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SEED DESICCATION TOLERANCE OF SOUTH INDIAN *RAUVOLFIA* SPECIES

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Abstract: *Rauvolfia* is an important medicinal genus, propagated through seeds with poor germination. Since water stress physiology influences cultivation and conservation of plant genetic resources, a study was attempted on seed desiccation tolerance of five South Indian *Rauvolfia* species. Seed desiccation tolerance of *R. hookeri* Srinivas. *et* Chithra, *R. micrantha* Hook. f., *R. serpentina* (L.) Benth. *ex* Kurz, *R. tetraphylla* L. and *R. verticillata* (Lour.) Baill. with respect to viability was tested by subjecting seeds to natural desiccation gradients and corresponding germination parameters were correlated. When desiccated to ≈5% moisture content, dormancy of about 30 days among all the five species was reduced to ≈6 days, along with an enhancement in initial seed germination from 56 ± 7.48 to 74 ± 8.86%. *Rauvolfia* seeds exhibited desiccation tolerance as an orthodox nature. Analysed results indicated slightly different desiccation pattern among seeds that suits their respective habitats which in turn are interpreted for standardized seed germination and storage.
Keywords: dormancy, germination index, moisture content, orthodox, peak value, seed germination

Introduction

Desiccation tolerance of seeds ensures extended germplasm conservation in a seed bank (Huang *et al*., 2014). Desiccation tolerant organisms survive the removal of water because cellular constituents are either protected or can be repaired and the timing for this type of damage was species dependent (Walters, *et al*, 2001). The ability to survive without water was recommended as the most amazing invention in plant biology (Bartels and Salamini, 2001). Tolerance of desiccation was enhanced by slow drying of immature orthodox (desiccation tolerant) seeds (Adams, *et al*., 1983; Chandler and Robertson, 1994; Kermode, 1995; Hong and Ellis, 1997). According to Calderon-Hernandez and Perez-Martinez (2018), studies on desiccation tolerance of species become a tool for decision making process for *ex situ* conservation. *Ex situ* conservation has become an important strategy that strengthens and integrates with *in situ* conservation in the context of global climate change. Most of the orthodox seeds can withstand dehydration to around 5%, even when maturation drying is not completed prior to shedding. Decrease in seed moisture content during development was

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accompanied by increase in desiccation tolerance and germinability, both reaching their maximum at the time of peak seed fall (Tompsett and Pritchard, 1993). Orthodox species acquire ability to tolerate desiccation at different stages of seed development and maturation which enables the species to survive extreme environments for quite long periods (Sanhewe and Ellis, 1996). According to Chin *et al.* (1989), seed moisture is a critical factor determining the viability of both recalcitrant and orthodox seeds and suggested to initially identify the seed type before prescribing a method of storage.

Rauvolfia, includes 80 species throughout the world (Mabberley, 2008), of which five are distributed in India. The species such as *R. hookeri* Srinivas. *et* Chithra and *R. micrantha* Hook. f. are endemic to southern Western Ghats. *R. Serpentine* (L.) Benth. *ex* Kurz distributed in the south-east Asian countries is an important traditional Indian medicine. *Rauvolfia tetraphylla* L. is introduced from West Indies and naturalized in India. *Rauvolfia verticillata* (Lour.) Baill. is distributed in India and China and is widely used as a Chinese medicine. Distributional range of these species spreads from 350 to 2000 m commonly face natural seed germination problems. In India their natural population is diminishing day by day due to the indiscriminate root collection by raw drug collectors and poor seed germination. Furthermore, their distribution in southern Western Ghats region is fragmented and comprises only few isolated populations. Poor seed germination was reported due to the presence of cinnamic acid derivatives in the seeds (Mitra, 1976). During development, seeds showed maturation drying and in general development of desiccation tolerance was associated with a net loss of seed water content which was postulated as an orthodox nature (Bindu and Anilkumar, 2014). Being distributed in a wide range of altitude difference, it become necessary to know whether any variation or similarity is accordingly exist among their germination after desiccation. It is also interesting to know how much vigour of seeds to germinate in the desiccated condition persists or is that any correlation in germination behaviour exists between species.

This study was for the optimised utilization of orthodox nature of *Rauvolfia* seeds based on physiological events revealed during fruit/seed development and the germination behaviour with respect to the desiccation pattern of *Rauvolfia* seeds. Through such studies on comparative germination among Indian *Rauvolfia* species, their conservation aspects were also discussed.

Methodology

Collection and processing

Ripened fruits of all the selected species were collected at the fully ripened condition from their natural habitats at an altitude ranging from 450 to 1100 m a.s.l. Fruits were immediately transported to the lab in sealed polythene bags. They were washed under tap water for removing pulp and impure seeds or chaffs, and submerged ones were then surface dried at the lab conditions.

Desiccation of seeds and determination of moisture content

Surface-dried seeds and embryos were used for determining the fresh moisture content (MC) by using the oven method, drying at 103°C for 17 hours (ISTA, 2008) with five replicates of five seeds in each. This method was used in every desiccation levels. For desiccation, the seeds were kept open in the lab condition at 30 ± 2°C/75 ± 10%RH as well as in the desiccators containing activated silica gel. Moisture content was monitored daily and germination was checked at every ≈ 5% reduction of seed moisture level.

Evaluation of germination

Germination was conducted in acid free germination paper rolls moistened with water and kept in germinator (30±2°C/80%RH). Seeds with 2mm root were considered as germinated and number of seeds germinated in each lot was counted and recorded on daily basis. Germination parameters such as germination index (GI), germination speed (GS), mean daily germination (MDG) and peak value (PV) were calculated according to Czabator (1962). Mean germination time (MGT) was calculated as per Ellis and Roberts (1981).

The germination and other parameters of desiccated seeds were compared with the control (fresh seeds) to evaluate the effect of desiccation in viability and vigour. Correlation between germination percentage of each species were also determined by using Microsoft excel 2017 and analysed by Karl Person's formula in Khan and Khanum (1994).

Results

Moisture reduction

Rate and duration of drying were different in each species and in *R. hookeri,* initial fresh moisture content of 25.86% was reduced at a comparatively faster rate than of any other *Rauvolfia* to become the lowest level of desiccation (4% MC) in 6 days desiccation. *Rauvolfia micrantha* took eight days for 5% MC and germination speed was linearly increased with the reducing moisture content. *Rauvolfia serpentina* and *R. verticillata* took 117 and 64 days respectively to reach 3.5 and 4.34% MC. While *R. tetraphylla* took 176 days to become 4.22% MC, GS was highest with the lowest MC. Generally the pattern of reduction of MC initially took little time but time taken for subsequent drying was comparatively longer (Fig.1).

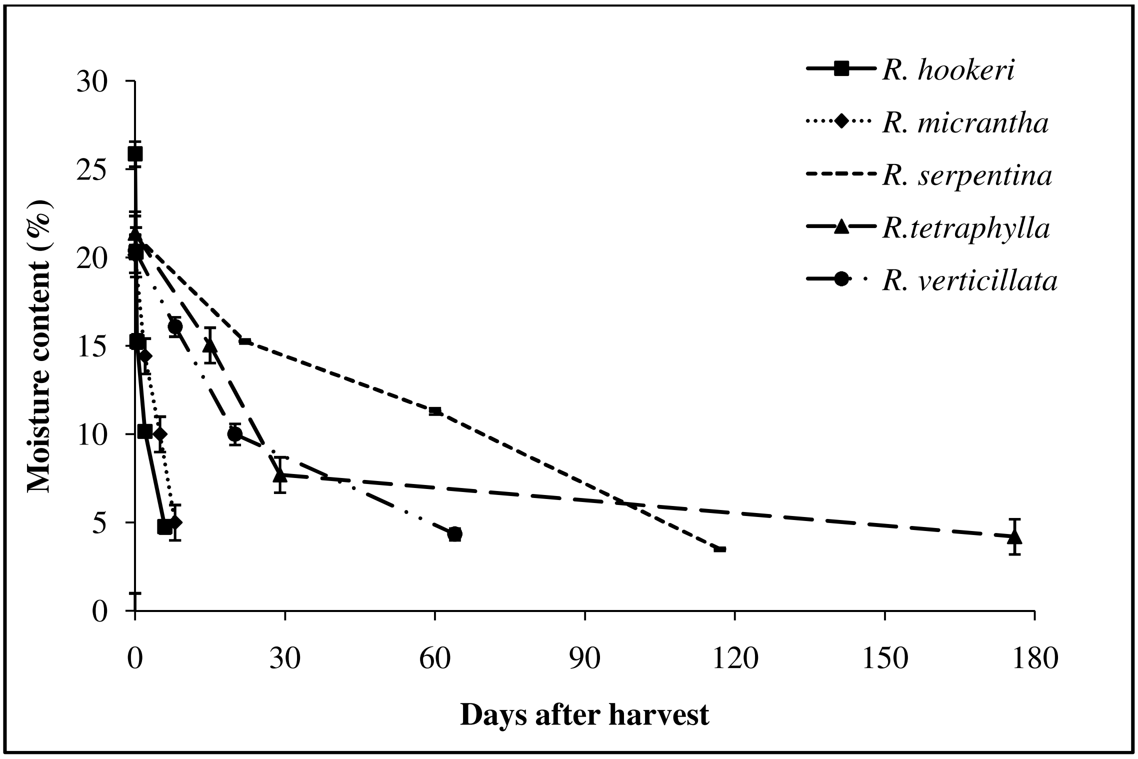

Fig 1: Rate of slow drying of seeds of *Rauvolfia* species in lab condition

Rauvolfia hookeri

In *R. hookeri,* initial fresh MC was found to be 25.86% with a mean germination percentage of 65 registered in an incubation period of 30 to 89 days (MGT = 41.94). After desiccation, the percentage of germination was reduced slowly to 51 with MC 15.26%. In the second day the MC was further reduced to 10% and the mean germination percentage was increased to its maximum (80%). Along with the MC reduction, the incubation period was also concurrently reduced from 89 days to 32 days but as a positive response, the mean germination percentage became enhanced. By the 6th day of continuous open room desiccation, the seeds of *R. hookeri* were desiccated to the minimum MC of 4.76% with germination of 75%. Correlation between days of desiccation and corresponding germination was found to be very low (*r*= 0.226).

Seed germination was sporadic and longest delay in germination was observed with fresh MC (25.86%) as well as at the 4th stage of moisture level (10.17%) of desiccation. Minimum value of MGT (17.62) and maximum GI (0.63) was reported with 20.31% MC. There was no strong relation (*r* = 0.226) between germination percentage and desiccation. There was significant difference in germination (Table 1) of the desiccated seeds to different levels (LSD 13. 83 at P< 0.05). Mean daily germination (MGT) was high towards the end of the desiccation trials even though the germination percent was comparatively high. All parameters supported the highest vigour of *R. hookeri* seeds with an average moisture content of 20.31%.

Table 1. Desiccation response of *R. hookeri* seeds to germination (%), Mean germination time (MGT), mean daily germination (MDG), peak value of germination (PV), germination index (GI) and germination speed (GS)

Desiccation period (days)	MC (%)	G (%)	MGT	MDG	PV	GI	GS
0	25.86 ± 0.71	65 ± 2.24	41.94 ± 0.67	0.07 ± 0.02	0.70 ± 0.06	0.05 ± 0.01	0.19 ± 0.03
2hrs	20.31 ± 1.40	63 ± 6.25	17.62 ± 0.79	0.25 ± 0.01	2.5 ± 0.20	0.63 ± 0.02	0.35 ± 0.02
4hrs	15.26 ± 0.41	50 ± 7.48	26.86 ± 1.04	0.14 ± 0.02	0.8 ± 0.07	0.11 ± 0.02	0.19 ± 0.02
2	10.17 ± 0.24	80 ± 2.45	38.10 ±0.59	0.16 ± 0.02	0.91 ± 0.03	0.15 ± 0.01	0.23 ± 0.02
6	4.76 ± 0.38	75 ± 2	25.21 ± 0.95	0.23 ± 0.09	1.28 ± 0.12	0.29 ± 0.02	0.27 ± 0.02
LSD (P<0.05)		13.83	0.824	2.44	0.334	0.05	0.073

Values: Mean ± Standard error (SE)

Rauvolfia micrantha

In *R. micrantha,* the initial MC was 20.14% with an average germination of 60% which was retained up to the next tested lower desiccation level of 14.42%. Seeds took 8 days to reach the lowest level of 5% MC and concurrently germination was increased to 90% (LSD 7.55 at P < 0.05). Germination percentage and number of drying days were in a significant relation with a linear regression of 0.89 (P < 0.05). Dormancy period was observed highest (30 days) in fresh seeds with MC 20.14% and it gradually decreased (6days) during desiccation. Mean germination time (MGT) was found minimum (7.22) with seeds having 5% MC (Table 2). Germination percentage was in high degree of negative correlation with MGT (– 0.99). Germination index was maximum (1.90) with seed MC (10%). In the course of desiccation, dormancy or the lag period was reduced gradually from 30 to 6 days along with decreased MGT and increased GI.

Table 2. Desiccation response of *Rauvolfia micrantha* seeds to germination (%), Mean germination time (MGT), mean daily germination (MDG), peak value of germination (PV), germination index (GI) and germination speed (GS)

Desiccation period (days)	MC (%)	G % (n=5)	MGT	MDG	PV	GI	GS
0	20.14 ± 0.26	60 ±1.70	20.86± 0.63	0.18 ± 0.01	0.86 ± 0.06	0.16 ± 0.01	0.17 ± 0.01
2	14.42 ± 0.26	60 ± 2.72	29.5 ± 0.5	0.19 ± 0.03	0.71 ± 0.02	0.14 ± 0.02	0.22 ± 0..05
5	10 ± 0.84	75 ± 2.24	11.75 ± 0.66	0.57 ± 0.04	3.33 ± 0.36	1.90 ± 0.12	0.68 ± 0.02
8	5 ± 0.28	90 ± 3.16	7.22 ± 0.50	0.47 ± 0.04	1.43 ± 0.13	0.67 ± 0.05	1.36 ± 0.16
LSD (P<0.05)		7.55	1.74	0.095	0.594	0.954	0.25

Values: Mean ± Standard error (SE)

Rauvolfia serpentina

Rauvolfia serpentina seeds with 21.27% initial MC showed only 30% germination after 28 days' lag period. On reduction of its MC to 15.26% after 22 DAH of open exposure, the germination percentage remained same but the lag period was reduced to 10 days. After 60 days of open room desiccation, at 11% moisture content, the germination percentage became the maximum of 70% without germination lag (Table 3). Seeds took 117 days to reach the minimum MC of 3.5% with 40% germination which was a highly significant reduction (LSD 7.10 at $P < 0.05$). Relation between germination percentage and days for desiccation were neither in a linear regression ($r^2 = 0.227$) nor in a significant way ($P < 0.05$).

In *R. serpentina,* seeds showed comparatively low germination percentage in all stages of desiccation (Table 5). Lag period was observed highest (28 days) in fresh seeds with 21.27% MC. Germination was maximum (70%) at 11.3% moisture content with minimum MGT (9.14) and maximum GI (1.76).

Table 3. Desiccation response of *Rauvolfia serpentina* seeds to germination (%), Mean germination time (MGT), mean daily germination (MDG), peak value of germination (PV), germination index (GI) and germination speed (GS)

Desiccation period (days)	MC (%)	G % (n=5)	MGT	MDG	PV	GI	GS
0	21.27 ± 0.74	30 ± 2.74	44.33 ± 0.32	0.05 ± 0.01	0.35 ± 0.04	0.02 ± 0.003	0.08 ± 0.01
22	15.26 ± 0.20	30 ± 0.73	16.06 ± 0.41	0.12 ± 0.01	1 ± 0.28	0.12 ± 0.02	0.21 ± 0.03
60	11.3 ± 0.41	70 ± 1.38	9.14 ± 0.31	0.47 ± 0.01	3.75 ± 0.19	1.76 ± 0.15	0.96 ± 0.02
117	3.5 ± 0.27	40 ± 3.54	10.84 ± 0.72	0.20 ± 0.02	2.5 ± 0.16	0.5 ± 0.09	0.51 ± 0.09
LSD (P<0.05)		7.102	1.40	0.042	0.572	0.265	0.14

Values: Mean ± Standard error (SE)

Rauvolfia tetraphylla

Rauvolfia tetraphylla with 21.09% initial MC registered an average of 60% germination within a longest period of germination *i.e*., 320 days. Seeds reached the minimum moisture level of 4.2% after a maximum desiccation time of 176 days of open storage. In *R. tetraphylla,* seeds of all desiccation levels retained high viability (Table 4). Maximum (85%) and minimum (40%) germination were found at 4.2% and 7.7% MCs respectively. At 4.2% MC, germination period was reduced considerably from 320 days of fresh seeds to 5 days. Days of drying had an inconsistent relation with germination percentage ($r^2 = 0.031^{ns}$). Mean germination time, MDG, PV, GI and GS were observed maximum (3.12, 1.7, 13.33, 22.66 and 2.93 respectively) with the seeds of maximum desiccated MC (4.22%).

Table 4. Desiccation response of *Rauvolfia tetraphylla* seeds to germination (G %), Mean germination time (MGT), mean daily germination (MDG), peak value of germination (PV), germination index (GI) and germination speed (GS)

Desiccation period (days)	MC (%)	G % (n=5)	MGT	MDG	PV	GI	GS
0	21.31 ± 0.86	60 ± 1.64	52 ± 0.57	0.02 ± 0.01	0.36±0.04	0.01±0.01	0.11±0.01
15	15.04 ± 0.39	85 ± 3.5	70.12 ± 0.51	0.04 ± 0.01	0.94±0.08	0.04±0.01	0.27±0.02
29	7.7 ± 0.16	40 ± 2.63	111.75 ± 0.66	0.02 ± 0.01	0.67±0.07	0.01±0.001	0.13±0.01
176	4.2 ± 0.22	85 ± 4.18	3.12 ± 0.47	1.7 ± 0.19	13.33 ± 0.70	22.66±0.82	2.93±0.26
LSD (P < 0.05)		9.398	1.68	0.282	0.335	1.23	0.382

Values: Mean ± Standard error (SE)

Rauvolfia verticillata

Rauvolfia verticillata had 20.4% MC initially with 50% germination and upon desiccation MC was reduced to 16.08% after 8 days of exposure with 55% average germination. Here, the days of drying were linearly associated with germination percentage with significant high regression value of 0.969 ($P < 0.05$). After 64 days seeds reached the minimum moisture level of 4.34% with 80% average germination. The lag period was reduced to 8 days with moisture reduction (10%) and at the last stage (4.34%), it suddenly increased to a maximum of 56 days. The incubation period was also increased with the moisture reduction but in the second stage of moisture reduction (16.08%) a slight decrease was found.

Mean germination time was maximum (59.75) with the seeds of lowest MC (4.34%) and minimum (10.73) in seeds with 16.08% MC. Maximum MDG, GI and GS values were maximum in seed with 16.08% MC (Table 5). Peak value was found maximum (1.2) in seeds having 10% MC.

Table 5. Desiccation response of *R. verticillata* seeds to germination (%), Mean germination time (MGT), mean daily germination (MDG), peak value of germination (PV), germination index (GI) and germination speed (GS)

Period of desiccation (days)	MC (%)	G (%) (n=5)	MGT	MDG	PV	GI	GS
0	20.4 ± 0.44	50 ± 1.70	27.8 ± 0.65	0.16 ± 0.02	0.74 ± 0.01	0.12 ± 0.12	0.03 ± 0.01
8	16.08 ± 0.35	55 ± 1.84	10.73 ± 0.59	0.34 ± 0.01	1.11 ± 0.07	0.38 ± 0.03	0.47 ± 0.01
20	10 ± 0.76	70 ± 3.15	19.71 ± 0.47	0.11 ± 0.01	1.2 ± 0.10	0.13 ± 0.01	0.35 ± 0.05
64	4.34 ± 0.20	80 ± 3.54	59.75 ± 0.58	0.12 ± 0.02	0.69 ± 0.01	0.08 ± 0.02	0.15 ± 0.03
LSD (P<0.05)		8.04	1.79	0.042	0.184	0.059	0.085

Values: Mean ± standard error (SE)

Correlation among species

There was no significant correation among the germination of fresh seeds of *Rauvolfia*, except in *R. serpentina* with *R. micrantha* and *R. verticillata* (Table 6). After desiccation, seed germination in all the species showed significant positive correlation as a matter of each species practical solution for seed dormancy (Table 7).

Table 6. Simple correlation among the initial germination percentage of *Rauvolfia* species

	R. hookeri	*R. micrantha*	*R. serpentina*	*R. tetraphylla*	*R. verticillata*
R. hookeri	1				
R. micrantha	0.5053^{ns}	1			
R. serpentina	-0.2326^{ns}	0.6918^{*}	1		
R. tetraphylla	0.8217^{ns}	0.2587^{ns}	-0.2355^{ns}	1	
R. verticillata	0.2576^{ns}	0.5252^{ns}	0.4018^{*}	-0.0049^{ns}	1

* - Significant at P<0.05; ^{ns} – Not significant

Table 7. Simple correlation among the average germination percentage of desiccated seeds of Rauvolfia species

	R. hookeri	*R. micrantha*	*R. serpentina*	*R. tetraphylla*	*R. verticillata*
R. hookeri	1				
R. micrantha	0.9983^{*}	1			
R. serpentina	0.9939^{*}	0.9927^{*}	1		
R. tetraphylla	0.9888^{*}	0.9901^{*}	0.9678^{*}	1	
R. verticillata	0.9982^{*}	0.9932^{*}	0.9898^{*}	0.9863^{*}	1

* - Significant at P<0.05

Discussion

The effect of desiccation on *Rauvolfia* seeds was studied by comparing the vigour parameters related to different degree of desiccation. All the five species of south Indian *Rauvolfia* are initially with MC range of 20.4 to 25.85%. Irrespective of the wide range of durations for moisture reduction to the lowest <5%, all seeds showed an enhancement in germination vigour.

Almost a linear pattern was followed in *R. hookeri* and *R. micrantha* while in other three species a drastic moisture reduction occurred at first phase followed by slow pace to reach the lower most moisture level in room conditions. Even though their moisture at the time of shedding was alike, the event of desiccation differed in terms of period *i.e*., 6 days in *R. hookeri* to 180 days in *R. tetraphylla*, for the moisture to become below 5%. According to Hong and Ellis (1997), slow drying of orthodox seeds allows them to go for more advanced stage in the maturation process as the seeds become more tolerant to desiccation. Walters *et al*. (2001) suggested rapid drying for the accurate assessment of the critical water content. In the case of *Rauvolfia* seeds, rapid drying did not support germination as it prefers slow drying for the completion of after-ripening process. *Rauvolfia tetraphylla* seed registered maximum viability (85%) with high germination index (22.66) at 4.2% moisture after 176th day of drying. Germination index (GI) is used as a tool for describing the relation between germination percentage and speed of germination (Kader, 2005).

The germination percentage was gradually increased during desiccation except in *R. serpentina* in which, moisture decline below 10% caused some reduction of the germination percentage, though, not less than the initial germination. In general, seed desiccation tolerance is a very remarkable ability for surviving dehydration particularly in the back drop of reports by Hong and Ellis (1990) on triggered germinability in orthodox Norway mapple (*Acer platanoides*) by the reduction in seed moisture content.

Irrespective to species specific differences among *Rauvolfia*, seeds tolerated desiccation without injury as reported in the case of papaya seeds which retained high germination with low moisture of 5%, as typical to orthodox seeds (Salomao and Mundim, 2000). In the case of *R. micrantha*, there was no deviation in the germination percentage from the linearity range with maximum GS value (1.36) of the seed with 5% MC but vigour was found high in seeds with 10% MC.

In *Rauvolfia* seeds, desiccation did not affect germination capacity but enhanced pace. Pammenter and Berjak (1999) listed a series of reasons for the orthodox seeds to survive dehydration, like reduction in the degree of vacuolation, insoluble reserve accumulation, reaction of the cytoskeleton, conformation of the DNA, chromatin and nuclear architecture, intercellular de-differentiation, ‘switching-off’ of metabolism, presence and efficient operation of antioxidant system, accumulation of protective molecules, *etc*. During the final stage of seed development, orthodox seeds reduce the volume of water-filled vacuoles and shut down their metabolic activities for acquiring desiccation tolerance. The LEA proteins accumulated during the later stages of embryo development in orthodox seeds like *Gossypium hirsutum* (Galau *et al*., 1986) had a protective role in the desiccation tolerance (Oliver and Bewley, 1997; Kermode, 1997). Drying of seeds to a certain level helps to minimize metabolic rate and by and large 5–10% moisture is reported to be optimum for retaining orthodox and sub orthodox seed viability (Bonner, 2008).

Rauvolfia seeds showed about thirty days of dormancy which on desiccation was evaded. Similar removal of physiological dormancy during desiccation in *Lannea microcarpa* and *Sclerocarya birrea* of Anacardiaceae family was reported by Pritchard *et al*, (2004). In *R. micrantha* and *R. verticillata,* there was no depression of germination capacity during the course of desiccation but was in a linear regression of high values, 0.891 and 0.969 respectively. The seeds of *R. hookeri, R. micrantha*, *R. tetraphylla* and *R. verticillata* are highly desiccation tolerant since maximum germination was reported after desiccation below 5% moisture content. *Rauvolfia serpentina* exhibited a decrease in germination below 10% moisture content which was not less than the initial one and also tolerated ultra-desiccation up to 3% moisture without losing viability. Similar pattern of desiccation response was reported by Pritchard *et al*. (2004) in *Lannea microcarpa* and *Sclerocarya birrea*. Farnsworth (2000); Oliver *et al*. (2000); Dickie and Pritchard (2002) proposed that desiccation tolerance may be an ancestral state of seeds while desiccation sensitivity being a derived trait. The ability to tolerate near complete desiccation was an important evolutionary step that play key role in dry land colonization (Dekkers et al, 2015).

Seed vigour was found slightly loosing when desiccated to <5% MC in all species except in *R. tetraphylla*, in which all germination parameters like MGT, MDG, PV, GI and GS including germination percentage were found maximum in seeds with lowest moisture content (4.2%). According to Matthews and Powell (2011) high MGT, low GS and low germination are signs of low vigour of seed lot as in the case of maize. Guy and Black (1998); Bailly *et al*. (2002) commented that an increase in MGT was a signal of seed ageing.

Generally it was derived from the experiments that average seed viability across all the studied *Rauvolfia* species was 56 ± 7.48% with an average 21.80 ± 1.04% MC. Salma *et al*., (2008) reported low germination percentage (25–50%) of *R. serpentina* and according to earlier reports this was due to the presence of cinnamic acid derivatives in the seeds (Mitra, 1976). After desiccation below 5% MC, the average germination percentage was increased up to 74 ± 8.86%. No correlation was observed when the initial germination of all the species were analysed, though on desiccation, there developed a high degree of positive correlation, *i.e*., all species showed similarity in seed germination behaviour. After drying at room conditions, the germination percentage changed significantly in all species of *Rauvolfia*. Interspecific correlation of germination subjected to desiccation among *Rauvolfia* seeds was high as matter of generic identity.

Conclusion

The relevance of seed desiccation responses among the *Rauvolfia* species largely rely on interpreting species ecology requirements and to aid restoration programme as in the case of widely depleted *R. serpentina* and poorly regenerated endemics like *R. hookeri* and *R. micrantha*. In the case of *R. verticillata* with comparatively wider distribution yet fewer individuals due to poor natural germination needs proactive seed management.

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