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Evaluation of emergence and vigour of Ashwagandha (*Withania somnifera* Dunal) seedlings under the influence of Sodium Hypochlorite ($NaHClO_3$) and different micro-environmental conditions

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ABSTRACT : Ashwagandha (Withania somnifera Dunal), a well recognised medicinal plant species, is widely used as tonic in various preparations of Ayurvedic, Unani and Modern Systems of Medicine. This species has consistent demand in pharmaceutical industries and being successfully cultivated in many tropical regions of India. Despite that the supply of 50% of the raw materials is met from the harvesting of wild populations. We examined the effect of different micro-environmental conditions (open, glasshouse and nethouse) and pre-sowing treatments of Sodium Hypochlorite ($NaHClO_3$: 5 & 10-minutes) on seedling emergence, growth and biomass in this species. The seeds treated with $NaHClO_3$ (5-minutes) showed maximum emergence in glasshouse condition (76.67%), which was significantly ($P<0.05$) higher than other conditions. However, seedlings growth and biomass in open condition were comparable to that in glasshouse condition. It is suggested that for large scale cultivation, the seedlings (in two-three-leaf stage) produced in the glasshouse should be transferred to the open fields. We offer these simple and low cost tools especially for unskilled and poor farmers interested in nursery development and cultivation of this species.

Keywords : Medicinal, micro-environments, pre-treatments, seedling emergence, growth, biomass, cultivation

INTRODUCTION

Genus Withania (Family-Solanaceae), comprises about 10 species worldwide, is distributed in east of the Mediterranean region, extending to South Asia mainly India, Sri Lanka and Pakistan (Gaur, 1999). Two species namely, *Withania coagulans* Dunal and *W. somnifera* Dunal occur in India. *Withania somnifera*, popularly known as Ashwagandha, is a 50-150 cm tall, erect evergreen tomentose self-pollinating shrub, which has consistent demand in pharmaceutical industries due to its unique medicinal properties (Anonymous, 1976). It occurs throughout the drier parts of India, mainly at low elevation ranges of Bombay, Gujarat, Madhya Pradesh, Rajasthan, Punjab, Uttar Pradesh. It thrives very well in waste places and roadsides. In Himalayan region, it occurs in Uttarakhand, Himachal Pradesh and Jammu & Kashmir upto 1650 m above mean sea level (amsl). This species is widely used as tonic in various preparations of Ayurvedic, Unani and Modern Systems of Medicine. Various parts of the plant are used to cure over 85 diseases (Butola *et al*., 2008). Leaves and roots contain many alkaloids and withanolids. Withaferin-A is the most important withanolids isolated so far which shows anti-biotic, anti-arthritic, anti-inflammatory and anti-tumour activities (Anonymous, 1976). Its fruits and seeds besides being medicinal (diuretic, hypnotic, etc.) are employed in curdling milk to prepare vegetarian cheese. The seeds are also used as a substitute of soap being rich in saponins.

Ashwagandha has high demand (500 tonnes, during 1999) in pharmaceutical industries, 50% of which is met through harvesting of wild population (NRIF, 2004). Its current domestic sales are approximately Rs. 100-120 million (Rawat and Garg, 2005). Extensive cultivation of this species is being done in many tropical regions of India. Report is also available revealing its successful cultivation in a temperate climate zone (Butola and Samant, 2007). It is mainly propagated through seeds. During our experimental trials in this species, it is experienced that the seeds when sown directly in the field yielded low and erratic germination and heterogeneous seedling stand. Different reports on this species suggest that the germination percentage can be improved by the application of pre-sowing chemical treatments (Kattimani *et. al*., 1999; Vakeswaran and Krishnasamy, 2003a,b) or by providing congenial growth environments (Obidoska *et al*., 2004; Butola and Samant, 2007; Panwar *et. al*., 2009). For successful propagation and mass multiplication of any plant species, the identification of suitable growth environments to achieve optimum seedling emergence, growth, yield and survival, is an important nursery practice (Nautiyal *et. al*., 2001, Butola and Badola, 2006a, 2008). In a crop's life cycle, the time from seed sowing to seedling establishment is considered a vital phase, which decidedly influences the final yield and post-harvest seed quality (Wurr and Fellows, 1983). Further, the probability of a seedling surviving to maturity largely depends upon the right time and place of germination (Thompson, 1973).

Keeping these points in mind, there is a dire need to explore low cost and simple technological tools particularly for nursery workers and poor farmers interested in developing mass planting stock of this species. Therefore, we designed the present study to examine the effect of different micro-environmental conditions and pre-treatments of a low cost surface disinfectant ($NaHClO_3$) on seedling emergence and vigour of this species.

MATERIALS AND METHODS

A. Plant materials

During the month of December (2008), mature fruits of Ashwagandha were collected from the medicinal plants garden of High Altitude Plant Physiology Research Centre (HAPPRC), located at 550 m amsl in Srinagar, district Pouri, Uttarakhand. Seeds were extracted from the fruits and air-dried for one week before storage in air-tight polybags until used for experimental trials.

B. Seedling emergence, growth and biomass

The experiment lasted for two months, *i.e.,* April-May, 2009. The percent moisture content of seeds was determined following ISTA (1985). Viability of the stored seeds was tested using tetrazolium (pH 6.0) according to the procedure of ISTA (1985). A lot of five hundred (#500) seeds were treated with Sodium Hypochlorite ($NaHClO_3$, 4% available) for 5 and 10-minutes. Seeds soaked with double distilled water for 24 hrs considered as control. Treated seeds (9 seeds per tray) were sown in Styrofoam seedling trays (30cm × 30cm) containing a potting mixture of garden soil (sandy loam), sand and leaf litter (1:1:1, v:v:v; pH: 5.5-6.4, organic carbon content: 1.0%-1.2%). A total of 27(3 treatments × 3 replicate × 3 conditions) trays were used. The seeded trays were placed in different micro-environmental conditions, *viz.,* Open (temperature: max. 29.5 ± 2.9°C and min. 13.8 ± 1.0°C); Glasshouse (temperature: max. 33.3 ± 4.0°C and mini. 15.33 ± 1.2°C) and Nethouse (temperature: max. 28.8 ± 2.8°C and mini. 12.6 ± 1.0°C). The trays were kept moist through watering with a fine sprayer as and when required. Seedlings were considered emerged when cotyledonary leaves appeared on the media surface. The experiment was monitored on daily basis and terminated when no more seedlings emerged for two weeks.

After 4 months of sowing, 30 seedlings from each condition (10 seedlings per treatment) were randomly harvested to assess growth and biomass. After recording different growth parameters, *viz*., shoot length and diameter, root length and diameter, leaf area, number of leaves and petiole length, the plants were slashed from collar to divide into above and below ground parts. These parts were oven-dried at 80°C for 24 hrs to assess total biomass (g/plant).

C. Data analysis

Data were analyzed statistically using MS excel, 2007. One way analysis of variance (ANOVA) and Fisher's least significant differences (F-LSD) was employed to calculate significant difference between means of different parameters (Snedecor and Cochran, 1967). Data in percentages were subjected to arcsine transformation before analysis of variance and then converted back to percentage for presentation.

RESULTS

At the time of experimentation, the viability and moisture content of Ashwagandha seeds were recorded to be 90.00 ± 2.00% and 7.06 ± 1.72%, respectively. Experimental results on seedling emergence in different micro-environmental conditions under the influence of $NaHClO_3$ are presented in Fig.1. Mean emergence percentage significantly ($P < 0.05$) varied between conditions. Maximum emergence was found in glasshouse condition (76.67%) in seeds treated with $NaHClO_3$ for 5-minutes and minimum in open condition (46.67%) in 10-minutes' treatment. As far as the effect of pre-treatment is concerned, 5 minutes' treatment of $NaHClO_3$ significantly improved mean emergence percentage in open (66.67% as compared to control, 53.33%) and glasshouse (76.67% as compared to control, 63.33%) conditions. However, 10-minutes' treatment proved inhibitory in these growing conditions. Mean emergence time did not varied significantly between treatments and conditions; it ranged between 15 and16 days in all the treatments and conditions Fig.2.

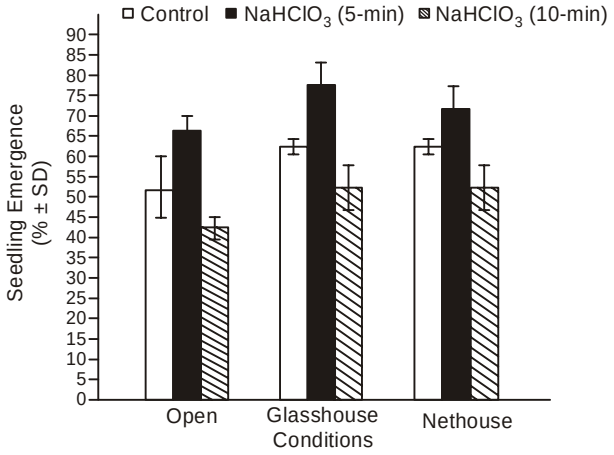

Fig.1. Mean emergence in *Withania somnifera* under the influence of $NaHClO_3$ and different micro-environmental conditions.

ANOVA Summary

	Conditions		
	Open	**Glasshouse**	**Nethouse**
P-value (LSD Between treatments)	< 0.05 (11.53)	< 0.001 (9.99)	> 0.05 (11.53)
P-value (LSD Between conditions)	< 0.05 (10.21)		

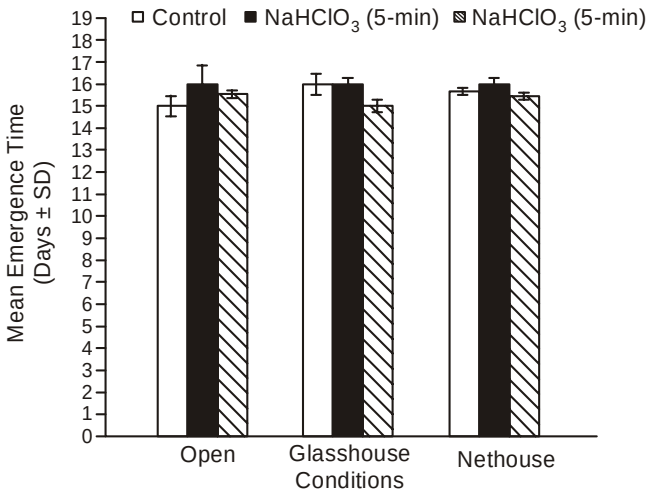

Fig.2. Mean emergence time in *Withania somnifera* under the influence of $NaHClO_3$ and different micro-environmental conditions.

ANOVA Summary

	Conditions		
	Open	**Glasshouse**	**Nethouse**
P-value (LSD Between treatments)	> 0.05 (0.59)	> 0.05 (0.24)	> 0.05 (0.50)
P-value (LSD Between conditions)	> 0.05 (0.63)		

The data pertaining to seedling growth and biomass in Ashwagandha are presented in Table 1, 2. Growth performance of seedlings significantly varied between treatments and growing conditions. Open condition was favorable to the plants raised through seeds treated with $NaHClO_3$ for 10-minutes, as shoot length (36.40 cm), shoot diameter (3.47 mm), leaf area (39.79 cm^2), petiole length (2.10 cm) and above ground dry weight (1.18 g) maximized in this condition. However, root length (18.38 cm), root diameter (8.21 mm), number of leaves (9.60), below ground dry weight (0.86 g) and total biomass (1.85 g) were found maximum in glasshouse condition in non-treated seeds.

Table 1 : Seedling growth in *Withania somnifera* under the influence of $NaHClO_3$ and different micro-environmental conditions.

Parameters/Treatments	**CONDITIONS**			***P*-value (LSD)**
	Open	**Glasshouse**	**Nethouse**	
Shoot length (cm)				
Control	32.58 ± 1.66	29.08 ± 1.06	27.98 ± 0.72	<0.001 (2.36)
$NaHClO_3$ (5-minute)	27.60 ± 0.96	25.52 ± 1.38	25.28 ± 1.08	
$NaHClO_3$ (10-minute)	36.40 ± 2.79*	21.80 ± 2.02	20.94 ± 1.19	
***P*-value (LSD)**	**<0.001 (3.46)**	**<0.001 (2.73)**	**<0.001 (1.81)**	
Shoot diameter (mm)				
Control	3.31 ± 0.33	2.72 ± 0.10	2.70 ± 0.08	<0.001 (0.36)
$NaHClO_3$ (5-minute)	2.65 ± 0.13	3.19 ± 0.45*	3.14 ± 0.40*	
$NaHClO_3$ (10-minute)	3.47 ± 0.29	3.20 ± 0.19*	3.12 ± 0.10*	
***P*-value (LSD)**	**<0.001 (0.29)**	**<0.05 (0.32)**	**<0.05 (0.27)**	
Root length (cm)				
Control	6.38 ± 0.34	18.38 ± 2.44	6.30 ± 0.21	<0.001 (2.42)
$NaHClO_3$ (5-minute)	7.30 ± 2.08	18.14 ± 5.58	6.38 ± 1.45	
$NaHClO_3$ (10-minute)	7.62 ± 0.55	11.40 ± 1.60	7.14 ± 0.53	
***P*-value (LSD)**	**>0.05 (1.41)**	**<0.05 (4.09)**	**>0.05 (1.01)**	
Root diameter (mm)				
Control	6.78 ± 0.50	8.21 ± 0.19	8.11 ± 0.10	<0.05 (0.53)
$NaHClO_3$ (5-minute)	6.04 ± 0.62	6.72 ± 0.58	6.59 ± 0.49	
$NaHClO_3$ (10-minute)	7.20 ± 0.36	7.54 ± 0.77	7.14 ± 0.52	
***P*-value (LSD)**	**<0.05 (0.57)**	**<0.01 (3.89)**	**<0.001 (0.47)**	
Leaf area (cm^2)				
Control	33.50 ± 12.4	22.82 ± 5.31	24.82 ± 4.79	>0.05 (6.46)
$NaHClO_3$ (5-minute)	30.64 ± 9.32	19.96 ± 1.83	20.29 ± 1.54	
$NaHClO_3$ (10-minute)	39.79 ± 4.76	22.52 ± 2.20	22.69 ± 2.14	
P-value (LSD)	>0.05 (10.54)	>0.05 (3.92)	>0.05 (3.55)	

(Contd...)

Parameters/Treatments	CONDITIONS			*P*-value (LSD)
	Open	Glasshouse	Nethouse	
Number of leaves				
Control	7.40 ± 0.55	9.60 ± 1.52	9.00 ± 0.71	>0.05 (1.04)
$NaHClO_3$ (5-minute)	6.60 ± 0.55	9.20 ± 1.48	8.40 ± 0.89	
$NaHClO_3$ (10-minute)	7.80 ± 0.84	9.60 ± 1.14	8.80 ± 0.45	
***P*-value (LSD)**	**<0.05 (0.74)**	**>0.05 (1.56)**	**>0.05 (0.79)**	
Petiole length (cm)				
Control	2.10 ± 0.14	1.52 ± 0.19	2.00 ± 0.18	<0.05 (0.21)
$NaHClO_3$ (5-minute)	1.72 ± 0.31	1.38 ± 0.08	1.76 ± 0.25	
$NaHClO_3$ (10-minute)	1.96 ± 0.36	1.70 ± 0.27	2.06 ± 0.44	
***P*-value (LSD)**	**>0.05 (0.32)**	**>0.05 (0.22)**	**>0.05 (0.34)**	

Table 2 : Seedling biomass in *Withania somnifera* under the influence of $NaHClO_3$ and different micro-environmental conditions.

Parameters/Treatments	CONDITIONS			*P* value(LSD)
	Open	Glasshouse	Nethouse	
Above ground dry weight (g)				
Control	0.82 ± 0.14	0.99 ± 0.07	0.78 ± 0.09	<0.001 (0.13)
$NaHClO_3$ (5-minute)	0.77 ± 0.03	0.68 ± 0.11	0.76 ± 0.02	
$NaHClO_3$ (10-minute)	1.18 ± 0.12*	0.71 ± 0.09	1.01 ± 0.09*	
***P*-value (LSD)**	**<0.01 (0.17)**	**<0.01 (0.14)**	**<0.01 (0.11)**	
Below ground dry weight (g)				
Control	0.44 ± 0.03	0.86 ± 0.10	0.43 ± 0.02	>0.05 (0.52)
$NaHClO_3$ (5-minute)	0.56 ± 0.10	0.44 ± 0.11	0.48 ± 0.06	
$NaHClO_3$ (10-minute)	0.62 ± 0.10	0.55 ± 0.01	0.56 ± 0.00*	
***P*-value (LSD)**	**>0.05 (0.12)**	**>0.05 (0.20)**	**<0.05 (0.057)**	
Total Biomass (g)				
Control	1.26 ± 0.17	1.85 ± 0.29	1.21 ± 0.11	<0.001 (0.77)
$NaHClO_3$ (5-minute)	1.34 ± 0.07	1.12 ± 0.22	1.24 ± 0.07	
$NaHClO_3$ (10-minute)	1.80 ± 0.20*	1.26 ± 0.09	1.57 ± 0.08*	
***P*-value (LSD)**	**<0.01 (0.25)**	**<0.01 (0.34)**	**<0.001 (0.13)**	

Abbreviations used : LSD = Least Significant Difference.

DISCUSSION

In present study, seedling emergence, growth and biomass significantly varied among different growing conditions. Glasshouse condition offered congenial environment for seedling emergence as compared to other conditions. In case of seedling growth and biomass, this condition did not perform consistently better for above ground parts over open condition. Significance of protected cultivation particularly of polyhouse for high seedling emergence and early true leaf emergence in some high altitude medicinal herbs, *viz*., *Picrorhiza kurrooa* Royle ex Benth., *Rheum emodi* Wall. ex Meissn., *Nardostachys jatamansi* DC., *Angelica glauca* Edgew., *Heracleum candicans* Wall. and *Aconitum heterophyllum* Wall., has been suggested by different workers (Nautiyal and Purohit, 2000; Nautiyal *et al.*, 2001, 2003; Chauhan and Nautiyal, 2005; Butola and Badola, 2008). Ashwagandha generally grows in hot climate and varied soil conditions even in rough sites and therefore, showed less advantage of protected cultivation. Temperature is the most important factor which greatly influences different phenological phases of any plant species. In present case, minimum seedling emergence was recorded in open condition. Fluctuation in temperature may be a prominent reason of the same. Butola and Samant (2006) also found similar results in germination of *Saussurea costus* while comparing laboratory and open conditions.

Besides growth environments, Sodium Hypochlorite considerably helped to enhance seedling emergence, growth and biomass. This chemical is commonly used as a seed disinfectant, targeting seed borne pathogens. In addition, it has proved useful for breaking seed dormancy, improving germination rate, enhancing seedling vigor in different medicinal plant species (Butola and Badola, 2004a,b, 2006b, 2007; Butola *et. al*., 2007).

CONCLUSIONS

The 5-minutes' treatment of $NaHClO_3$ and glasshouse condition yielded maximum emergence in Ashwagandha. However, seedlings growth and biomass in open condition were comparable to that in glasshouse condition. Therefore, it can be concluded that the seeds treated with the above chemical should be sown in glasshouse for mass scale production of healthy seedlings. But for large scale cultivation, the seedlings (in two-three-leaf stage) produced in glasshouse should be transferred to the open fields. These methods being simple and low cost may be readily adopted by unskilled and poor farmers for developing healthy planting stock and cultivation of this species.

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