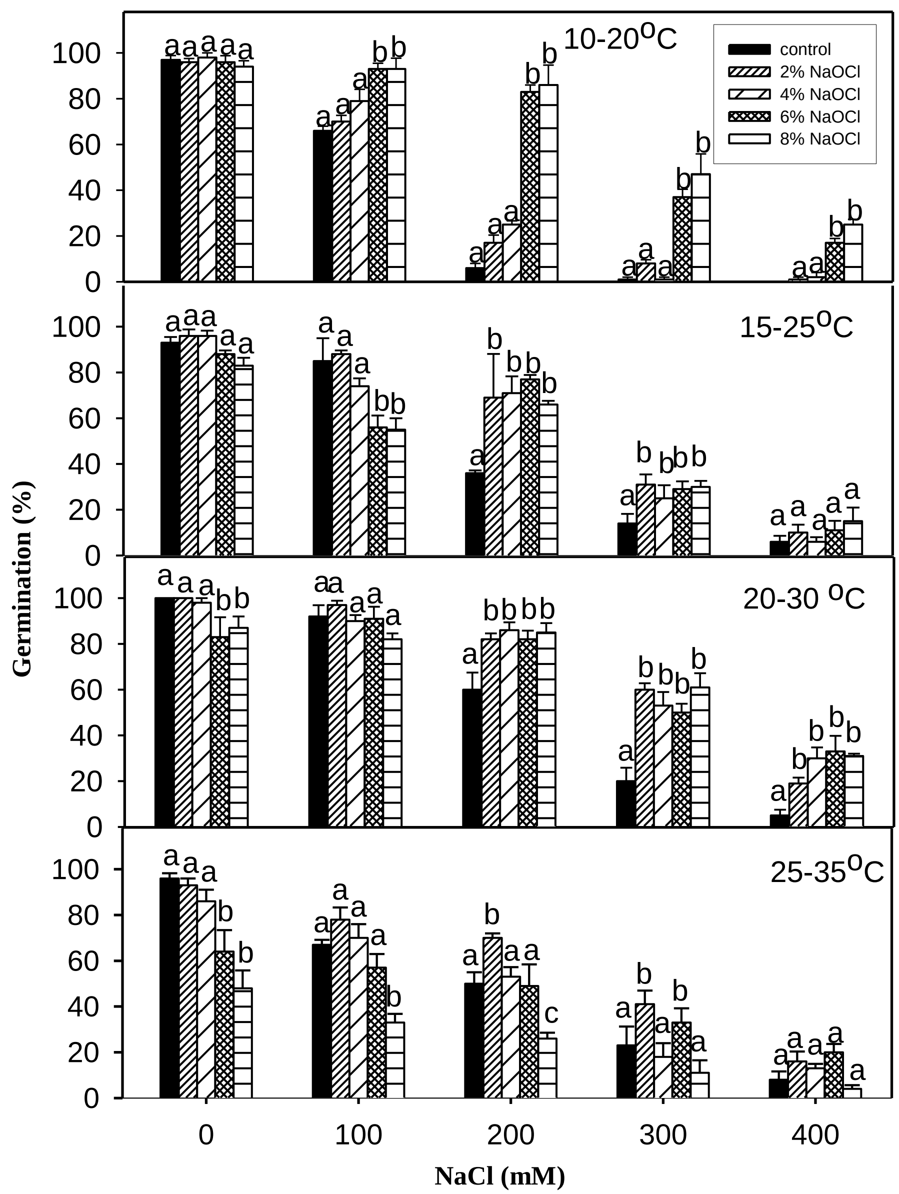

Fig. 2. Germination percentage of *Limonium stocksii* seeds immersed in different salinity concentrations with various percent of Na-hypochlorite. Letters indicate Bonferroni test within each salinity treatment.

Table 4. Rate of germination (Mean ± SE) of *L. stocksii* in different salinities with various concentrations of Sodium hypochlorite (NaOCl) at various temperature regimes.

NaOCL (%)	Salinity (mM)	Thermo period (night-day)			
		10-20 ºC	15-25 ºC	20-30 ºC	25-35 ºC
0	0	44.55±1.13	43.50± 1.52	49.45± 0.19	47.80± 1.27
	100	29.25± 9.29	35.80± 4.01	45.35± 2.35	32.90± 5.91
	200	02.65± 0.98	14.05± 4.54	26.25± 3.49	23.15± 6.59
	300	00.35± 0.35	05.25± 1.56	08.35± 2.35	10.20± 3.75
	400	00.00± 0.00	01.80± 0.73	01.80± 0.98	03.35± 1.48
2	0	37.85±3.90	46.00±2.60	49.70±0.13	46.30±1.62
	100	21.75±6.55	38.85±0.85	47.80±0.84	38.05±3.23
	200	05.10±1.11	28.00±8.19	38.45±1.28	32.25±5.78
	300	02.30±0.47	10.80±1.65	26.05±1.16	17.65±2.31
	400	00.15±0.15	03.20±1.06	08.20±1.01	06.95±1.99
4	0	39.70±4.70	45.80±2.06	48.70±1.04	42.25±3.11
	100	25.15±4.26	31.90±2.09	44.05±1.23	33.90±3.58
	200	06.55±2.74	27.95±7.21	40.15±1.98	23.90±3.54
	300	00.25±0.25	08.75±2.33	23.45±2.82	08.10±2.75
	400	00.55±0.55	01.50±0.51	12.50±2.23	05.55±0.81
6	0	38.85±1.05	42.35±1.07	41.35±4.42	31.00±4.35
	100	32.80±1.23	23.70±3.12	44.75±2.56	25.95±2.74
	200	22.70±0.95	31.80±0.82	37.40±1.72	21.00±4.22
	300	8.15±2.05	10.70±1.01	21.45±1.96	13.90±2.79
	400	02.90±0.37	03.65±1.60	14.30±3.18	07.65±1.73
8	0	32.30±4.69	38.80±2.37	42.80±2.43	22.75±3.91
	100	25.05±2.91	23.10±3.17	40.45±1.08	14.70±1.52
	200	23.95±3.66	27.05±5.20	41.05±1.86	10.25±1.09
	300	10.65±2.74	11.20±1.15	26.55±2.57	04.80±2.53
	400	03.55±1.26	05.40±2.18	12.25±0.60	01.75±0.74

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References

Abdul-Baki, A.A. 1974. Pitfall in using Sodium hypochlorite as seed disinfectant in ^{14}C incorporation studies. *Physiologia Plantarum*, 53: 768-771.

Anonymous. 2002. SPSS: SPSS 11 for Windows update. SPSS Inc. USA.

Bewley, J.D. and M. Black. 1994. *Seeds: Physiology of development and germination.* Plenum Press, New York.

Bloomfield, S.P. and M. Arthur. 1991. Comparative testing of disinfectants and antiseptic products using proposed European suspension test methods. *Applied Microbiology*, 13: 233-239.

Böhm, J. 2003. Terrestrial Orchid Seed Handling: Growing orchids of temperate climates from seed. In: *Seed Pretreatment Protocol*. Institute of Physiology, Medical Faculty, University of Saarland, Homburg-Saar, Germany.

Clevering, O.A. 1995. Germination and seedling emergence of *Scirpus lacustris* L., and *Scirpus maritimus* L., with special reference to the restoration of wetlands. *Aquatic Botany*, 50: 67-83.

Drew, R.L.K. and P.A. Brockelhurst. 1984. The effect of Sodium hypochlorite on germination of lettuce seed at high temperature. *Journal of Experimental Botany*, 35: 975-985.

Ervin, G.N. and R.G. Wetzel. 2002. Effects of Sodium hypochlorite sterilization and dry cold storage on germination of *Juncus effusus* L. *Wetlands*, 22: 191-195.

Fieldhouse, D.J. and M. Sasser. 1975. Stimulation of pepper seed germination by Sodium hypochlorite treatment. *Horticultural Science*, 10: 622-623.

Frank, A.B. and K.L. Larson. 1970. Influence of oxygen, Sodium hypochlorite and dehulling germination of green needle grass seed (*Stipa viridula* Trin.). *Crop Science*, 10: 679-682.

Fushing, L., J. Peron and N. Blanchard. 1998. Effect of different pre-treatments to overcome the dormancy of seakale (*Crambe maritima* L.) seeds. *Acta Horticulture,* 467: 289-295.

Galletta, G.J., J.R. Ballington and A.D. Draper. 1989. Pre-germination treatment of seeds of species and hybrids in *Rubus* with Sodium hypochlorite. *Acta Horticulture*, 263: 289-295.

Hsiao, A.I. 1979. The effect of Sodium hypochlorite and Gibberellic acid on seed dormancy and germination of wild oats (*Avena fatua*). *Canadian Journal of Botany*, 57: 1729-1734.

Hsiao, A.I. and A.W. Quick. 1984. Action of Sodium hypochlorite and hydrogen peroxide on seed dormancy and germination of wild oats, *Avena fatua* L. *Weed Research*, 24: 411-419.

Hsiao, A.I. and J.A. Hans. 1981. Application of the Sodium hypochlorite seed viability test to wild oat populations with different dormancy characteristics. *Canadian Journal of Plant Science,* 61: 115-122.

Hsiao, A.I., A.D., Worsham and D.E. Moreland. 1981. Effect of Sodium hypochlorite and certain plant growth regulators on germination of witchweed (*Striga asiatica*) seeds. *Weed Science*, 29: 98-100.

Igbinnosa, I. and S.N.C. Okonkwo. 1992. Stimulation of germination of seeds of cowpea witchweed (*Striga gesneriodes*) by Sodium hypochlorite and some growth regulators. *Weed Science*, 40: 25-28.

Ilahi, I. and F. Hussain. 1988. Germination improvement of *Reptonia buxifolia* (Falc.) A. DC., with physico-chemical factors. *Pakistan Journal of Forestry*, 38: 69-78.

Kanecko, Y. and Y. Morohashi. 2003. The effect of Sodium hypochlorite treatment on the development of α-amylase activity in mung bean cotyledons. *Plant Science*, 164: 287-292.

Khan, M.A. and B. Gul. 2002. *Some ecophysiological aspects of seed germination in halophytes*. In: *Halophyte Utilization and Regional Sustainable Development of Agriculture*. (Eds.): X. Liu and M. Liu. Metereological Press, Beijing, pp. 56-68.

Khan, M.A. and I.A. Ungar. 1997. Effect of light, salinity, and thermoperiod on the seed germination of halophytes. *Canadian Journal of Botany*, 75: 835-841.

Mackinnon, P.J. and M.R. Alderton. 2000. An investigation of the degradation of the plant toxin, ricin by Sodium hypochlorite. *Toxicon*, 38: 287-291.

Major, R.L. and L.N. Wright. 1974 Seed dormancy characteristics of sideoats gramagrass (*Bouteloua curtipendula* (Michx.) Torr.). *Crop Science*, 14: 37-40.

Miyoshi, K. and M. Mii. 1998. Stimulatory effects of Sodium and calcium hypochlorite, pre-chilling and cytokinins on the germination of *Cypridium macranthos* seed *In vitro*. *Physiologia Plantarum,* 102: 481-486.

Ogawa, K. and M. Iwabuchi. 2001. A mechanism for promoting the germination of *Zinnia elegans* by hydrogen peroxide. *Plant & Cell Physiology*, 42: 286-291.

Vujanovic, V., M. St-Arnaud, D. Barabe and M. Thieault. 2000. Viability testing of orchid seed and the promotion of colouration and germination. *Annals of Botany,* 86: 79-86.

Yildiz, M. and E. Celal. 2002. The effect of Sodium hypochlorite solution on *In vitro* seedling growth and shoot regeneration of flax (*Linum usitatissimum). Natureweissenshaften*, 89: 259-261.

Zia, S. and M.A. Khan. 2003. Effect of germination regulating chemicals in alleviating salinity enforced germination inhibition of *Limonim stocksii*. *Pakistan Journal of Botany*, 36: 928-938.

Zia, S. and M.A. Khan. 2004. Effect of light, salinity and temperature on the germination of *Limonium stocksii*. *Canadian Journal of Botany,* 82: 151-157.

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