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INFLUENCE OF NITRATE SALT SOLUTIONS ON SEED GERMINATION DYNAMICS OF *WITHANIA COAGULANS* (STOCKS) DUNAL, AN IMPORTANT MEDICINAL PLANT OF THE INDIAN THAR DESERT

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Abstract

The effect of different concentrations (0.10, 0.25, 0.50, 0.75 and 1.0%) of nitrate salt solutions, *viz.* $Ca(NO_3)_2$, $Co(NO_3)_2$ and KNO_3, on various aspects of fresh and one-year-old seeds and the seedling growth of *Withania coagulans* (Stocks) Dunal, is a critically endangered medicinal plant of the Indian Thar desert was dealt with. The maximum germination percentage in fresh and one-year-old seeds was observed in 1.0 and 0.10% $Co(NO_3)_2$ solutions, respectively. The maximum root: shoot ratio in seedlings from fresh and one-year-old seeds was observed in 0.75 and 0.50% of $Ca(NO_3)_2$ solutions, respectively.

The demand for medicinal plants has increased globally due to the resurgence of interest and acceptance of herbal medicine, and is being met largely through collection of these plants from wild populations and the rate of exploitation often exceeds that of the local natural regeneration (Sharma *et al.* 2006). Thus, there is an urgent need to develop conservation strategies and implement regeneration techniques for the over-exploited medicinal plant species. Seed-based multiplication is the most effective and convenient means for regeneration of most of the desert species.

Withania coagulans (Stocks) Dunal (Solanaceae) is a critically endangered medicinal plant (Jain *et al*. 2009) and used in the Unani System of medicine as Tukhm-e-Hayath, this small shrub, popularly known as Indian cheese maker, is commercially important for its efficacy in the treatment of ulcers, rheumatism, and dropsy, constipation and sexual debility. The seeds are reported to be sedative, emetic and stomachic, blood purifier and febrifuge, diuretic and bitter tonic in dyspepsia and also promote growth of infants (Hemalatha *et al.* 2004). In the Indian sub-continent, its berries are used as a blood purifier, twigs are chewed for cleaning of teeth, and smoke of the plant is inhaled for relief in toothache (Gupta 2013). In northern India, traditional healers use its dry fruits for the treatment of diabetes.

Seed germination and seedling vigour are prerequisites for successful establishment of plant stands (Rauf *et al.* 2007). Seed dormancy is a mechanism by which seeds inhibit their germination and wait for more favourable conditions (Finkelstein *et al*. 2008), whereas seed germination is a mechanism, in which morphological and physiological alterations result in activation of the embryo. Before germination, seeds absorb water, leading to expansion and elongation of seed embryos (Hermann *et al.* 2007).

Nitrate can act as a source of N and enhance seed germination (Atia *et al.* 2009) by adjusting K^+/Na^+ ratio and increasing ATP production and seed respiration (Zheng *et al*. 2009). The present study is focused on morphological parameters of seeds such as shape, size, colour, weight and on

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the influence of different nitrate salts on seed germination and seedling growth in *W. coagulans* under controlled laboratory conditions.

The mature fruits of *Withania coagulans* were purchased from authorized Ayurvedic shop during February - March 2016 and 2017. The seeds were cleaned and stored in plastic containers with parad tablets (a mercury compound) to protect them from insects in the controlled laboratory conditions. Seed morphological characters such as shape, size, colour and weight were recorded, based on the examination of 100 seeds, with the help of dissecting microscope and Vernier calliper. Seed viability was tested by the tetrazolium method, following Porter *et al.* (1947).

The seeds were first cleaned and treated with 0.1% $HgCl_2$ for 30 sec and then kept under running tap water for 3 - 4 hrs for removing injurious substances, if any. Different pre-treatments were then provided to fresh as well as one-year-old seeds for enhancing germination percentage and facilitating seedlings growth. Sterilized Petri dishes lined with single layer of filter paper moistened with distilled water were used for conducting experiments to study seed germination in alternate white light (1000 lux) and dark (12 hrs) obtained from 3 fluorescent tubes of 40 Watts each fitted at a height of half meter from Petri dishes in seed germinator at 28ºC temperature. In each set of three Petri dishes, 10 seeds pre-soaked for 24 hrs with different concentrations (0.10, 0.25, 0.50, 0.75, 1.0%) of $Ca(NO_3)_2$, $Co(NO_3)_2$ and KNO_3 were placed in each dish. Distilled water alone was used for the control set. Filter paper was moistened daily with 0.2 ml of distilled water. Observations were recorded daily for 15 days. Germination percentage and the root and shoot length of seedlings were analysed after 15 days. The experiment was repeated for confirming the observations and the data obtained were subjected to ANOVA using RBD, as suggested by Gomez and Gomez (1984).

Study of seed morphology is important to understand its adaptation variability in desert condition (Verma and Kasera 2008). Morphological information on seeds of *W. coagulans* is presented in Table 1. The seeds were ear-shaped and pale yellow in colour. Their mean length and breadth were 0.349 ± 0.07 and 0.307 ± 0.04 mm, respectively. The weight of 100 seeds was 0.277 g. The fresh and one-year-old seeds showed 93.33 and 63.33% viability, respectively.

Table 1. Morphological parameters of *W. coagulans* seeds.

Parameters		Values
Shape		Ear
Length (mm)		0.349 ± 0.07
Breadth (mm)		0.307 ± 0.04
Colour		Pale yellow
Weight of 100 seeds (g)		0.277
Viability (%)	Fresh	93.33
	One-year-old	63.33

In most of the desert plant species germination process can be enhanced to some extent by appropriate pre-treatment of seeds (Bose and Sharma 2000). Among various chemicals, nitrates play an important role in breaking seed dormancy (Kasera and Sen 1992). Nitrate inhibits seed dormancy by decreasing the levels of ABA in seeds (Finkelstein *et al*. 2008). The fresh and one-year-old seeds of *W. coagulans* showed maximum germination percentage *i.e*. 46.66 and 53.33 with 1.0 and 0.10 $Co(NO_3)_2$ concentrations, respectively (Table 2). Observations of Jayakumar *et al*. (2009) that cobalt at low concentration improves germination find support from the present findings.

Table 2. Effect of different concentrations of nitrate solution pre-treatments on seed germination, seedling growth, root/shoot lengths (R/S ratio) in fresh and one-year-old seeds of *W. coagulans*.

Nitrate solutions	Conc. (%)	Germination (%)		Seedling growth (cm)				R/S ratio	
				Root		Shoot			
		Fresh	Old	Fresh	Old	Fresh	Old	Fresh	Old
	0.0	3.33	10.0	1.2	0.93	2.9	2.5	0.41	0.37
	0.10	23.33	6.66	1.171	1.5	2.7	3.15	0.433	0.47
$Ca(NO_3)_2$	0.25	6.66	3.33	1.25	1.6	1.95	0.8	0.641	2.0
	0.50	23.33	16.66	1.35	1.78	2.04	2.26	0.664	2.26
	0.75	3.33	30.0	1.0	1.85	3.0	2.43	3.33	0.76
	1.00	10.0	36.66	0.53	1.74	1.16	2.85	0.457	0.61
CD		1.82^{**}	ns	ns	ns	1.27^{**}	1.20^{**}	0.21^{**}	0.95^{**}
	0.0	3.33	10.0	1.2	0.93	2.9	2.5	0.41	0.37
	0.10	10.0	53.33	0.366	1.08	0.966	1.92	0.378	0.56
$Co(NO_3)_2$	0.25	10.0	30	1.233	1.13	2.233	1.87	0.552	0.60
	0.50	40.0	36.66	1.025	1.0	2.2	1.53	0.465	0.65
	0.75	30.0	26.66	0.544	0.8	1.011	1.71	0.538	0.46
	1.00	46.66	43.33	0.664	0.87	1.064	2.0	0.624	0.43
CD		ns	ns	ns	ns	ns	ns	ns	0.05^{**}
	0.0	3.33	10.0	1.2	0.93	2.9	2.5	0.41	0.37
	0.10	-	13.33	-	1.0	-	2.0	-	0.5
KNO_3	0.25	3.33	6.66	0.2	2.4	0.2	1.95	1.0	1.23
	0.50	-	33.33	-	1.41	-	2.07	-	0.68
	0.75	3.33	16.66	0.2	1.56	0.2	3.16	1.0	0.49
	1.00	6.66	20.0	0.55	1.81	2.0	2.2	0.275	0.82
CD		2.26^{**}	ns	0.19^{**}	ns	1.04^{**}	ns	ns	0.21^{**}

- = No germination, ns = Non-significant, and ** = Significant at ($p < 0.01$).

The maximum length of root (1.35 cm) and shoot (3.0 cm) was observed in 0.50 and 0.75% $Ca(NO_3)_2$ solutions, respectively in freshly purchased seeds (Table 2). In one-year-old seeds, maximum root (2.4 cm) and shoot (3.16 cm) lengths were recorded in 0.25 and 0.75% of KNO_3 solutions, respectively (Table 2). The highest R/S (root/shoot) ratio in fresh (3.33) and one-year-old seed (2.26) were observed in 0.75 and 0.50% $Ca(NO3)_2$, respectively (Table 2). Calcium plays an essential role in preserving the structural and functional integrity of cell membrane and stabilizing the cell-wall structure in plants (Rengel 1992, Howladar and Rady 2012). Lal and Kasera (2014) found $Ca(NO_3)_2$ and KNO_3 to be most suitable for optimizing germination and seedling development in *Commiphora wightii*. Likewise, Cetinbas and Koyuncu (2006) could cause effective germination with treatment of KNO_3 in *Prunus avium*. Swami *et al*. (2011)

reported maximum root and shoot lengths in one-year-old seeds of *Withania somnifera* with treatment of 0.25% $ZnNO_3$ and 1.0% KNO_3. Gashi *et al*. (2012) observed maximum germination of *Ramonda serbica* seeds treated with KNO_3.

Statistical analysis has shown that values of shoot length and R/S ratio were significant at $p < 0.01$ for the fresh as well as in one-year-old seeds treated with $Ca(NO_3)_2$; the same applies to germination percentage of fresh seeds treated with KNO_3. Present study reveals that seeds of *W. coagulans* are nitrophilous, showing a remarkable enhancement of germination when pre-treated with various nitrate solutions. Lower concentrations (0.10%) of $Co(NO_3)_2$ promoted the germination in old seeds, while higher ones (1.0%) in fresh seeds. It may therefore be concluded that $Co(NO_3)_2$ offers the best treatment for the large-scale multiplication of *W. coagulans.*

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