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Effect of seed storage conditions on seed germination and vigor of *Withania somnifera*

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Abstract
The present communication describes seed germination behaviour of *Withania somnifera*, a commercial medicinal herb, under different storage conditions *viz*; temperature and duration. Seeds stored for 2 months showed significantly lower germination percentage (44.0% - 58.0%) indicating the requirement of after-ripening after the harvest. Maximum germination percentage (99.0%), emergence index (0.41), germination energy (7.09), germination speed (18.61) and seedling vigour index I (619.76) & II (66.09) were observed in seeds stored at 0^0C for 4 months. Similarly, earliest onset and completion of germination and minimum mean germination time to germinate was also observed in seeds stored at 0^0C for 4 months. However, germination was not observed in seeds stored for 10 months at either of the temperatures.

Keywords: Ashwagandha, germination percentage, temperature, onset of germination, seedling vigour

Introduction
Seeds are uniquely equipped to survive as a viable regenerative organism until the time and place is right. Preservation of quality seed stock in storage for the next season is as important as producing quality seeds every year thus, retaining potential germinability and vigour of seeds is of major concern under ambient storage conditions. Storage environment indeed is very important for extending the life span of seeds, several factors *viz*; temperature, nature of the seeds, seed moisture content, relative humidity influence seed longevity during storage. Hence, a close relationship exists between the loss of seed viability during storage and the storage environment (Roberts 1988). Increase in temperature and moisture content may promote fungal growth, insect infestation, enhances metabolic activities of the seed which in turn results into decline in seed germination capacity (Harrington 1972). Therefore, seeds stored under adverse conditions results in the production of aged seeds exhibiting a variety of symptoms ranging from reduced viability or germinability (sometimes to zero germination) to more or less full viability (i.e. no obvious decline in germinability) but with abnormal development of the seedlings i.e. poor vigor (Bewley and black 1985) [1]. Hence, storage of seeds in ideal conditions is essential for improving the life span of the seeds as it preserves seed quality, vigor and viability by slowing down the pace of physio-biochemical processes so that it can be used for future use by farmers and breeders (McDonald and Copeland 2005) [12].
With an increase use of conventional synthetic drugs and health hazards they are posing to mankind there is a never-ending quest to find an alternative that could maintain the health of people without causing them much harmful side effects. Thus, this leads to the exploration of herbal medicines. Amongst the extensive use of various herbal plants; *Withania somnifera* commonly known as Ashwagandha, Asgandh, Indian ginseng and Indian Winter Cherry appears to be high in demand due to its aphrodisiac, anti-inflammatory, astringent properties which helps to treat bronchitis, asthma, ulcer, emaciation, insomnia, senile dementia etc like dreadful diseases. Wide use of the plant in various ailments and diseases has increased the demand of this medicinal plant globally due to the resurgence of interest and acceptance of herbal medicines. Most of the demand is being met through collection of large quantities of medicinal plants from wild populations (Sharma *et al.* 2006; Pushpangadan and Nair 2001; Rao and Rajasekharan 2002) [18, 14]. Such approaches are especially crucial in case of those plants where demand is very high.
In nature, Ashwagandha plants are self-propagated through seeds. The propagation in nature is not sufficient to ensure the survival of this plant since, the germination potential of the seeds have been reported to be very low (Vakeswaran and Krishnaswamy, 2003 b) [20]. Throughout availability of the plant can only be sustained by standardizing proper storage mechanism which is indeed a precondition to strengthen species conservation programme and allow the judicial use of seeds as valuable genetic material. However, no scientific information is

available on storage behaviour and ideal storage conditions for seeds of this commercial medicinal species i.e. *Withania somnifera* Dunal. Therefore, there is a need to find the optimum temperature and adequate packaging material for storing seeds so that minimum standard of germination is maintained for longer period. In addition, improving the quality of seed is an approach which is likely to produce significant benefits in almost all circumstances.

Material and methods

Freshly harvested seeds of *Withania somnifera* were procured from the herbal garden of the Department of Medicinal and Aromatic Plants, UHF Nauni, Solan (altitude 1200 m) during the last week of August. Thereafter, seeds were air dried to an optimum moisture level (9 %) and stored in plastic jars at four different temperatures *viz;* 0,5,10ºC and ambient temperature (20 ± 30^{0}C) for five different durations *viz*; 2,4,6,8 and 10 months. Seeds stored at ambient conditions serve as control and were tested bimonthly for various seed quality attributes. Uniform and healthy seeds were surface sterilized with 0.1% mercuric chloride for 2 minutes to remove any strains of bacteria and fungi and then washed thoroughly with distilled water. Therefore, for bimonthly tests 50 seeds in four replicates each were allowed to germinate in petri dishes lined with Whatman no.1 filter paper, in seed germinator at 25 ± 2^{0}C and 80% relative humidity in continuous light. The filter papers were moistened daily using distilled water. Seeds were considered germinated upon radicle emergence; daily count of germination was taken and the day when the first seed showed germination was considered as time taken for onset of germination and the day when the last seed showed germination was considered as time taken for completion of germination. Final count was recorded on 30th day from sowing.

The germination percentage and emergence index was calculated by following formulae (ISTA 2015):

$$\text{Germination \%} = (\text{Number of seeds germinated}/\text{Total number of seeds}) \times 100$$

$$\text{Emergence index} = \text{Emergence (dn)}/\text{ day of emergence (N)}$$

Mean germination time (MGT) and Seedling vigour index (SV-I and SV–II) was calculated according to the following formulae (Das *et al.* 2017) [2]**.**

$$\text{MGT (days)} = \Sigma\,[\text{The number of germinated seeds on day i (ni) X incubation time (d)}] \,/\, \text{N (total number of seeds germinated)}$$

SV I =Germination percentage x seedling length (cm)

SV II = Germination percentage x seedling dry weight (mg)

Germination speed (GS) was worked out as per Gairola *et al.*, 2011 [5] and Germination energy (GE) by Maguire 1962 [11] as per following formulae:

$$GS = n1 + n + n3\ldots\ldots nx \text{ (number of seeds germinated on day 1 to 30 th day)} / \text{number of days.}$$

$$GE = \text{Germination percentage}/\text{ Day of completion of germination.}$$

Statistical analysis

The data pertaining to the experiment were subjected to statistical analysis by analysis of variance method as suggested by Gomez and Gomez (1984) [6].

Results

Seeds stored at different durations and temperatures were tested bimonthly for evaluation of various germination attributes and data is presented. The perusal of data in Table 1 shows that storage environment has significant effect on germination percentage and emergence index of seeds. These parameters are the reflection of vigor of the seed. Higher the values more vigorous will be the seed. It increases with decreasing storage temperature. The perusal of data in table 1 shows that storage temperatures and durations are major factors which significantly affect seed germination and emergence index. Seeds kept at 0^{0}C for 4 months gave maximum germination percentage and emergence index i.e. 99.0% and 0.41 respectively, which was very closely followed by 96.0 % and 0.39 in seeds stored at 5^{0}C for 4 months. Seeds stored under ambient conditions registered a rapid decline in germination percentage and emergence index (83.0 %) and in 4 months (0.16) which clearly states the effect of high temperature on seeds. Seeds stored for 8 months recorded a minimum (30.0 %) germination percentage and (0.12) emergence index at ambient temperature. Nevertheless, seeds being stored at low temperature seeds did not germinate at either of the four storage temperature after 10 months of storage, thereby indicating the total loss of germination ability of seed.

Table 1: Effect of storage temperatures and durations on germination percentage and emergence index

Storage duration (month)	Germination percentage					Emergence index				
	Temperature (^{0}C)					Temperature (^{0}C)				
	0	5	10	Ambient Temp.	Mean	0	5	10	Ambient Temp.	Mean
2	44.00	50.00	58.00	53.00	51.25	0.12	0.18	0.28	0.25	0.21
4	99.00	96.00	92.00	83.00	92.50	0.41	0.39	0.24	0.16	0.30
6	74.00	68.50	60.50	54.50	64.38	0.35	0.33	0.22	0.14	0.26
8	61.00	51.00	42.50	30.00	46.13	0.21	0.18	0.16	0.12	0.18
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	69.50	66.38	63.25	55.13		0.27	0.25	0.21	0.17	

CD0.05

T	0.89	0.88
D	0.89	0.88
TxD	1.77	3.76

Seeds sown after 4 months of storage at 0^{0}C exhibited earliest germination i.e. after 6.0 days of sowing after which was followed by 6.75 days in seeds stored for 6 months at 0^{0}C. Most delayed onset of germination i.e. 17.00 days after

sowing was observed in seeds stored for 8 months at ambient temperature (Table 2). Storage temperatures and durations altogether affect the time taken by seeds to complete their germination. Therefore, seeds kept at 0^0C for 4 months of storage took minimum time for germination (14.00 days) which, was significantly at par (16.75 days) for seeds stored at 0^0C and 5^0C for 4 and 6 months, respectively. However, maximum time i.e. 29.50 days taken to complete the germination was registered by seeds stored for 8 months under ambient conditions. Similarly, the effect of storage environment was also evident from the total germination time taken by seeds to germinate (Fig.1). Minimum MGT (10.07 days) was observed in seeds stored at 0^0C for 4 months, which was followed by 14.71 days, registered by seeds stored for 6 months at the same temperature. Maximum MGT (30.55 days) was recorded in seeds stored at ambient temperature after 8 months of storage (Fig. 1).

Table 2: Effect of storage temperatures and durations on Onset of germination (Days) and Completion of Germination (Days).

Storage duration (month)	Onset of Germination (Days)					Completion of germination (Days)				
	Temperature (^{0}C)					Temperature (^{0}C)				
	0	5	10	Ambient Temp.	Mean	0	5	10	Ambient Temp.	Mean
2	16.00	13.75	8.50	9.50	11.94	24.75	22.00	19.00	21.50	21.81
4	6.00	7.00	9.00	11.00	8.25	14.00	16.75	19.00	22.75	18.13
6	6.75	9.50	12.75	15.00	11.00	16.75	20.50	22.00	25.50	21.19
8	8.50	12.50	16.75	17.00	13.56	23.00	25.00	28.75	29.50	26.44
Mean	9.31	10.69	11.63	13.13		19.63	21.06	22.07	24.81	

CD0.05

T	0.89	0.88
D	0.89	0.88
TxD	1.77	3.76

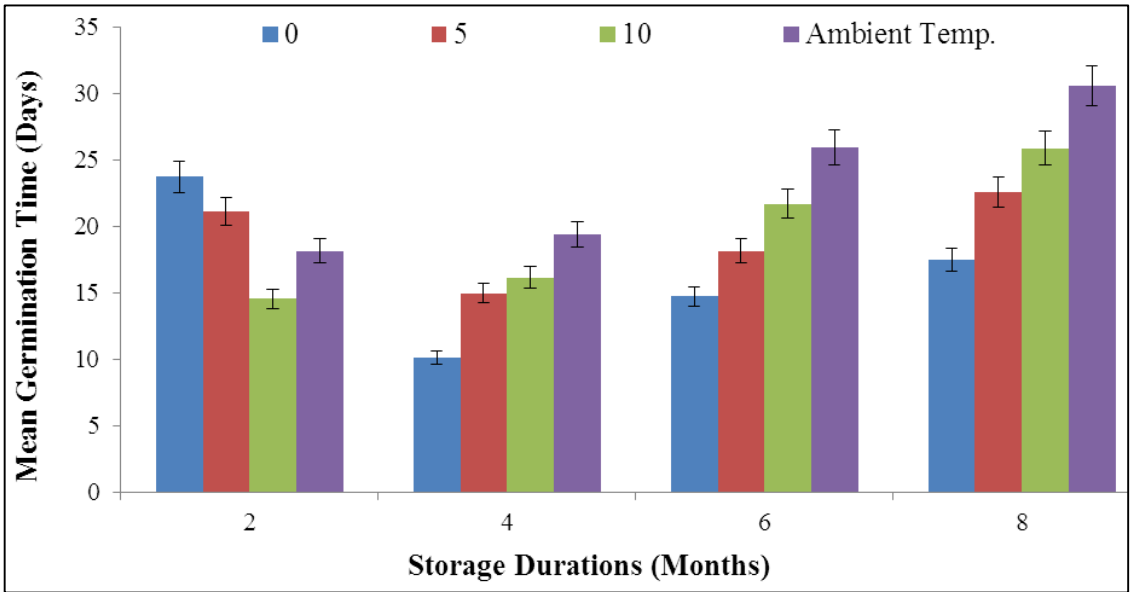

Fig 1: Effect of storage temperatures and durations on Mean Germination Time (Days)

Among all the treatment combinations, seeds stored at 0^0C for 4 months gave maximum germination speed (7.09), which was followed by seeds stored at same duration at 5^0C (5.65). Whereas, minimum speed of germination was registered by seeds stored for longer durations i.e. 8 months at ambient conditions (1.07). Seeds stored at 0^0C for 4 months showed maximum germination energy (18.61); followed by seeds stored at 5^0C (17.24) at same duration of storage. Minimum germination energy (2.77) was observed in seeds stored under ambient conditions for 8 months (Table 3).

Table 3: Effect of storage temperatures and durations on germination speed and germination energy

Storage duration (month)	Germination speed					Germination energy				
	Temperature (^{0}C)					Temperature (^{0}C)				
	0	5	10	Ambient Temp.	Mean	0	5	10	Ambient Temp.	Mean
2	1.84	2.32	3.35	2.69	2.55	2.73	3.06	5.62	4.75	4.04
4	7.09	5.65	4.61	3.39	5.18	18.61	17.24	15.53	14.81	16.55
6	4.63	3.69	2.60	2.55	3.37	10.44	9.87	7.68	5.93	8.48
8	2.82	2.09	1.53	1.07	1.88	7.32	5.98	3.33	2.77	4.85
Mean	4.09	3.44	3.02	2.42		9.77	9.04	8.04	7.07	

$CD_{0.05}$

T	0.28	T	0.34
D	0.28	D	0.34
TXD	0.56	TXD	0.57

Storage conditions had significant effect on seedling vigour index during the storage period (Table 4). Seeds stored at 0^0C for 4 months of storage recorded significantly higher and maximum SV I (619.76) and SV II (66.09) compared to other treatment combinations. This was followed by SV I (455.21) and SV II (50.85) for seeds stored at 5^0C at same storage duration indicating that 5^0C was second best storage temperature. Minimum SV I (28.90) and II (5.18) were found in seeds kept for 8 months of storage at ambient temperature.

Table 4: Effect of storage temperatures and durations on Seedling Vigour Index I and II.

Storage duration (month)	Seedling Vigour Index I					Seedling Vigour Index II				
	Temperature (^{0}C)					Temperature (^{0}C)				
	0	5	10	Ambient Temp.	Mean	0	5	10	Ambient Temp.	Mean
2	183.33	210.91	289.33	255.57	234.79	16.04	24.42	37.11	29.81	26.84
4	619.76	455.21	376.26	297.41	437.16	66.09	50.85	46.91	41.65	51.37
6	382.74	256.73	180.52	112.82	233.20	37.69	34.31	29.39	24.93	31.58
8	150.08	97.32	50.57	28.90	81.72	21.15	11.00	8.42	5.18	11.44
Mean	333.98	255.04	224.17	173.67		35.24	30.14	30.46	25.39	

$CD_{0.05}$

T	23.86	T	4.56
D	23.86	D	4.56
TXD	47.71	TXD	9.10

Discussion

The perusal of data revealed that storage conditions have significant effect on all the germination attributes of the seeds. Minimum values for onset of germination and completion of germination and maximum values for mean germination time was observed in seeds stored at 0^0C after four months of storage. The better performance of seeds stored at low temperature probably is due to the slow rate of deteriorative processes during storage and nevertheless low temperature exerts a stimulating effect on completion of pre germinative and germination promoting metabolic activities *viz*; ABA degradation (Egley and Paul 1982) [3], changes in membrane permeability (Francis and Coolbear 1987) [4]. Thus, such changes in turn have helped the seeds to achieve threshold status of all germination promoting substances which has enabled the seeds to protrude radicle early as compared to control. Our results, were in harmony with seeds of *Thymus daenensis* stored at -20^0C (Rowshan *et al.* 2013) [16] and at -20^0C in *Hyptis pectinata* (Jesus *et al.* 2016) [9]. The investigation on the effect of storage duration revealed that germination percentage and emergence index were low after 2 months and attained its peak value after 4 months of storage. A decreasing trend was observed after 6 and 8 months of storage. Further, the germinal capacity of the seeds became zero after 10 months of storage. Lower germination percentage after 2 months of storage seems to be due to some magnitude of dormancy present in younger seeds. Freshly harvested seeds of many wild species do not undergo germination immediately and need some period of after-ripening of embryo, due to which they attain peak germination after four months of storage. This was also reported by Shanmugararnam *et al.* 2013 in *Withania somnifer*a. Seeds stored under ambient conditions showed poor germination potential after successive storage durations. Various plant species including *Swertia chirayta* shows loss in germination capacity and viability of seeds when stored at room temperature (Pradhan and Badola, 2012). The major reason for loss in germinability of the seeds stored at ambient conditions seems to be DNA degradation during ageing or impaired transcription or lipid peroxidation (McDonald and Copeland 2005) [12]. Non germinability of seeds stored for 10 months at each of the four tested temperatures convincingly indicates that the storage conditions highly affects the seed germination attributes which declines with increasing storage duration irrespective of storage conditions as reported in medicinal specie *Swertia chirayta* (Pradhan and Badola 2012) [13]. The ageing or deterioration of seeds is a progressive process accompanied by reduction in enzyme activity (α – amylase and hydrolytic enzyme) activity, amount of carbohydrate storage and tendency of proteins to undergo denaturisation during long term storage which progressively depress the germination capacity and growth of seedlings (Kapilan and Thiagarajah, 2015) [10].

Therefore, storage of seeds at 0^0C for 4 months resulted in maximum germination percentage and vigorous seedlings indicating the most favourable storage conditions for *Withania somnifera*. The reasons seem to be accumulation of least deteriorative metabolic activities which result in maximum germination potential. Under favourable storage conditions, the initiation of decline in germination and seedling parameters may be from few months to many years depending on storage conditions (Shelar *et al.* 2008) [19].

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