

NITRATES IMPROVED SEED GERMINATION PERFORMANCE IN *COMMIPHORA WIGHTII* (GUGGAL), A DATA DEFICIENT MEDICINAL PLANT FROM THE INDIAN ARID ZONE

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Abstract: The present article deals with seed germination behaviour of *Commiphora wightii* (Guggal) by using various concentrations (0, 5, 10, 15 & 20 mg L⁻¹) of different nitrate solutions such as NH₄NO₃, Co(NO₃)₂, Ca(NO₃)₂ and KNO₃ under nursery conditions. At present, plant is considered under data deficient category. The seeds were collected from four different sites of three districts in western Rajasthan, viz. Jodhpur, Jaisalmer and Barmer. Results revealed that seeds presoaked in Ca(NO₃)₂ and KNO₃ were found to be most suitable for germination under nursery conditions as compared to others. The best results for germination initiation, germination duration (days), germination percentage, peak value (PV), root and shoot lengths, collar diameter and biomass yield were also observed maximum in these two nitrate pretreatments.

Keywords: *Commiphora wightii*, nitrate solutions, germination initiation day, peak value (PV), data deficient, medicinal plant

Introduction

Commiphora wightii (Arnott) Bhandari (Family Burseraceae) locally known as Guggal, is a perennial branched shrub or small medium sized tree up to 1.5-3.0 m in height, with crooked and knotty branches ending in sharp spines (Fig. 1). The stem is covered with silvery white, papery bark that peels-off as flakes from the older parts of stem, whereas the younger branches are pubescent and glandular [ANONYMOUS, 2008]. The plant almost remains leafless except in rainy season. It is a very slow growing species on rocky substratum and grows in shallow, gravelly, unfertile soil, hilly terrains, open canopies and adaptable to high temperature (45 °C) in arid and semi-arid climates with an annual rainfall 225-500 mm [KUMAR & SHANKAR, 1982; KULHARI & al. 2012]. The plant produces fruits throughout the year, but maximum fruit production takes place from January to April. Fruits are ovoid, single, or 2-3 in a bunch, bright red when riped. Seeds are ovoid, bilobed, sometimes trilobed or rarely tetralobed. Immature seeds are reddish brown, while matured ones are yellowish- white and black [PRAKASH & KASERA, 2000; LAL & KASERA, 2010]. The plant is a source of oleo-gum resin, which exudes from bark, is an effective drug in several diseases in Indian Systems of Medicine.

Presently, over-exploitation, poor seed production, rare seed germination, slow growth rate, lack of cultivation, excessive and unscientific tapping method, over-grazing by domestic animals, mining activities and invasion of alien species, etc. are some of the major reasons to its destruction in natural habitats and make this plant an endangered species of

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Indian Thar desert [KASERA & PRAKASH, 2005; REDDY & al. 2012]. Recently, IUCN declared its status as under “Data Deficient Category ver. 2.3” [IUCN, 2012].

The regeneration of this plant takes place vegetatively either by stem cuttings or air-layering, but through seeds is extremely poor in nature [YADAVA & al. 1999; KASERA & PRAKASH, 2005]. A large number of chemical substances such as various nitrate solutions have been reported by many researchers for breaking dormancy in seeds, enhancing their permeability, inducing and hastening the germination and thereby acting as chemical regulator for seed germination. Chemical substances may behave as germination stimulator or inhibitor and their effects on inhibitions and germination may vary [SEN, 1977; SWAMI & al. 2011]. In view of this, in the present investigation an attempt has been made to study the morphological parameters of seeds as well as effect of different concentrations, *i.e.* 5, 10, 15 & 20 mg L⁻¹ of various nitrate solutions such as NH₄NO₃, Co(NO₃)₂, Ca(NO₃)₂ and KNO₃ for enhancing seed germination potentialities as well as seedling parameters of *Commiphora wightii*, an important data deficient medicinal plant from the Indian Thar desert.



Fig. 1. *Commiphora wightii* – plant growing in natural habitat

Materials and methods

Mature ripened fruits of *C. wightii* were collected from four sites (Fig. 2) of western Rajasthan, viz. Machia Biological Park (MBP), Jodhpur (site-I; 11 km away from the University Campus in west direction); Beriganga, Jodhpur (site-II; 34 km in north-east); Kiradu, Barmer (site-III; 250 km in south-east) and Aakal Wood Fossils Park (AWFP), Jaisalmer (site-IV; 300 km in east direction from the University Campus, Jodhpur) during January-April (2011 & 2012). The pulp of air-dried fruits was removed gently through hand rubbing and seeds were washed in running tap water to remove any adhering pulp and other growth inhibitors (Fig. 3). Afterwards, the air-dried seeds were stored in plastic container with parad tablets to protect them from insects. Two types of seeds, viz. black and whitish-black have been observed from mature fruits (Fig. 4). The black seeds are viable, and a single seed produces more than one seedling due to its polyembryonic nature. However, whitish-black seeds were non-viable due to absence of embryo. Seed viability was tested by the tetrazolium method [PORTER & al. 1947]. Experiments were carried out at the University Campus during February-March 2011 & 2012 under nursery (*in-vivo*) conditions. The fresh black seeds were sown under *in-vivo* conditions in thermo cups, containing sand, clay and FYM (Farm Yard Manure) in 1: 1: 1 ratio at 0.5-1.0 cm depth.

Before sowing, seeds were presoaked for 24 h in different concentrations (5, 10, 15 & 20 mg L⁻¹) of nitrate solutions such as NH₄NO₃, Ca(NO₃)₂, Co(NO₃)₂ and KNO₃. For control experiments, seeds were presoaked in distilled water for same duration. Two seeds were sown in each thermo cup and total 45 replicates were maintained for each pretreatment. Irrigation was provided as and when required according to environmental conditions. To the germinated seeds, the seed counting process was begun with the day on which they were sown in soil mixture ratios to the end of last germination of seeds. A seed was considered to germinate when seedling was emerged out from soil surface. The germination initiation day, germination duration (days) and germination percentage values were recorded till last seed germination. While, seedling parameters such as root and shoot lengths, collar diameter and total dry weight were measured after completion of the three months of setting the experiments. The Peak Value (PV) was calculated by using the following formula:

$$PV = \frac{\text{Final germination percentage}}{\text{Number of days that took to reach the peak germination}}$$

Seed morphological parameters and the mean value of two years data pertaining to effect of different concentrations of nitrate solutions on seed germination are presented in the Tab. 1, 2 and 3. Data of three-months-old seedlings with various parameters were generated for analyses of variance using Randomized Block Design (RBD) in accordance with GOMEZ & GOMEZ (1984).

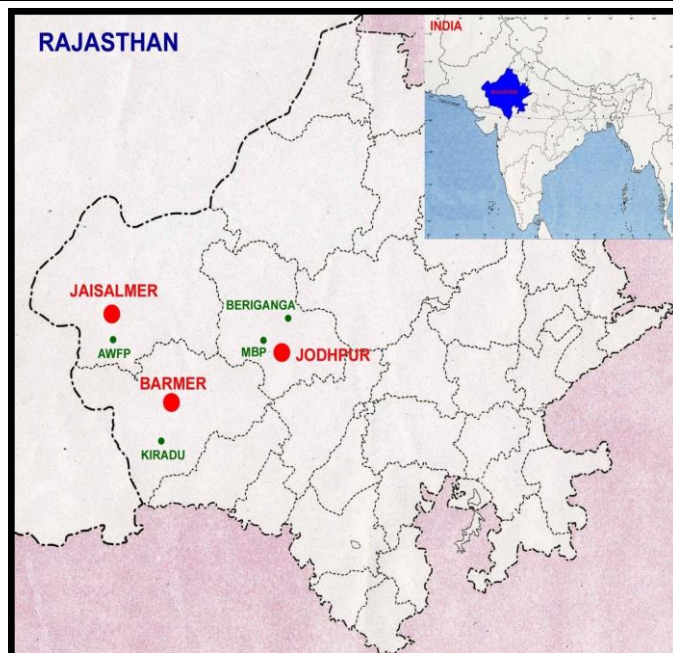


Fig. 2. Map of four selected sites of western Rajasthan for collection of *C. wightii* germplasm. MBP = Machia Biological Park; and AAFP = Aakal Wood Fossils Park

Results

The following experiments were conducted in the present investigations.

Weight, size, viability and ratio of black & whitish-black seeds

It is evident from Tab. 1 that there were significant variations in morphological parameters of seeds such as weight, size, viability and ratio of black and white seeds, collected from sites-I-IV. The seeds collected from site-III were heaviest in weight and largest in size, while those from site-I were lightest and smallest (Fig. 3). The highest viability and ratio of black and whitish-black seeds were observed in seeds collected from site-IV, while minimum from site-I.

Tab. 1. Morphological parameters of *C. wightii* seeds collected from sites-I-IV

Sites	Weight of 100 seeds (g)	Size (mm)			Viability (%)	Black and whitish-black seeds ratio
		Length	Breadth	Thickness		
I	2.557	5.1	4.5	3.2	53.33	0.563
II	2.790	5.5	4.7	3.4	61.66	0.693
III	4.426	6.8	5.8	4.1	68.33	0.617
IV	2.619	5.9	5.5	3.6	81.66	3.426
CD	0.240*	0.815*	0.452*	0.317*	18.763*	0.748*

* = significant at $p < 0.05$.

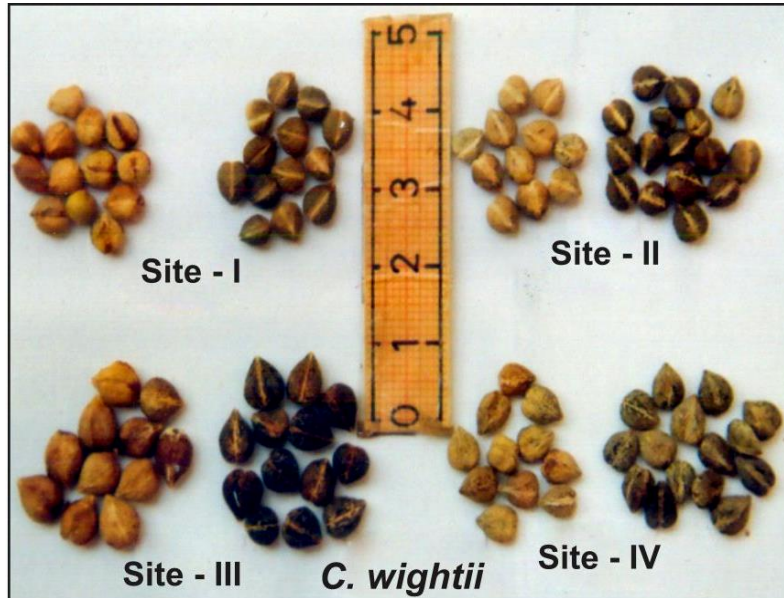


Fig. 3. Variation in colour and morphological parameters of *C. wightii* seeds collected from sites I-IV (adapted from LAL & KASERA, 2012)

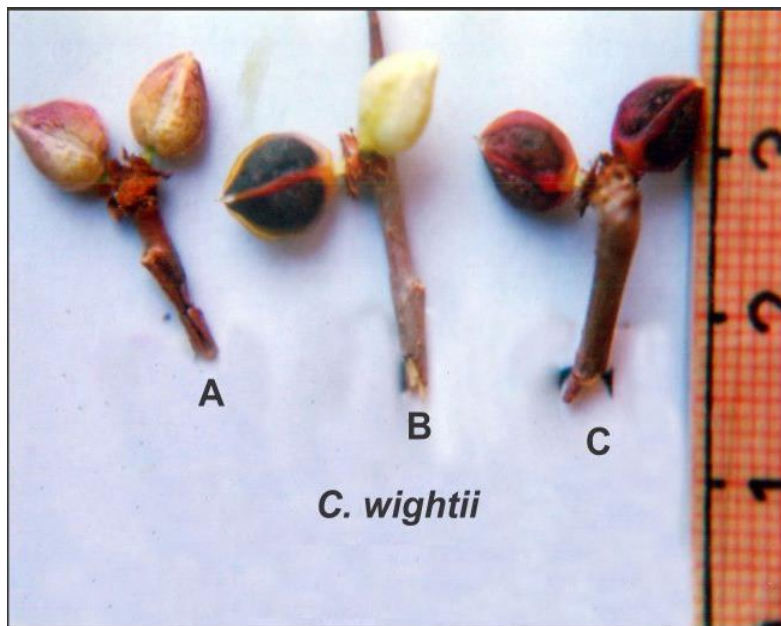


Fig. 4. Twig of *C. wightii* with immature reddish-brown (A), mature black and yellowish-white (B), and mature black (C) fruits, bearing black and whitish-black seeds

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Tab. 2. Effect of different concentrations of nitrate solutions on seed germination behaviour in *C. wightii* under nursery conditions from sites-I-IV

Treatments	Concentrations (mg L ⁻¹)	Germination initiation day				Germination duration (days)				Germination percentage				Peak value			
		Site-I	Site-II	Site-III	Site-IV	Site-I	Site-II	Site-III	Site-IV	Site-I	Site-II	Site-III	Site-IV	Site-I	Site-II	Site-III	Site-IV
Control	0	10.5	8.5	5.5	5.0	18.5	18.5	15.0	17.0	11.11	33.88	19.44	46.11	0.92	2.88	1.94	5.42
NH ₄ NO ₃	5	-	9.0	6.5	5.2	-	19.0	15.5	16.0	-	28.33	18.33	43.73	-	2.36	1.67	4.79
	10	-	9.5	8.5	5.5	-	18.0	13.5	13.5	-	21.11	16.11	38.33	-	1.92	1.57	4.65
	15	-	10.0	-	5.5	-	16.0	-	12.5	-	19.33	-	38.11	-	1.12	-	4.49
	20	-	-	-	6.5	-	-	-	12.0	-	-	-	37.22	-	-	-	4.32
Co(NO ₃) ₂	5	8.0	8.5	6.0	5.5	17.0	16.5	14.5	13.5	11.66	39.44	21.66	46.66	1.11	4.15	2.10	5.62
	10	9.5	9.0	7.5	5.5	16.5	15.0	13.0	12.0	10.55	31.66	18.88	40.55	0.97	3.39	1.89	4.87
	15	9.5	9.3	-	5.7	13.0	14.5	-	12.0	8.89	28.33	-	38.22	0.56	2.72	-	3.60
	20	-	9.5	-	6.0	-	12.4	-	10.0	-	27.22	-	37.22	-	2.12	-	3.20
Ca(NO ₃) ₂	5	6.5	6.0	6.0	5.0	14.5	17.5	17.5	14.5	20.55	42.77	53.88	73.11	2.42	4.75	5.99	9.57
	10	8.0	5.5	6.5	4.0	14.5	16.0	16.5	13.5	14.44	37.72	35.00	59.44	1.52	3.92	3.68	8.17
	15	8.2	5.3	7.0	4.0	13.0	15.0	16.0	12.0	13.33	33.33	31.22	58.11	1.22	3.45	2.94	7.93
	20	8.5	5.0	7.5	4.0	11.5	14.0	14.5	11.5	12.22	32.77	29.44	57.22	1.10	3.15	2.55	6.35
KNO ₃	5	6.0	5.0	6.5	4.0	16.0	20.0	17.0	13.5	23.73	51.66	40.55	70.55	2.92	5.11	4.20	10.08
	10	6.0	5.0	6.5	4.0	14.5	17.5	15.5	12.5	16.11	40.55	31.66	56.66	1.79	4.92	3.39	8.09
	15	6.2	5.3	6.3	4.3	13.5	15.0	15.0	12.0	14.11	38.66	29.11	51.33	1.33	4.27	3.02	7.44
	20	6.5	5.5	6.0	4.5	12.0	13.5	13.5	12.0	13.33	38.33	27.22	48.33	1.15	3.75	3.55	6.75
CD		1.036*	1.388*	1.198*	1.126*	1.507*	2.228*	2.061*	2.390*	6.897*	11.557*	7.727*	9.585*	0.170*	0.280*	0.231*	0.335*

* = significant at p<0.05; and - = no germination.

Tab. 3. Effect of different concentrations of nitrate solutions on various seedlings parameters of three months old seedlings of *C. wightii* under nursery conditions from sites-I-IV

Treatments	Concentrations (mg L ⁻¹)	Root length (cm)				Shoot length (cm)				Collar diameter (mm)				Total dry weight (g plant ⁻¹)			
		Site-I	Site-II	Site-III	Site-IV	Site-I	Site-II	Site-III	Site-IV	Site-I	Site-II	Site-III	Site-IV	Site-I	Site-II	Site-III	Site-IV
Control	0	21.4	23.9	24.6	26.2	9.7	10.9	9.3	11.2	3.2	3.8	3.7	3.5	0.272	0.345	0.348	0.366
NH ₄ NO ₃	5	-	21.9	22.5	23.9	-	11.7	9.9	10.8	-	3.7	3.5	3.9	-	0.294	0.301	0.310
	10	-	16.6	16.1	22.1	-	10.8	8.9	9.6	-	3.3	3.5	3.9	-	0.254	0.280	0.279
	15	-	15.6	-	22.1	-	10.1	-	9.6	-	3.2	-	3.8	-	0.248	-	0.250
	20	-	-	-	19.9	-	-	-	9.4	-	-	-	3.6	-	-	-	0.237
Co(NO ₃) ₂	5	22.5	27.6	23.4	25.5	9.7	10.9	10.3	10.7	3.6	3.7	3.7	3.7	0.268	0.365	0.348	0.402
	10	19.3	25.1	19.5	19.5	8.2	10.1	9.3	10.5	3.5	3.7	3.6	3.6	0.261	0.326	0.312	0.340
	15	18.7	20.3	-	18.9	8.0	9.9	-	9.9	3.1	3.5	-	3.6	0.213	0.314	-	0.315
	20	-	19.4	-	18.8	-	9.4	-	9.7	-	3.3	-	3.2	-	0.290	-	0.303
Ca(NO ₃) ₂	5	26.3	31.7	29.7	37.3	11.9	12.8	11.9	14.2	3.8	4.2	4.5	4.0	0.418	0.479	0.596	0.602
	10	22.4	29.6	27.6	33.8	10.4	12.7	11.0	13.8	3.6	4.2	4.3	3.8	0.366	0.420	0.515	0.535
	15	22.0	26.5	25.9	32.1	10.1	11.6	10.3	12.7	3.4	4.0	4.1	3.6	0.350	0.385	0.475	0.512
	20	21.8	24.7	25.8	30.2	9.9	11.5	10.8	11.5	3.3	3.9	3.9	3.5	0.326	0.389	0.460	0.457
KNO ₃	5	28.8	34.1	33.6	39.4	10.7	12.5	10.9	13.3	4.0	4.8	4.9	4.3	0.456	0.482	0.532	0.547
	10	27.0	31.6	29.1	35.2	10.2	12.5	10.5	12.2	3.8	4.5	4.6	4.1	0.393	0.432	0.468	0.473
	15	25.3	27.5	28.1	33.5	9.8	11.4	9.9	11.5	3.6	4.3	4.4	3.8	0.356	0.421	0.435	0.454
	20	23.6	26.9	27.5	33.4	9.5	11.0	9.5	11.2	3.4	4.2	4.3	3.7	0.331	0.413	0.424	0.433
CD		4.854*	5.648*	5.848*	4.854*	1.235*	2.328*	0.696*	0.985*	0.089*	0.608*	0.525*	1.267 ^{ns}	0.048*	0.042*	0.011*	0.029*

* = significant at p<0.05; ns = non -significant; and - = no germination.

Germination initiation day and duration

The seeds pretreated with all the concentrations of KNO_3 germinate earlier as compared to others (Tab. 2). Results revealed that all concentrations of NH_4NO_3 totally inhibited germination from site-I, while 15 and 20 mg L^{-1} from site-III and only 20 mg L^{-1} from site-II. The higher concentrations of $\text{Co}(\text{NO}_3)_2$ also inhibited germination from sites-I & III. Further, increasing concentrations of all nitrates delay in germination from all sites. The early germination was observed on 4th day after seed sowing from site-IV in 10, 15 & 20 mg L^{-1} $\text{Ca}(\text{NO}_3)_2$ and in 5 & 10 mg L^{-1} KNO_3 and delayed up to 10.5th day from site-I in control. The values of germination duration (days) were decreased with increasing concentrations of nitrates from all sites. The maximum value (20 days) was found with 5 mg L^{-1} of KNO_3 from site-II, while minimum (10 days) in 20 mg L^{-1} of $\text{Co}(\text{NO}_3)_2$ from site-IV.

Germination percentage and Peak value

Data presented in Tab. 2 revealed that under controlled conditions seeds produced maximum seedlings (46.11%) from site-IV followed by site-II and minimum from site-I. Further, results revealed that maximum germination percentage values from sites-I and II were recorded in 5 mg L^{-1} of KNO_3 . The same concentration of $\text{Ca}(\text{NO}_3)_2$ showed maximum germination from sites-III and IV. $\text{Ca}(\text{NO}_3)_2$ and KNO_3 showed positive results with higher concentrations only. It is evident from Tab. 2 that maximum peak values were reported in 5 mg L^{-1} of KNO_3 from sites-IV followed by site-III in 5 mg L^{-1} of $\text{Ca}(\text{NO}_3)_2$ and minimum from site-I in 15 mg L^{-1} of $\text{Co}(\text{NO}_3)_2$. The peak values were also decreased with increasing concentrations of nitrate solutions.

Root, shoot lengths and collar diameter

Results revealed from Tab. 3 that maximum root length was found from site-IV in 5 mg L^{-1} of KNO_3 and minimum from site-II in 15 mg L^{-1} of NH_4NO_3 . The highest shoot length was observed in 5 mg L^{-1} of $\text{Ca}(\text{NO}_3)_2$ from site-IV and lowest in 15 mg L^{-1} of $\text{Co}(\text{NO}_3)_2$ from site-I. The maximum values of collar diameter were observed from site-III in 5 mg L^{-1} of KNO_3 and minimum from site-I in 15 mg L^{-1} of $\text{Co}(\text{NO}_3)_2$.

Biomass yield (g plant^{-1})

The sum of root and shoot dry weights were considered as biomass of plant. The maximum biomass yield was observed from site-IV followed by site-III in 5 mg L^{-1} $\text{Ca}(\text{NO}_3)_2$, whereas minimum in 15 mg L^{-1} of $\text{Co}(\text{NO}_3)_2$ from site-I (Tab. 3). The lower concentration (5 mg L^{-1}) of $\text{Ca}(\text{NO}_3)_2$ and KNO_3 were found to suitable for maximum biomass yield. Further, the biomass yield decreased significantly with increasing concentrations of nitrate pretreatments.

Data pertaining two years for various parameters of seed germination and seedling growth behaviour were significant at 95% level at all sites, except for collar diameter at site-IV, which were non-significant.

Discussion

Nitrogenous compound in various forms has been used to stimulate germination [CHOUDHARY & al. 1996; MC INTYRE & al. 1996]. They play a critical role in increasing the physiological efficiency [BHARGAVA & BANERJEE, 1994] and influence germination may be due to change in water relationship [NIKOLAEVA, 1977]. Ammonium nitrate was proved to inhibit or promote seed germination dependent on species type [SINGH & AMRITPHALE, 1992]. CHOUDHARY & KUMAR (2003)

reported that the higher concentration of NH_4NO_3 inhibited seed germination in *Plantago ovata*. HASSAN & al. (2011) also reported that higher concentrations of NH_4NO_3 delayed germination in *Striga hermonthica*. In the present studies, NH_4NO_3 showed very poor results as compared to control from all sites. The maximum germination (43.73%) was reported from site-IV in 5 mg L^{-1} and minimum (16.11%) from site-III in 10 mg L^{-1} solutions. Further, with increased concentrations, it completely inhibit seed germination from site-I. Our results were confirmative with the above findings.

Cobalt generally considered toxic to cells and it can cause various toxic effects on plant such as inhibition of seed germination, plant growth and yield reduction [SHAUKAT & al. 1999]. JAYAKUMAR & al. (2009) reported that cobalt at low concentrations has sobbed beneficial values in soybean germination. KHAN & KHAN (2010) observed that higher concentrations of cobalt (200 and 400 ppm) are detrimental to seed germination in chickpea. The results are in accordance with all these above-mentioned findings. Only, the lower concentration, *i.e.* 5 mg L^{-1} was found to be beneficial for improving germination as compared to control.

Calcium plays an essential role in protecting plants such as preserving the structural and functional integrity of cell membrane, stabilizing plant cell wall structure, regulating ion transport & selectivity and controlling ion-exchange behaviour as well as enzyme activities [RENGEL, 1992; HOWLADAR & RADY, 2012]. GEHLOT & KASERA (2011) observed cent percent germination in *Withania coagulans* when seeds were pretreated with 0.50% $\text{Ca}(\text{NO}_3)_2$ and also reported that higher concentrations retarded it. In the present studies, $\text{Ca}(\text{NO}_3)_2$ pretreatments showed the highest germination, *i.e.* 73.11% from site-IV in 5 mg L^{-1} and lowest 12.22% from site-I in 20 mg L^{-1} solutions. Our results are confirmative with above all these findings.

Use of KNO_3 has been an important seed treatment in seed-testing laboratories for many years without a good explanation for its action [HARTMANN & al. 1997]. The ion is bound to pyruvate kinase and other essential enzymes, regulating respiration and carbohydrate metabolism [SALISBURY & ROSS, 1991]. SINGH & al. 1998 reported 40% germination in 0.25% KNO_3 in *Commiphora wightii*. TIWARI & CHAUHAN (2007) reported that KNO_3 significantly enhanced seed germination (32-36%) in *Rhododendron niveum*. KARIMMOJENI & al. (2011) reported that 0.02 M concentration of KNO_3 increased germination up to 61.0% in *Lepidium latifolium*, while higher one retarded it. LAL & KASERA (2012) observed that seeds of *C. wightii* pretreated with 5 mg L^{-1} of KNO_3 were most favorable for optimizing germination and seedling development under *in-vivo* conditions. The results confirm all these findings presented above.

In the present investigation, the lower concentration (5 mg L^{-1}) of $\text{Ca}(\text{NO}_3)_2$ and KNO_3 performed best results of seedling parameters such as root & shoot lengths, collar diameter and total dry weight. KSHETRAPAL & SHARMA (1992) reported maximum root length in stem cuttings of *C. wightii* with 0.3% solution of KNO_3 . GEHLOT & KASERA (2011) also reported maximum root and shoot lengths in *Withania coagulans* with 0.25 and 0.10% solutions of KNO_3 and $\text{Ca}(\text{NO}_3)_2$, respectively. This may be due to nitrate salts with an osmotic role on water uptake, which exerted a nutritional effect on protein synthesis as suggested by MC INTYRE & al. (1996).

Conclusions

Results obtained from the present studies revealed that morphological parameters of seeds such as weight and size does not affect the seedling emergence percentage, whereas the viability and ratio of black & whitish-black seeds affected them significantly. The seeds of *C. wightii* pretreated with 5 mg L⁻¹ of Ca(NO₃)₂ and KNO₃ were found to be most suitable for optimizing germination and seedlings development under *in-vivo* conditions. The lower concentrations of nitrate solutions promote the germination as well other parameters of seedlings, while higher ones retarded them. Further, the lower concentration (5 mg L⁻¹) of Ca(NO₃)₂ showed best growth of shoot length and total dry weight, while the same concentration of KNO₃ performed with root length and collar diameter. Finally, it is concluded that KNO₃ and Ca(NO₃)₂ significantly influenced all parameters of seed germination at all sites. Hence, for large-scale cultivation and conservation of this plant, seeds collected from sites-II (Jodhpur), III (Barmer) and IV (Jaisalmer) should be pretreated with 5 mg L⁻¹ of Ca(NO₃)₂ and KNO₃ before sowing under nursery conditions.

Acknowledgements

The first author is grateful to the University Grants Commission (UGC), New Delhi for financial assistance in the form of NET-SRF [No. F. 17-78/08 (SA-I) dated 9th July 2008]. The authors are also thankful to the Professor and Head, Department of Botany, JNV University, Jodhpur for providing necessary facilities. Thanks to Dr. David N. Sen [Professor and Head (Retired), Department of Botany, Jai Narain Vyas University, Jodhpur] for valuable suggestions and guidance in improvement of this paper.

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Received: 27 March 2014 / Revised: 20 November 2014 / Accepted: 24 November 2014