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GERMINATION, VIABILITY AND VIGOUR OF FRESH AND AGED SEEDS OF SOME ENDANGERED MEDICINAL PLANT SPECIES OF WESTERN HIMALAYAS

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SUMMARY

Fresh and aged (one year old) seeds of endangered medicinal plant species of Western Himalayas were evaluated for germination, viability and vigour attributes. It was observed that aged seeds of *Achillea millefolium*, *Gentiana kurroo* and *Podophyllum hexandrum* registered a substantial loss of 20, 75 and 25 per cent germinability, respectively. Further, emergence index declined by 5, 90 and 40 per cent in aged seeds of *Achillea, Gentiana* and *Podophyllum* respectively. A higher proportion of strong seedlings was recorded when raised from current year collection. Further, significant correlations were observed between germination percentage and speed; emergence index and vigour index; vigour and viability. However, negative correlation of electrical conductivity (EC) was observed with germination percentage and speed.

Key words **: *Achillea millefolium*, emergence index, *Gentiana kurroo*, germination, *Podophyllum hexandrum*, viability, vigour index.**

INTRODUCTION

Achillea millefolium, *Gentiana kurroo* and *Podophyllum hexandrum* are endangered medicinal plant species of Western Himalayas and are in great demand by pharmaceutical industries for their active ingredients. These species are overexploited on account of the importance of their ingredients. Medicines prepared from the valuable alkaloid gentiopicrin from *G. kurroo*, podophyllotoxins and podophyllic acid from *P. hexandrum* and volatile oil from *Achillea millefolium* are used for cure of rheumatic diseases, cancers and hepatic disorders. These species mostly inhabit and grow in inaccessible far-flung areas (Chauhan 1999). Due to vagaries of weather, poor seed setting, poor seed germination, low seedling survival, coupled with non judicious exploitation by drug industries, these herbs have become endangered. Further, due to restricted availability of seeds, natural regeneration of these valuable species does not seem to pave the way for mass multiplication or covering larger areas under these non-conventional crops. Cultivation practices on these medicinal plants suffer a lot due to lack of reliable information on viability and vigour of fresh and aged seeds. Little information is available on germination and viability of seeds of some medicinal plant species (Nautiyal *et al.* 1987, Kant *et al.* 1998 and Gu *et al.* 1999). However, for conservation or multiplication of these valuable species it is necessary to generate reliable and indepth information on viability, vigour and germination characteristics in fresh and aged seeds of above species in order to decrease the dependence on the fresh collection of seeds. Present investigation was, therefore, undertaken with the objective to obtain reliable information on the comparative status of viability and germination characteristics of fresh and one-year-old (aged) seeds.

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MATERIALS AND METHODS

Seeds of three herbal species namely *Achillea millefolium, Gentiana kurroo* and *Podophyllum hexandrum* were used in the present study. A one time collection of seeds of *Achillea* was done in the month of July 2001 and 2002, whereas seeds of *Podophyllum* and *Gentiana* were collected from their natural sites during the last weak of August and September, respectively. Thereafter, seeds were cleaned, air-dried, sorted and stored in plastic jars at room temperature (19ºC average temperature). Uniform and healthy seeds of *A. millefolium, G. kurroo and P. hexandrum* were surface sterilized with 0.1 per cent $HgCl_2$ for 2, 2 and 10 minutes respectively. Seeds were thoroughly washed with distilled water and tested for viability and vigour characteristics. Viability was tested by tetrazolium choride (TTC) test (Moore 1985). The best combination of concentration and duration of incubation in tetrazolium chloride was standardized for the above species. For quantification of viability, the seeds of *Achillea*, *Gentiana* and *Podophyllum* were soaked for 48 hrs and slit longitudinally. These were then incubated in 0.25% tetrazolium chloride for 24 hrs in case of *Podophyllum* and 48 hrs in *Achillea* and *Gentiana.* After incubation these seeds were homogenized in 100% methanol and the final volume adjusted to 10 ml. Optical density (OD) was recorded spectrophotometrically at 485 nm. Germination test was performed according to ISTA (1976). Standard practices were followed to calculate emergence index, germination speed and vigour index. The formula used were :

$$\text{Emergence Index (EI)} = \frac{dn}{n},$$

where dn = emergence (number of seeds germinated on a particular day *i.e.* n) and n = day of emergence

$$\text{Germination speed} = \frac{\text{Germination percentage}}{\text{Day of completion of germiantion}}$$

Vigour index = Germination per cent × shoot length

For seedling vigour normal seedlings from standard germination tests were further classified as strong and weak seedlings by visual observations and expressed as percentage. The erect and sturdy seedlings with well developed seedlings parts were kept in the category of strong seedling, whereas seedlings with poorly developed parts under weak category.

Root length, shoot length and fresh and dry weight of seedlings were recorded for *Achillea*, *Gentiana* and *Podophyllum* on 8th, 20th and 210th day after sowing respectively. Electrical conductivity (EC) of the seedlings was measured with conductivity meter and expressed as dSm^{-1}. Fifty normal seeds of known weight were soaked in 250 ml of deionized water for 24 hrs at 25ºC and electrical conductivity measured. The design of experiment was completely randomized with three replications of fresh and aged seeds of each species.

RESULTS AND DISCUSSION

In the present study, seed viability was tested with the help of tetrazolium chloride (TTC) test and the best combination of teterazolium chloride conc. and duration of incubation in TTC was standardized for different species. The studies revealed that 0.25 per cent TTC for 48 hrs was the best combination for *Achillea millefolium* and *Gentiana kurroo* whereas 0.25 per cent TTC for 24 hrs was found the best for *Podophyllum* (Fig. 1).

Ageing of seeds caused loss of viability and germination characteristics in seed of all the species (Table 1). Seeds of *A. millefolium, G. kurroo* and *P. hexandrum* registered a substantial loss of 20, 75 and 25 per cent germination capability, respectively after one year of storage thereby indicating the necessity to investigate the treatments which may help in restoring the germination potential of aged seeds. Seeds germinability of several species have been reported to decline with age during storage (Viana 1990, Aguair-IB-de *et al.* 1996, Kozlowski *et al.* 1997, Formanowiczova *et al.* 1998).

Seedling emergence was significantly delayed by 9 and 32 days, respectively in aged seeds of *Gentiana* and *Podophyllum*. Germination was completed within 9 and 150 days, respectively in fresh seeds of *G. kurroo* and *P. hexandrum* in comparison to 15 and 177 days in aged seeds. Ageing in seeds of *Achillea* and *Gentiana* has not been found to prolong germination period but nevertheless substantially reduced germination percent. Emergence index declined considerably by 5, 90 and 40 per cent respectively in aged seeds of *Achillea*, *Gentiana* and *Podophyllum* (Table 1). Maximum decline (90%) was observed in *Gentiana kurroo* whereas the minimum

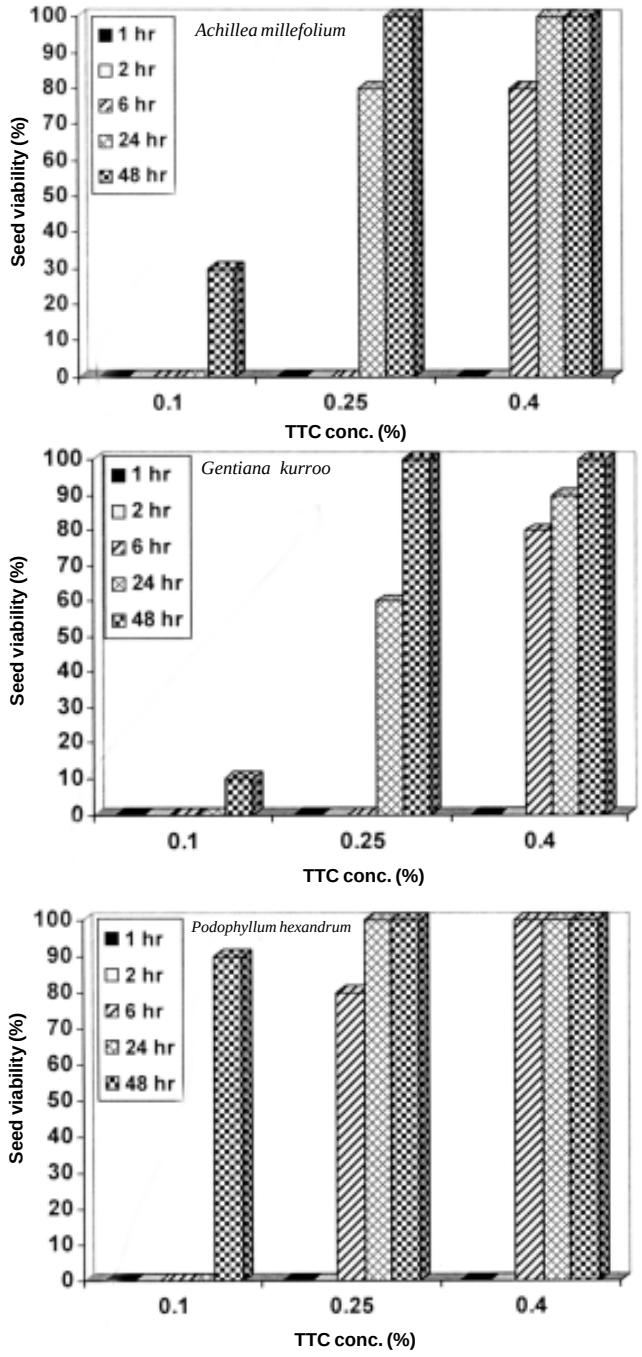

Fig. 1. Seed viability in the three species of endangered medicinal plants (standardised using TTC test)

(5%) in *Achillea millefolium*. Further, germination speed also decreased significantly in aged seeds of *G. Kurroo* and *P. hexandrum*. The germination speed was 9.04 and 1.33 for current year and aged seeds of *Gentiana* and 0.53 and 0.34 for *Podophyllum*, respectively.

One-year-old seeds of *Achillea*, *Gentiana* and *Podophyllum* registered a substantial loss of viability of 20, 45 and 40 per cent, respectively. Seed weight (air dried) and moisture content also declined with increasing age, decline being maximum in *Gentiana kurroo*. Vigorous seeds germinated rapidly thereby indicating the loss of vigour characteristics after one year of storage (Table 1). Seeds undergo several structural, biochemical and genetic alterations with age (Osborne 1982). These include reduced capacity for synthesis of proteins, lipids and RNA, injury to membranes and chromosomes and decreased repair systems (Osborne 1980, Priestley *et al.* 1980, Thakur *et al.* 1997).

Vigour and germination tests conducted to compare the performance of seedlings raised from seeds of 2001 and 2002 indicated the loss of vigour and viability attributes with ageing especially in *Gentiana* and *Podophyllum*. Percentage of strong seedlings was higher when raised from current year (2002) seeds as compared to the aged (2001) seeds. Vigour index was also significantly higher in current year seeds of *Achillea* and *Gentiana*. This reflects better physiological health of fresh seeds in comparison to old seeds.

In the present study the seeds of all the species namely *A. millefolium*, *G. kurroo* and *P. hexandrum* seemed to loose vigour and germination ability upto 1 year. The loss of germination ability and vigour were found to be highest in *Gentiana kurroo* in comparison to *Achillea* and *Podophyllum* under similar storage conditions *i.e.* average temperature of 19ºC and relative humidity 60%. The minimum loss was observed in *Achillea millefolium*. The loss of viability and germination ability during the storage period reflects deterioration of membrane properties. The disruption of membrane due to ageing can lead to diverse metabolic changes which differently contribute to seed deterioration and loss of viability and vigour. The species that can resist changes in membrane is able to maintain viability and quality of seeds (Bewley and Black 1985). Seeds of the medicinal plant species *A. millefolium* in the present study maintained better seed quality upto one year as compared to *Gentiana kurroo* and *Podophyllum hexandrum*. This is an important and desirable tract since these inhabit far flung areas, making collection of seeds every year difficult and expensive. Therefore, there is need to standardize storability

Table 1. Viability and vigour characteristics of fresh and aged seeds of three endangered medicinal plant species.

S. No.	Character	*Achillea millefolium*			*Gentiana kurroo*			*Podophyllum hexandrum*		
		2001	2002	$t_{0.05}$	2001	2002	$t_{0.05}$	2001	2002	$t_{0.05}$
1.	Germination percent	65.00	80.00	4.95	20.00	80.00	12.34	60.00	80.00	2.20
2.	Day of emergence	3.00	3.00	NS	15.0	6.00	6.97	166	1.34	4.04
3.	Day of completion	6.00	6.00	NS	15.0	9.00	3.91	177	150.00	2.56
4.	Emergence index	21.66	26.67	NS	1.33	13.33	6.79	0.36	0.59	21.38
5.	Germination speed	12.50	13.33	NS	1.33	9.04	7.07	0.34	0.53	3.33
6.	Strong seedlings (%)	77.50	87.50	NS	95.00	80.00	5.03	80.00	90.00	NS
7.	Week seedlings (%)	22.50	12.50	3.36	5.00	20.00	5.35	20.00	10.00	3.38
8.	Root length (mm)	5.12	4.45	NS	4.00	2.96	NS	18.00	19.33	NS
9.	Shoot length (mm)	5.82	6.07	NS	3.00	4.60	NS	10.67	10.33	NS
10.	Vigour index	376.0	501.75	4.15	72.00	377.40	20.38	640.2	678.37	NS
11.	Seedling fresh weight (mg)	1.50	1.42	NS	1.00	2.40	3.97	94.5	132.93	2.68
12.	Seedling dry weight (mg)	0.12	0.11	NS	0.10	0.40	7.21	21.05	26.00	NS
13.	Electrical conductivity (dsm^{-1})	0.011	0.010	NS	0.012	0.11	5.37	35.4	0.350	NS
14.	100 seed weight (mg)	16.00	22.00		15.00	26.00		66.66	69.23	
15.	Viability (%)	80.00	100.00		21.87	43.18		16.00	22.00	
16.	Moisture content (%)	21.87	43.18		66.66	69.23		27.06	27.87	

techniques so as to decrease the dependence on collection of fresh seeds each year.

Correlation for some selected vigour and viability characteristics was worked out for the above species (Table 2, 3 and 4). A significant and positive (0.686) correlation was estimated between germination percentage and germination speed in *Achillea* (Table 2). The emergence index and vigour index also exhibited a significant positive correlation for both collection years. Positive correlation between emergence index, standard germination and strong seedlings for different cereal and vegetable crops have also been reported by Delouche and Baskin (1973) and Mian and Coeffey (1971).

In *Achillea* germination percentage and germination speed showed a negative correlation with electrical conductivity (EC). EC indicates the amount of electrolytes, which leach out from seeds as they deteriorate. Ageing induced damage to the integrity of the cell membrane results in increased leaching of electrolytes. The greater leakage of solutes from old than young seeds implies that the integrity of plasmalemma and tonoplast is lost during ageing (Priestley 1986). Several other physiological and biochemical reactions leading to deterioration of seeds have been reported in *Acer plantanoides* and bell pepper (Pukacka and Kuiper 1988, Thakur *et al.* 1997).

In *G. kurroo* standard germination exhibited a positive and significant correlation with viability, percentage of strong seedlings and seed weight (Table 3). However, a significant but negative correlation was established with EC indicating the loss of membrane integrity in aged seeds. Seed moisture content was related positively to germination speed (Table 3). This could be helpful for predicting the better establishment of seedlings in the field. In *G. kurroo* correlation between seed viability and moisture content was positive although non-significant for fresh as well as aged seeds. Seedlings raised from vigourous seeds acquired more dry weight indicating probably

Table 2. Correlation coefficients (r) for viablility and vigour characteristics of fresh and aged seeds of *Achillea millefolium.*

S. No.	Character	2	3	4	5	6	7	8	9	10	11
1.	Germination percent	0.331	0.686**	0.322	0.284	0.201	-0.219	-0.335	-0.483*	0.258	-0.441
		-0.604	0.182	-0.133	0.524	-0.123	-0.507*	-0.394	0.107	0.44*	0.474*
2.	Emergence index		0.339	0.244	0.365	0.903*	0.532*	-0.453	-0.416	0.051	0.154
			-0.144	0.416	0.336	0.318	0.884**	0.577	0.251	0.041	-0.593
3.	Germination speed			0.358	0.551*	0.241	-0.158	-0.138	0.684**	0.129	-0.408
				0.347	0.723**	0.184	-0.36	-0.213	0.480*	0.138	0.090
4.	Strong seedlings (%)				0.238	-0.176	-0.467	-0.189	-0.45	0.168	-0.561*
					0.187	0.386	0.294	0.210	-0.078	0.180	-0.487*
5.	Week seedlings (%)					0.398	-0.260**	0.723	0.436	-0.413	0.075
						-0.334	-0.391	-0.175	0.026*	-0.591*	0.291
6.	Vigour index						0.671	-0.594	-0.376	0.251	-0.018
							0.278	-0.263	0.448	0.241	-0.020
7.	Seedling dry weight (mg)							-0.035	0.019	0.529*	-0.077
								-0.482*	0.081	0.520*	-0.582*
8.	Electrical conductivity (dsm^{-1})								0.214	0.083	0.049
									-0.229	0.061	-0.231
9.	100 seed weight (mg)									-0.266	0.483
										0.480	0.297
10.	Viability (%)										0.820**
											0.700*
11.	Moisture content (%)										1.00
											1.00

Upper value is for the aged and lower for the fresh seeds.

Table 3. Correlation coefficients (r) for viablility and vigour characteristics of fresh and aged seeds of *Gentiana kurroo.*

S. No.	Character	2	3	4	5	6	7	8	9	10	11
1.	Germination percent	0.048	0.442	0.464	-0.156	-0.332	0.296	-0.711	-0.732	0.611	0.097
		0.042	-0.174	0.058	0.066	-0.104	0.171	0.124	0.700	0.620	0.071
2.	Emergence index		0.231	0.511*	0.418	-0.206	0.011	-0.583*	0.128	0.041	0.040
			0.260	0.057	0.636	-0.732	0.204	-0.352	-0.207	0.011	0.038
3.	Germination speed			0.160	-0.256	-0.338	-0.427	-0.172	-0.256	0.259	0.819**
				0.174	0.121	-0.044	0.533*	0.478*	0.172	0.216	-0.626*
4.	Strong seedlings (%)				0.274	-0.673*	0.205	-0.799	0.013	0.116	-0.285
					0.054	-0.032	-0.320	0.374	0.427	0.118	-0.545*
5.	Week seedlings (%)					0.399	-0.455*	-0.235	-0.228	0.232	0.123
						-0.127	0.313	-0.207	0.072	0.212	-0.268
6.	Vigour index						-0.466*	0.351	0.284	0.062	-0.263
							-0.436*	0.105	0.184	0.061	0.015

S. No.	Character	2	3	4	5	6	7	8	9	10	11
7.	Seedling dry weight (mg)							-0.148	0.100	0.065	-0.632*
								-0.314	0.279	0.062	-0.157
8.	Electrical conductivity (dsm^{-1})								0.447	0.394	-0.215
									-0.250	-0.390	0.650**
9.	100 seed weight (mg)									0.795	-0.024
										0.695	0.282
10.	Viability (%)										0.190
											0.202
11.	Moisture content (%)										1.00
											1.00

Upper value is for the aged and lower for the fresh seeds.

Table 4. Correlation coefficients (r) for viablility and vigour characteristics of fresh and aged seeds of *Podophyllum hexandrum.*

S. No.	Character	2	3	4	5	6	7	8	9	10	11
1.	Germination percent	0.277	-0.689**	-0.131	-0.498	0.080	0.160	0.396	0.621*	0.247	0.230
		0.497	0.561	0.125	-0.077	-0.034	0.159	0.221	0.286	0.615*	-0.288
2.	Emergence index		0.025	0.066	0.449	0.734**	0.213	0.810**	0.400	-0.023	-0.083
			0.486*	0.568*	-0.154	0.155	0.155	-0.183	0.021	-0.028	-0.619
3.	Germination speed			-0.145	0.414	-0.096	0.239	-0.331	-0.045	-0.036	-0.456
				0.612	-0.363	0.047	0.799**	0.702	0.747	-0.260	-0.180
4.	Strong seedlings (%)				0.601*	0.516*	-0.385	0.296	0.205	0.170	0.117
					-0.074	-0.180	0.657*	0.172	0.358	0.020	0.006
5.	Week seedlings (%)					0.627*	0.178	0.418	0.196	0.080	-0.285
						-0.297	0.358	0.416	0.377	0.420	0.006
6.	Vigour index						0.224	0.698*	0.185	0.334	-0.101
							0.035	0.229	0.288	0.420	-0.603
7.	Seedling dry weight (mg)							0.051	-0.088	0.066	0.308
								0.645*	0.700	0.061*	-0.126
8.	Electrical conductivity (dsm^{-1})								0.629*	0.054	-0.690
									0.729*	-0.040	0.480
9.	100 seed weight (mg)									-0.233	0.283
										0.330	0.120
10.	Viability (%)										0.457*
											0.520
11.	Moisture content (%)										1.00
											1.00

Upper value is for the aged and lower for the fresh seeds.

judicious utilization of available resources. Further, emergence index was positively correlated with strong seedlings. Thus, vigorous seeds exhibit higher emergence index and give rise to strong seedlings.

Germination percentage was positively correlated with emergence index for young as well as aged seeds of *Podophyllum* (Table 4). Germination speed exhibited a negative correlation (r = -0.689**) with germination percentage for aged seeds, however, a positive relationship for fresh seeds was registered. The emergence index was strongly correlated (r = 0.734**) with vigour index indicating that seeds with higher vigour index could predict the better establishment of seedlings in the field (Dahiya *et al.* 1997). Further, fresh seeds exhibited faster speed of germination. Germination percentage, viability and 100 seed weight showed a significant positive correlation for fresh as well as aged seeds of *Podophyllum*. The presence of more dry matter or resources and storage material in seeds is associated with more germination and viability. A positive correlation (0.799**) was observed between germination speed and seedling dry weight for fresh seeds. The seed viability and moisture content had significant positive correlation indicating recalcitrant type behaviour of *Podophyllum* seeds.

The results indicated a substantial loss of germination, viability and vigour characteristics in one year old seeds of these important endangered medicinal plant species of Western Himalayas. Decline in germinability of seeds of few medicinal species has been reported by Kozlowski *et al.* (1997). Disruption of membranes, loss of respiratory substrate and other deteriorating processes associated with ageing could lead to diverse metabolic changes resulting in seed deterioration, loss of germinability, viability and vigour. Therefore, the present investigation will go a along way in evolving the appropriate storage methods which may help in restoring the germination potential and vigour of these medicinally important endangered species. Further, it will also pave the way for ensured availability of seeds for next year if setting of seeds or its availability due to one or the other factor is poor in the next year. Nevertheless this will reduce the dependence on fresh collection of seeds from difficult and inaccessible areas.

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