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EFFECT OF WATER STRESS ON SEED GERMINATION AND SEEDLING GROWTH IN SIX MEDICINAL PLANT SPECIES IN TARAI REGION, UTTARAKHAND

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ABSTRACT
The influence of water stress (0, -5, -10 and -15 bar) on seed germination and seedling growth in *Lepidium sativum* Linn., *Spilanthes acmella* Linn., *Matricaria chamomilla* Linn., *Ammi majus* Linn., *Andrographis paniculata* Nees., and *Cassia tora* Linn. in Tarai region of Uttarakhand, India were examined under laboratory conditions with triplicates. The per cent seed germination was maximum and minimum in 0 and – 15 bar water stress, respectively in all the species. The per cent germination was maximum (0 bar) in *L. sativum* (89.0) and minimum in *M. chamomilla* (46.0%). In high water stress (-15 bar), *M. chamomilla* was failed to germinateand it was 53.33 % in *A. paniculata* indicating more sensitive and tolerant species, respectively in comparison to other species. In the remaining species, radicle and plumule length (cm) and total seedling dry weight (mg) of 15 days old seedlings were reduced significantly with increasing water stress in all the species and the per cent reduction (percentage of maximum) was highest in *C. tora* (68.07), *A. paniculata* (81.27) and *S. acmella* (66.99), respectively. Thus, indicating that these species are more susceptible/intolerant to water stress compared to rest of the species.

INTRODUCTION

Medicinal plants offer alternative remedies with tremendous opportunities to generate income, employment and foreign exchange for developing countries (Rawat and Uniyal, 2004). Medicinal plants are rich source of secondary metabolites, biosynthetically derived from primary metabolites butrestricted to specific taxonomic genera of plant kingdom and specific part(s) of plant body. Secondary plant products are of major interest because of their biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological and pharmaceutical (Talreja *et al.,* 2012). Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to preventor cure several human diseases (Dhar *et al.,* 1999; Savithramma and Sudrasanamma, 2006). Consumption of herbal medicines is widespread and increasing in recent years and nearly 80% population of developing countries relies on traditional system of medicine (WHO, 2005).

India has been considered as a treasure house of valuable medicinal and aromatic plant species and it was estimated about 9,500 plant species are being used in traditional system of medicine (Mishra, 2006). Environmental factors influence the characters, composition, growth and development of individual plants and plant communities. When any of these environmental factors exceed the optimum tolerance results stress in plants (Lawlor, 2002).Biological stress is an adverse force or a condition that inhibits the normal functioning and well-being of plants (Jones *et al*., 1989). Tolerance to abiotic stresses is very complex due to the intricate of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Razmjoo *et al*., 2008). In recent times, water stress is a global issue to ensure survival of plant growth and development (Nakayama *et al*., 2007) and in the present study, an attempt is made on six different plant species having great medicinal and economic value in both regional as well as international market were selected to examine the effect of water stress on seed germination and seedling growth under controlled conditions.

MATERIALS AND METHODS

Study site and collection of seeds

The seeds of all selected species were collected at the time of maturity from Medicinal Plants Research and Development Center (MRDC), Haldi, G.B. Pant University of Agriculture and Technology, Pantnagar and healthy and non-infected seeds were separated, kept in cotton bags and stored under laboratory conditions, Department of Biological Sciences, College of Basic Sciences and Humanities, G B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

Seed Germination and Seedling Growth

The seeds were sterilized by soaking in 0.1 per cent $HgCl_2$ solution for 5 min washed thoroughly with tap water and finally with sterilized distilled water. Mannitol was used to maintain desired levels of water stress (osmotic potential) using the formula given by Helmerick and Pfeifer (1954). Uniform

and healthy sterilized seeds were placed on double layered filter papers in Petri dishes (9 cm dia.). Petri plates were kept for germination under water stress (0, -5 bar, -10 bar and -15 bar) by using mannitol solution with three replications. Radicle emergence is considered as an index of seed germination (Rao and Singh, 1985). The germination of seeds was recorded at an interval of 24 h up to 15 days. After 15 days, three seedlings from each Petri dish (a total of nine seedlings) were selected randomly and their length (cm) and total seedling dry weight (mg) were measured.

The Response Index (RI)

The Response Index (RI) was calculated as per the formula given byWilliamson and Richardson (1988) for the magnitude of inhibition versus stimulation by environmental factor *i.e.,* water stress on seed germination, radicle length, plumule length and total seedling dry weight using following formula:

1. When germination of treatments (T) is lower than the control (C):

RI = (T/C) – 1

2. When germination of treatments (T) is higher than the control (C):

RI = 1 – (C/T).

If RI > 0 Treatment stimulated germination

If RI = 0 No effect

If RI < 0 Treatment inhibited germination

Statistical Analysis

The data was presented as arithmetic means of three replicates ± standard error and analyzed statistically by using two factorial C.R.D. programme and ANOVA. Standard error and Critical Differences (CD) were evaluated at 1% level of significance by the method of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Seed germination

Seed germination is an important factor for the survival of plant species given the variable conditions of most natural ecosystems. Each plant species has a specific range of environmental requirements necessary for germination (Baskin and Baskin, 1989). Environmental factors such as light, soil moisture, temperature and pH are known to affect seed germination (Chachalis and Reddy, 2000). When any of these environmental factors exceed the optimum tolerance, the result is stress, which in turn influences developmental, structural, physiological and biochemical processes of seed germination. At the time of sowing, inadequate soil moisture results in irregular seed germination and unsynchronized seedling emergence, thereby affecting the establishment of a stand, with negative effects on the yield (Mwale *et al.,* 2003; Okcu *et al*., 2005). Seed germination and seedling growth are affected by both genetic and environmental factors, and different species have evolved different mechanisms to adapt to adverse conditions. As a result, the seed germination and seedling growth of different species can be varied under a similar environment.

In the present study, the per cent seed germination and the rate of germination at the end of the experiment (after 15 days) decreased and delayed, respectivelywith increasing water stress *i.e.,* from 0 bar to -15 bar in all the species (Table 1 and Fig. 1). The per cent germination under control (0 bar) was maximum in *L. sativum* (89.0) followed by *A. paniculata* (84.0%) and minimum in *M. chamomilla* (46.0%). At high

Table 1: Effect of water stress on seed germination (%) in different medicinal plant species (Mean ± S.E.).

Species	Control(0 bar)	-5 bar	-10 bar	-15 bar	Means within species
C. tora	70.00 ± 0.58	43.00 ± 1.15	31.67 ± 0.88	14.34 ± 1.15	39.50
A. paniculata	84.00 ± 1.55	70.00 ± 1.86	58.67 ± 1.45	53.33 ± 0.88	66.50
L. sativum	89.00 ± 1.53	59.00 ± 1.20	41.67 ± 1.45	33.33 ± 1.86	55.91
M. chamomilla	46.00 ± 1.50	28.00 ± 1.73	13.39 ± 0.88	00.0 ± 0.0	21.83
A. majus	63.33 ± 1.76	33.67 ± 2.03	23.34 ± 1.76	18.42 ± 1.87	34.66
S. acmella	73.67 ± 0.88	55.69 ± 1.45	40.63 ± 1.33	38.77 ± 1.67	50.42
Means with in Treatment	70.50	48.23	34.89	25.49	
	'F' value		Cd at 5%	Cd at 1%	
Species (a)	847.023**		1.565	2.089	
Treatment (b)	1885.786**		1.278	1.705	
Species x Treatment (a*b)	18.339**		3.132	4.178	
CV	4.257				

** Significant at 1% level.

Table 2: Response Index (RI) for seed germination, radicle length, plumule length and total seedling dry weight in different medicinal plant species due to water stress.

Species	Seed germination			Radicle length			Plumule length			Total seedling dry weight		
	-5 bar	-10 bar	-15 bar	-5 bar	-10 bar	-15 bar	-5 bar	-10 bar	-15 bar	-5 bar	-10 bar	-15 bar
C. tora	-0.55	-0.71	-1.00	-0.18	-0.46	-0.68	-0.19	-0.35	-0.55	-0.28	-0.45	-0.56
A. paniculata	-0.23	-0.35	-0.43	-0.11	-0.30	-0.42	-0.39	-0.69	-0.81	-0.13	-0.36	-0.53
L. sativum	-0.36	-0.52	-0.64	-0.14	-0.37	-0.48	-0.17	-0.25	-0.49	-0.15	-0.35	-0.50
M. chamomilla	-0.33	-0.69	-1.00	-0.49	-0.72	-1.00	-0.28	-0.49	-1.00	-0.21	-0.47	-1.00
A. majus	-0.45	-0.64	-0.75	-0.18	-0.28	-0.47	-0.23	-0.36	-0.45	-0.28	-0.36	-0.41
S. acmella	-0.22	-0.43	-0.53	-0.06	-0.19	-0.33	-0.17	-0.36	-0.62	-0.11	-0.29	-0.61

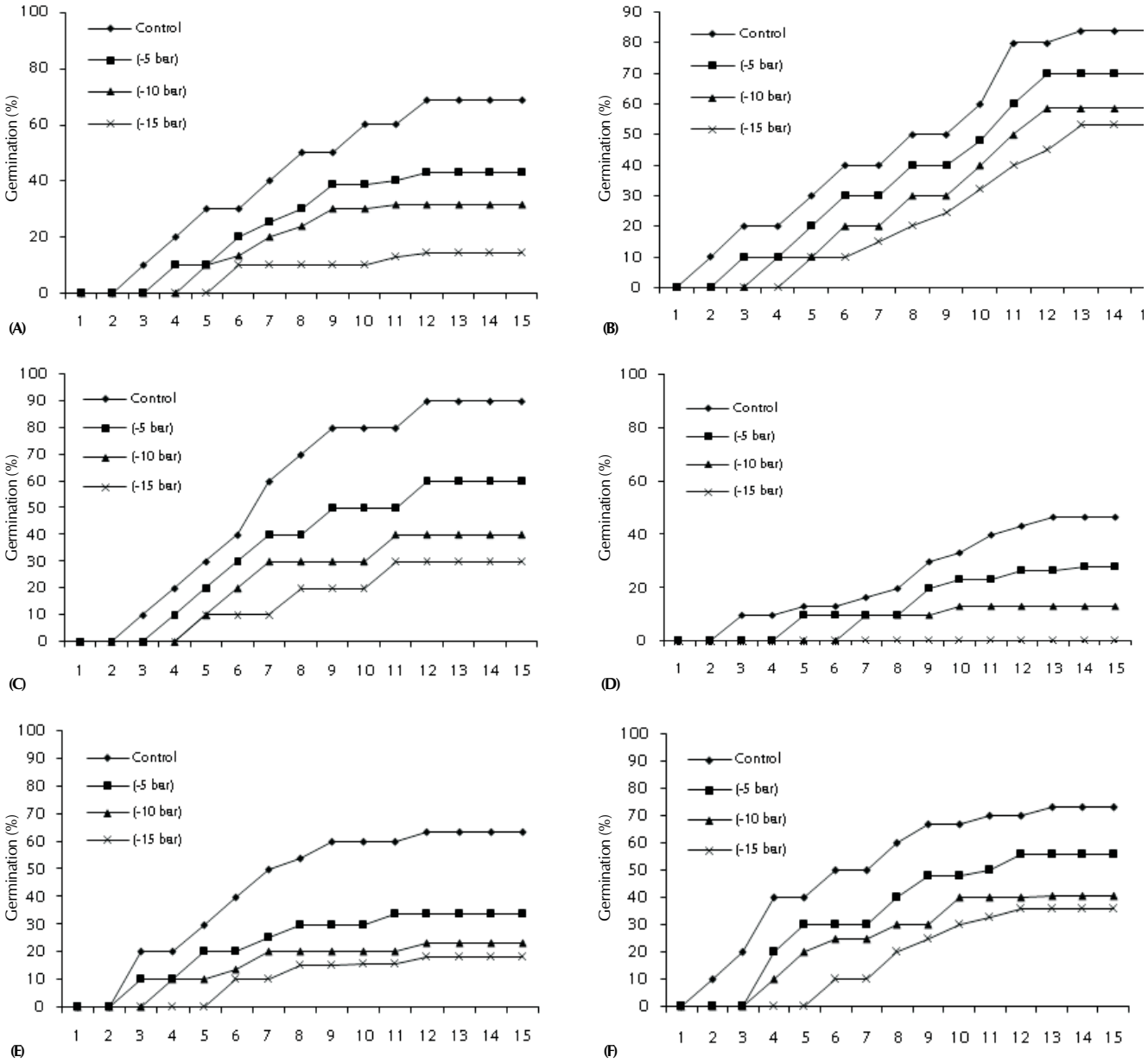

Figure 1: Effect of water stress on rate of seed germination in different medicinal plant species. (A) *C. tora,* (B) *A. paniculata,* (C) *L. sativum,* (D) *M. chamomilla,* (E) *A. majus,* and (F) *S. acmella*.

water stress (-15 bar), the per cent seed germination was decreased in all the species except in *M. chamomilla*in which the seeds failed to germinate,indicating intolerant to water stress. In the remaining species, it was maximum in *A. paniculata* (53.33 %), indicating more tolerant to high water stress and intermediate response in the rest of the species. The ANOVA values for seed germination in different species and water stress indicated that the differences between species, treatment and species × treatments were significant at $P < 0.01$.The per cent reduction (percentage of maximum or control) was maximum at high water stress (-15 bar) in all the species. The per cent reduction was significantly higher in *M. chamomilla*(100.00) and the rest of the species followed the order: *C. tora* (79.51) > *A. majus* (70.91) >*L. sativum* (62.55) >*S. acmella* (47.37) and >*A. paniculata* (36.51) (Fig. 2A). The Response Index (RI) was negative with different level of water stress in all species (Table 2). In high water stress (-15 bar), the RI values ranged from -1.00 in *C. tora* to -0.43 in *A. paniculata* (Table 2). Similar observations i.e., reduction in per cent seed germination and delayed rate of germination were also made by Bokhari *et al.* (1975) in *Bouteloua gracilis* (blue grama*), Agropyron smithii* (western wheatgrass) and *Buchloe dactyloides* (buffalo grass) at water stress greater than -3, -7 and -11 bars, respectively; Rao *et al.* (1984) in a pioneer Himalayan tree species (*Populus ciliata* Wall.), and King and Oliver (1994) in *Digitarias anguinali*. Mohassel *et al*. (2012) stated that prickly lettuce had a high germination percentage at appropriate moisture condition, but germination rate and

Table 3: Effect of water stress on radicle length (cm), plumule length (cm) in different medicinal plant species (Mean±S.E.).

Radicle length (cm) Species	Control(0 bar)	-5 bar	-10 bar	-15 bar	Means within species		Plumule length (cm) Control(0 bar)	-5 bar	-10 bar	-15 bar
Means within species										
C. tora	2.60± 0.15	2.13 ±0.18	1.40 ±0.15	0.83± 0.12	1.742	11.36± 0.22	9.23± 0.15	7.37± 0.18	5.07± 0.09	8.26
A. paniculata	2.83 ± 0.20	2.53 ±0.15	1.97± 0.09	1.63±0.19	2.242	5.50 ± 0.17	3.33 ± 0.12	1.70 ± 0.15	1.03±0.09	2.89
L. sativum	3.03 ±0.15	2.60±0.16	1.90±0.12	1.57±0.18	2.275	8.50± 0.20	7.03± 0.15	6.33± 0.20	4.27±0.12	6.53
M. chamomilla	1.17±0.20	0.60±0.12	0.33±0.06	0.00	0.525	2.10±0.15	1.50±0.18	1.07±0.15	0.00±0.00	1.17
A. majus	2.63 ±0.13	2.17±0.23	1.90±0.17	1.40± 0.15	2.025	4.07± 0.26	3.13 ± 0.19	2.60± 0.15	2.20±0.21	3.00
S. acmella	6.27±0.12	5.90±0.21	5.03±0.18	4.23±0.12	5.358	1.90± 0.18	1.57 ± 0.20	1.20±0.16	0.73±0.15	1.35
Means with in	3.09	2.66	2.09	1.61		5.57	4.30	3.38	2.21	
Treatment			'F' value	Cd at 5%	Cd at 1%		'F' value	Cd at 5%	Cd at 1%	
Species (a)			2317.087**	0.095	0.127		3055.483**	0.149	0.198	
Treatment(b)			563.821**	0.077	0.104		1109.141**	0.121	0.162	
Species x Treatment (a*b)			8.008**	0.189	0.253		70.019**	0.297	0.396	
CV	4.890		4.682							

** Significant at 1% level.

percentage decreased severely with decreasing available water and increasing water potential up to 0.5 MPa. Monfared *et al.* (2012) examined the effects of water stress on germination of *Lactuca serriola* seedsand reported that the germination percentage was decreased with increasing water potential. Thus, the present study results indicated that both seed germination (%) and rate of germination was significantly affected by water stress in all the species. Interestingly, the seeds failed to germinate in *M. chamomilla*,indicating most susceptible/intolerant and *A. paniculata*more resistant/tolerant to high water stress.

Seedling growth

At the end of experiment (after 15 days), the average radicle and plumule length (cm) and seedling dry weight (mg) was significantly affected by different level of water stress (Tables 3 and 4). The radicle and plumule length (cm) was maximum under control (0 bars) and minimum under - 15 bar in all species. Since the seeds of *M. chamomilla* failed to germinate under -15 bar water stress (Table 1) could not produce seedlings. In rest of the species, the radicle length (cm) was maximum and minimum in *S. acmella* (6.27 at 0 bar) and *C. tora* (0.83 at -15 bar), respectively. The per cent reduction (percentage of maximum) under high stress in different species followed the order: *C. tora* (68.07) >*L. sativum* (48.18) >*A. majus* (46.76) >*A. paniculata* (42.40) and >*S. acmella* (32.53) (Fig. 2B). The plumule length (cm) was maximum in *C. tora* (11.36 at 0 bar) and minimum in *S. acmella* (0.73 at -15 bar) and the per cent reduction (percentage of maximum) under high stress in different species followed the order :*A. paniculata* (81.27) >*S. acmella* (61.57) >*C. tora* (55.36) >*L. sativum* (49.76) and >*A. majus* (45.94) (Fig. 2B). The seedling dry weight (mg) was maximum in *C. tora* (14.57 at 0 bar) and minimum in *S. acmella* (1.26 at -15 bar) and the per cent reduction (percentage of maximum) under high stress in different species followed the order: *S. acmella* (60.99) >*C. tora* (56.96) >*A. paniculata* (53.03) >*L. sativum* (50.00) and >*A. majus* (41.46) (Fig. 2D). The RI values were negative in different levels of water stress in all the species (Table 2). The RI values at -15 bar water stress ranged from -1.00 (*M. chamomilla)* to -0.33 (*S. acmella)* in radicle length; -1.00 (*M. chamomilla)* to -0.45 (*A. majus)* in plumule length; and -1.00 (*M. chamomilla)* to -0.41 in (*A. majus)* in seedling dry weight (Table 2). The ANOVA values for radicle and plumule length and seedling dry weight in different species indicated that the differences between species, treatments and species × treatments were significant at $P < 0.01$.

In the present study, the seedling growth under un-stressed condition (0 bar) was the maximum and minimum under high water stress (-15 bar). Thus, increasing water stress resulted in decreased in seedling growth in all the species.Similar observations were also madein pea (*Pisum sativum) (*Gamze *et al*., 2005), two alfalfa cultivars(*Medicago sativa*L.) (Wang *et al*., 2009), on different alfalfa cultivars (*Medicago sativa*L.) (Hamidi and Safarnejad, 2010),leguminous species (Wu *et al*., 2011) and different varietiesof *Sesamum indicum* (Keshavarzi, 2012) by using PEG. Considerable reduction in root and shoot lengths of seedlings of finger millet with increasedchemical stress (Al and Cd concentration) (Hemalatha *et al*., 2011).

Table 4:Effect of water stress on total seedling dry weight (mg) in different medicinal plantspecies (Mean ± S.E.).

Species	Control (0 bar)	-5 bar	-10 bar	-15 bar	Means within species
C. tora	14.57 ± 0.23	10.43 ± 0.30	7.97 ± 0.48	6.27 ± 0.12	9.80
A. paniculata	6.60 ± 0.31	5.77 ± 0.27	4.17 ± 0.20	3.10 ± 0.32	4.90
L. sativum	5.00 ± 0.29	4.24 ± 0.15	3.24 ± 0.19	2.50 ± 0.29	3.74
M.chamomilla	2.03 ± 0.15	1.60 ± 0.12	1.07 ± 0.18	0.00 ± 0.00	1.17
A. majus	6.27 ± 0.12	4.50 ± 0.17	4.00 ± 0.23	3.67 ± 0.09	4.60
S. acmella	3.23 ± 0.19	2.87 ± 0.15	2.27 ± 0.12	1.26 ± 0.18	2.40
Means within Treatment	6.28	4.90	3.78	2.80	
	'F' value		Cd at 5%	Cd at 1%	
Species (a)	944.831**		0.275	0.368	
Treatment(b)	358.516**		0.225	0.301	
Species x Treatment (a*b)	32.267**		0.551	0.735	
CV	7.556				

** Significant at 1% level.

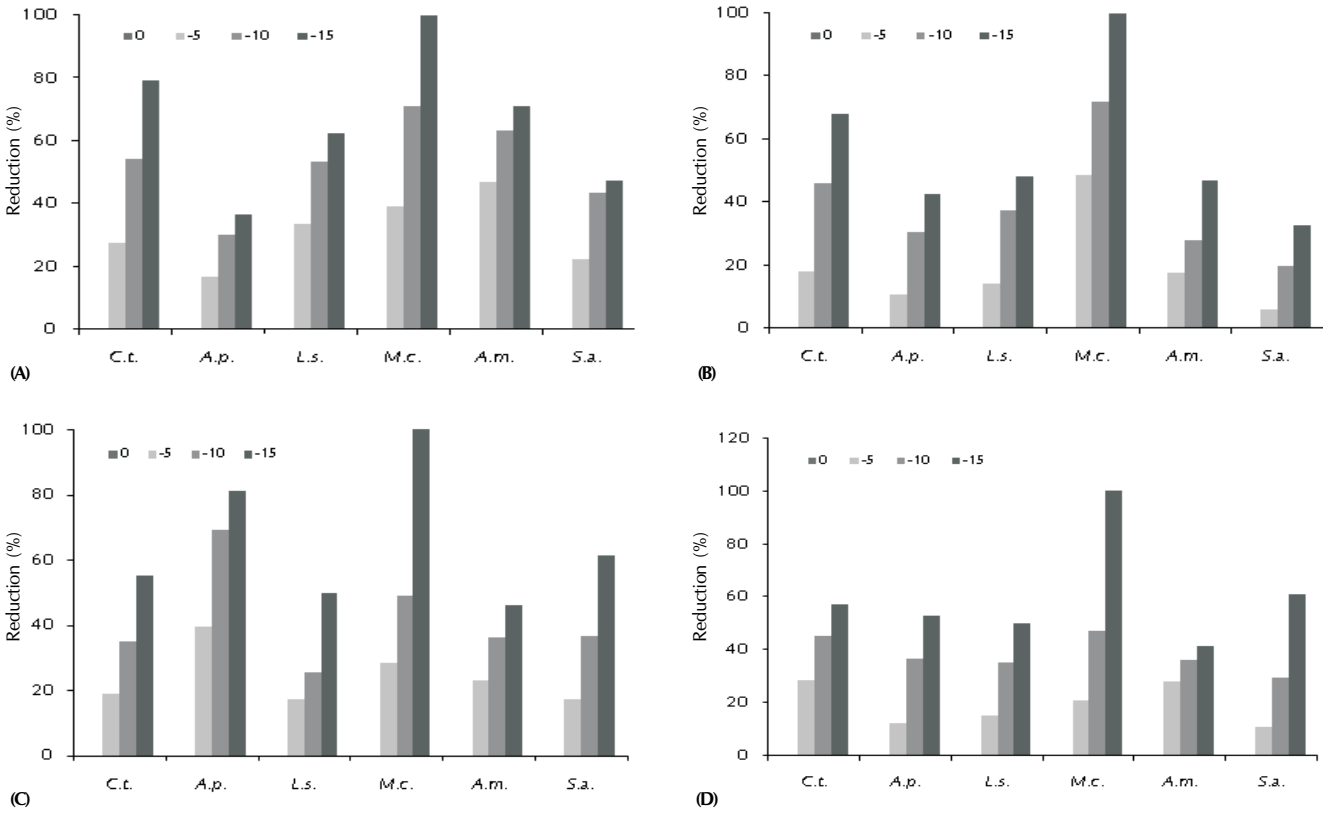

Figure 2: Effect of water stress on per cent reduction in different medicinal plant species. (A) Seed germination, (B)radicle length, (C)plumule length, and (D)seedling dry weight. *C.t. = Cassia tora, A.p. = Andrographis paniculata, L.s.= Lepidium sativum, M.c. = Matricaria chamomilla, A.m. = Ammi majus, and S.a.= Spilanthes acmella.*

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