Seed Storage Behaviour in *Rauvolfia serpentina*

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***Abstract:* - *Rauvolfia serpentina* Benth. (of *Apocynaceae*) commonly known as Sarpagandha is one of the most valuable medicinal plants in Indian system of medicine. No information is available on seed morphology, dormancy and seed storage behavior of the species which is required for conserving the seeds in seed banks. Therefore, an experiment was conducted with seed collected from Sushila Tiwari Herbal Garden, Rishikesh, Uttarakhand, India. Studies were conducted on seed germinability (using germination tests) and seed viability (using topographical tetrazolium chloride test) in relation to desiccation and chilling tolerance using a factorial combination of four moisture contents (5, 7, 10 and 12% moisture content in seeds on fresh weight basis), three storage temperatures (seeds stored in ambient condition, at 15°C and at –20°C) and five storage periods (0, 3, 6, 9 and 12 months). Perusal of data revealed that no significant loss in germination was observed in seeds with 7% and 10% moisture content under ambient as well as other storage conditions, suggesting that seeds of *Rauvolfia serpentina* are desiccation as well as chilling tolerant and exhibited orthodox seed storage behavior. The seeds are ideal for *ex- situ* conservation in seed banks/ gene banks for a longer period.**

***Key words*: *Rauvolfia serpentina*, Orthodox seeds, Seed storage, Germination and Moisture content.**

I. INTRODUCTION

Rauvolfia serpentina is one of the important medicinal plants of India as used in Ayurvedic system of medicine (Biswas and Mukherjee 2003). This is a perennial under shrub, growing to a height of 60-90 cm. The fruit is a drupe, 0.5 cm in diameter and shiny black when fully ripe. The root-bark, which constitutes 40-60% of the whole root, is rich in alkaloids. Almost every part of this plant has some medicinal value. The root of *Rauvolfia* is generally used in medicine to cures Kapha and Vata disorders. *Rauvolfia* is mainly used for the treatment of various central nervous system disorders associated with psychosis, schizophrenia, insanity, insomnia, and epilepsy. Similarly, Extracts of the roots are valued for the treatment of intestinal disorders, particularly diarrhoea and dysentery and also as anathematic. Mixed with other plant extracts, they have been used in the treatment of cholera, colic and fever the root was believed to stimulate uterine contrition and recommended for use in child-birth in difficult cases.

The storage of seeds in seed banks/gene banks is generally considered the safest, most inexpensive and most convenient method of conservation as seeds occupy little space, and also require little attention over considerable period of time (Linington and Pritchard 2001, Engelmann and Engels 2002). Conservation of germplasm only in field condition is risky as it can be lost because of genetic erosion, pest or disease or adverse weather conditions. However, storage of seeds in seed banks for *ex-situ* conservation needs a thorough understanding of post harvest seed physiology and seed storage biophysics as seeds exhibiting orthodox seed storage behavior can only be stored in seed banks for a longer period of time without losing the seed viability. Orthodox seeds are desiccation tolerant and can be dried to 5-7 % moisture content (fresh weight basis) and stored at sub-zero temperature. Seed survival in desiccation tolerant seeds can be quantified by seed viability equations, as seed viability depends upon chemical composition of seed, moisture content and storage temperature (Roberts 1973, Ellis and Roberts 1980, Ellis *et al*. 1988, Pritchard and Dickie 2004, Hong *et al.* 2005). Orthodox seeds retain viability for several decades in gene bank condition as 90-95 % of their original water is lost during seed maturation.

No information is available on seed morphology, dormancy, and germination characteristics and seed storage behavior in *R. serpentina* which is essential for storing the seeds in seed bank for germplasm conservation. Therefore, experiments were conducted with the objectives to study (i) seed morphology and anatomy (ii) effect of different temperatures on seed germinability and (iii) storage behaviour of seeds (with different moisture content) stored in different storage temperatures so as to examine the effect of moisture content on seed viability and desiccation and chilling tolerance. The seeds with varying (targeted) moisture content were stored in three different storage temperatures and seed germinability and seed viability was tested at three months interval up to 12 months. The Tz test was also standardized so as to study seed deterioration pattern in the embryos and to correlate the seed viability with seed germinability after desiccation and storage in three different storage temperatures.

II. MATERIALS AND METHODS

Mature fruits of *Rauvolfia serpentina* were collected from Sushila Tiwari Herbal Garden, Rishikesh, Uttarakhand, India. Seeds were extracted from the fruits by pressing the seeds in water and dried under ambient laboratory condition. Seed moisture content (mc) was determined in three replicates of 4g seeds each after drying for 17 hr in an oven at 103°C (ISTA 2008) and moisture content expressed as percentage of fresh weight. Due to heterogeneity in seed size, the lot was purified by gravity separator and air and screen cleaner machine and medium sized seeds which constitute the bulk have been used for present studies.

Freshly extracted and dried seeds having moisture content of 6.7% were germinated in 25, 30, and 35°C incubation temperatures using sand so as to determine the dormancy status, optimum temperature for germination and time taken for germination. Observations were recorded on final per cent germination (normal seedlings) and seed vigor (as reflected by seedling length). A seed was considered germinated when all the essential structure of the seedlings were visible so as to differentiate between normal and abnormal seedlings. Seed viability tests were also standardized and conducted so as to correlate the viable seeds with germinable seeds, as a viable seed may not germinate due to dormancy.

Preconditioning of two hundred (50×4) seeds done by scarifying the seeds with concentrated sulphuric acid H_2SO_4 for ½ hour and soaked overnight in water for 17 (±1) hr at 20^{o}C. The outer seed coverings were removed and softened hard endosperm were cut slightly and kept in 1% TZ solution for 24 hours at 42^{o}C. The data were correlated with standard germination test. Seeds with 6.7% moisture content were again dried over silica gel in desiccators till they reached 5% moisture content. For preparing seeds with higher moisture content seeds were placed in saturated salt solution and repeatedly weighed till they reached the targeted moisture content following the protocol suggested by Sun (2002). Seeds having moisture content of 5, 7, 10 and 12% were tested for seed germinability at 30°C as well as seed viability and subsequently stored in hermitically sealed containers in three storage temperatures, viz. (a) ambient storage condition of the laboratory, where temperature varied from 15°C during winter to 37°C during summer, (b) at 15°C storage temperature in a temperature controlled room and (c) at –20°C in a refrigerator following the methodology given by Hong and Ellis (1996).

Germination studies were conducted using sand at 30°C incubation temperature at three months interval up to12 months. Seeds stored at –20°C and seeds with 5, 7, and 10% moisture content were kept at room temperature (25-30°C) with prevailing humidity for about 24 hr before putting for germination tests to prevent imbibitional damage(Ellis *et al.* 1990, Scande *et al*. 2001). Observations were recorded on final per cent germination (normal seedlings), time taken for 50% germination and seed vigor (as reflected by seedling length).

III. RESULTS AND DISCUSSION

1) *Effect of temperature on percentage germination***:** Seeds obtained from freshly harvested fruits were germinated in different incubation temperatures, viz. constant temperature of 25, 30, and 35°C. Seeds having hard seed coat, pretreated with $GA_3$200ppm followed by Concentrated H_2SO_4 for 6 minutes at optimum 25°C incubation temperature were found significantly less effective in percent of germination as compared to 30^{0}C temperature (71%), whereas, 35^{0}C resulted lowest among all. (Table-1).

Table 1: Effect of different temperature on percent germination of seeds of *Rauvolfia serpentina.*

Incubation temperature (^{o}C)			
	25	30	35
Germination (%)	58.00 (49.65)	71.00 (57.47)	34.00 (35.64)
CD	2.85		

*Values in parenthesis are the arc-sine values

2) *Effect of dehydration and hydration on seed germinability and seed viability:*
Desiccation or hydration of seeds did not influence percent germination of seeds as no significant difference in germination or seed viability was observed due to dehydration or hydration. Perusal of the data on seed germination and seed viability (Table 2) reveals high correlation between percent germination and per cent viability (completely stained embryo). The unstained embryo were correlated with dead seeds while partially stained embryo produced abnormal seedlings with root missing (radical unstained) or deformed cotyledons.

Table2: Viability of seeds in *Ravolfia serpentina* after tetrazolium tests.

Lot	**Viable seeds**			**Non-viable seeds**			
	TS	**ES**	**Total**	**MEU**	**RU**	**TU**	**Total**
Lot 1	39.50 (38.93)	48.00 (43.85)	87.50 (69.58)	3.75 (11.14)	4.75 (12.57)	4.00 (11.49)	12.50 (20.68)
Lot 2	47.00 (43.27)	47.25 (43.42)	94.25 (76.23)	2.25 (8.59)	2.25 (8.59)	1.25 (6.33)	5.75 (13.86)
CD at 5%	3.66	3.21	6.37	0.86	0.86	1.16	1.69

Note: TS= Totally stained; ES= Embryo stained; MEU= Major portion of the embryo unstained; RU= radicle unstained; TU= Totally unstained.

3) *Effect of desiccation, storage temperature and storage period on seed germinability:*

The percent mean germination was reduced during ambient storage as compared to initial germination at the time of storage. Similarly, germination during storage was adversely affected in seeds having higher moisture content compared to lower moisture content. However no significant difference in mean germination was observed in seeds having 7% and 10% moisture content. Seeds with 5% moisture content did not show significant reduction in germination up to 3 months of storage as germination at the time of storage was 81.75% which was reduced to 77.25% after 6 months in ambient storage. However at 7% and 10% moisture, no significant reduction in germination was recorded at 6, 9 and 12 months of storage in seeds. Seeds with 5% moisture content decline rapidly as seeds showed hard seededness compared to seeds with 7% moisture contents (Fig.1and table-3). Significant reduction in germination was observed at 6 months of storage which was constant and no reduction was observed from 6 months onwards. No significant difference in germination was recorded in seeds with 10 and 7 % moisture content from 6 to 12 months of storage. Similar findings on Seed Storage Behaviour in Berberis Aristata have been reported by Debarati Mukhopadhyay *et al*., (2013).

Decline in germination was much faster in seeds with having 5 and 12% moisture as germination was 93.50% and 95.25 % at the time of storage which reduced to 62.50 and 69.50% respectively after 12 months in ambient condition. In general decline in germination was less in seeds stored at 15^{o}C compared to ambient storage condition (Fig 2 and Table 4). No significant reduction in germination was observed in seeds with 10% and 7% moisture even after 12 months of storage. However significant reduction in germination was observed in seeds with 5 and 12% moisture content (Fig 2 and Table3).

Table -3 Effect of moisture content and storage period on germination percentage of *Ravolfia serpentina*

Moisture content	Treatment	Temperature (°C)			
		Ambient	**15°C**	**-20°C**	**Mean**
M.C. 5%	0M	93.50 (75.28)	95.25 (77.45)	95.50 (77.77)	94.75 (76.83)
	3M	81.75 (64.79)	82.25 (65.10)	83.75 (66.22)	82.58 (65.37)
	6M	77.25 (61.73)	70.25 (56.94)	73.25 (58.87)	73.58 (59.18)
	9M	73.50 (59.04)	67.75 (55.40)	66.25 (54.48)	69.16 (56.31)
	12Months	62.50 (52.23)	57.25 (49.16)	55.50 (48.15)	58.41 (49.85)
	Mean	**77.70** (62.62)	**74.55** (60.81)	**74.85** (61.10)	**75.70** (61.51)
M.C 7%	0M	94.75 (76.76)	95.25 (77.45)	95.75 (78.16)	95.25 (77.46)
	3M	80.75 (63.98)	83.50 (66.04)	87.50 (69.33)	83.91 (66.45)
	6M	77.75 (61.85)	73.50 (59.02)	78.25 (62.20)	76.50 (61.02)
	9M	72.75 (58.56)	68.50 (55.85)	67.50 (55.24)	69.58 (56.55)
	12Months	68.75 (56.01)	58.50 (49.89)	57.25 (49.17)	61.50 (51.69)
	Mean	**78.95** (63.43)	**75.85** (61.65)	**77.25** (62.82)	**77.35** (62.63)
M.C. 10%	0M	95.50 (77.77)	95.25 (77.45)	95.75 (78.16)	95.50 (77.79)
	3M	83.50 (66.04)	85.25 (67.41)	88.25 (70.00)	85.66 (67.82)
	6M	78.25 (62.20)	74.75 (59.83)	80.75 (64.02)	77.91 (60.02)
	9M	72.25 (58.21)	69.25 (56.32)	73.25 (58.86)	71.58 (57.80)
	12Months	69.75 (56.63)	60.75 (51.21)	66.25 (54.48)	65.58 (54.11)
	Mean	**79.85** (64.17)	**77.05** (62.45)	**80.85** (65.11)	**79.25** (63.91)
M.C. 12%	0M	95.25 (77.45)	94.25 (76.13)	95.75 (78.16)	95.08 (77.25)
	3M	85.25 (67.41)	84.75 (67.02)	82.50 (65.32)	84.16 (66.58)
	6M	77.50 (61.68)	72.25 (58.21)	69.50 (56.47)	73.08 (58.79)
	9M	71.50 (57.73)	65.75 (54.18)	65.25 (53.89)	67.50 (55.27)
	12Months	69.50 (56.56)	54.25 (47.44)	52.75 (46.57)	58.83 (50.19)
	Mean	**79.80** (61.17)	**74.25** (60.59)	**73.15** (60.08)	**75.73** (61.61)

	CD at 5%
Temperature (A)	0.63
Moisture content (B)	0.73
Months (C)	0.82
A×B	1.27
A×C	1.42
B×C	1.64
A×B×C	2.84

Storage of seeds at –20°C proved beneficial as the seeds were not only desiccation tolerant as well as chilling tolerant (Fig-3; table3). No significant reduction in germination was observed after 12 months of storage as temperature and moisture content interaction was not significant. However slight decline in mean germination was observed after 12 months of storage. Germination in seeds with 12% moisture content declined from 95.75% to 52.75% after 12 months of storage at –20°C, suggesting that higher moisture content > 10% was having adverse effect on seed viability or seeds were chilling sensitive at higher moisture content. Considering the above, it can be concluded that seeds showed purely orthodox storage behavior and can be stored at freezing temperature after lowering its moisture content (to 5-7%) for a longer period of time. Storage temperature and seed moisture content are the most important factors affecting seed longevity, with moisture content usually more influential than temperature. Roberts (1972) described the relationship of temperature and moisture content to the period of seed viability of certain crop species exhibiting orthodox storage behavior. Harrington (1972) proposed thumb rule and stated that the life of seed is halved (1) for each one percent increase in moisture content (2) for each 5°C increase in seed storage temperature. Although reduction in moisture content and storage temperature increases the life span of true orthodox seeds, but it cannot be prolonged indefinitely by progressively drying the seeds (Ellis 1998, Probert and Hay 2000) and moisture content below which longevity could not be improved is considered the critical moisture content (Ellis and Hong 2007, Vertucci *et al*. 1994).

The existence of the critical moisture content is the most important point in the seed storage debate and drying below certain moisture content will not improve seed longevity (Probert and Hay 2000, Buitink and Hoekstra 2004). Increased longevity upon drying is related to an increased intracellular viscosity and dehydration induced increase in cytoplasm viscosity leads to a decrease in the rate of detrimental reaction in seeds leading to an increase in seed longevity. Understanding these processes is an issue of economic importance and a major concern for seed quality assurance and also for *ex-situ* conservation of seeds in seed banks.

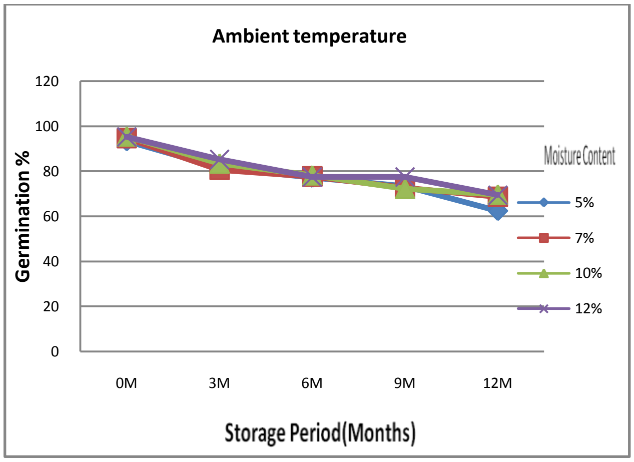

Fig.1 Effect of moisture content (%) and storage period (months) on germinability at ambient temperature in *Ravolfia serpentina.*

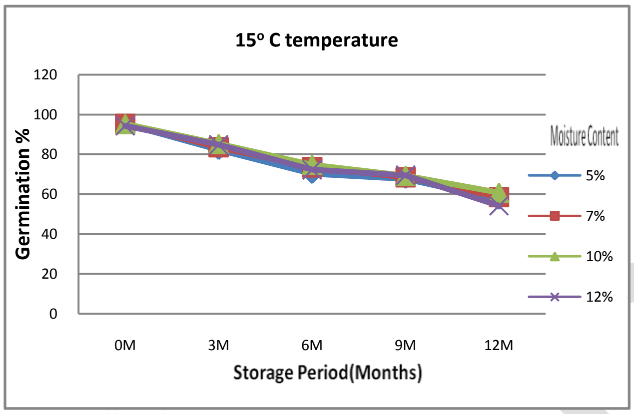

Fig.2 Effect of moisture content (%) and storage period (months) on germinability at 15° C temperature in *Ravolfia serpentina*.

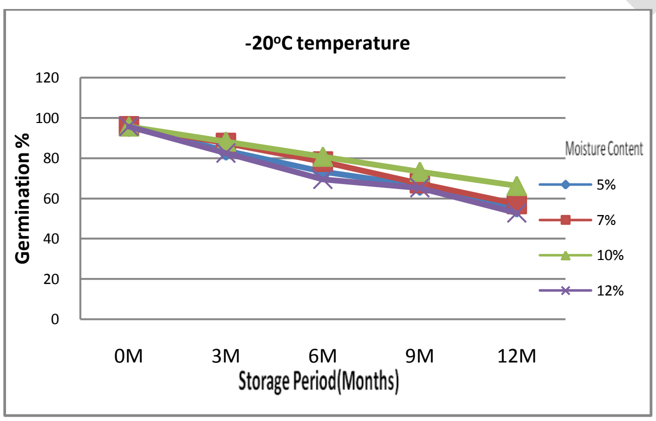

Fig.3 Effect of moisture content (%) and storage period (months) on germinability at -20° C temperature in *Ravolfia serpentina*.

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