Medicinal Plants
Vol. 11 (4), December 2019, 464-474

doi : 10.5958/0975-6892.2019.00061.3

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Research Article

Seed germination and storage studies in *Decalepis hamiltonii* Wight and Arn.: A threatened medicinal plant

B.L. Navya[1], G.R. Smitha[2]*, H.S. Yogeesha[2], P.E. Rajasekharan[2], Vishnuvardhan[1] and H.M. Pallavi[3]

[1]*College of Horticulture, UHS Campus, GKVK Post, Bengaluru-560 065, India*
[2]*ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru-560089, India*
[3]*College of Horticulture, Yalachahalli Horticulture Farm, SH 117, Yelawala, Mysore-571130, India*

Received: November 18, 2019; Accepted: December 19, 2019

ABSTRACT

Decalepis hamitonii Wight and Arn. is an important RET medicinal climber which is over exploited from its natural habitat for its high valued roots. The roots are extensively used in folk and *Ayurvedic* medicines mainly as blood purifier and hepatoprotective compound due to the presence of 2-hydroxyl-4-methoxy benzaldehyde (HMB). The work on propagation and storage of seeds in this plant species is meagre for extending the cultivation and conservation. Therefore, the present study was taken up. The two factorial experiment was conducted on the effect of media (top paper, between paper and cocopeat) and temperature (20/ 30°C, 20°C, 30°C) on seed germination. Results revealed that the seeds placed at 20/ 30°C, tested on between paper method has recorded maximum germination percentage (96.00%), root length (6.48 cm) and seedling vigour index (955.77). Whereas, shoot length (10.40 cm), seedling length (16.14 cm), fresh (91.16 mg) and dry weight (8.76 mg) of seedling was maximum in the seeds placed at 30°C temperature sown in cocopeat. No germination was recorded when the seeds were placed at 20°C temperature sown in cocopeat. In an another experiment comprising of three factorial storage study on effect of moisture content (8% and 6%), packaging material (aluminium pouch, polythene cover and butter paper cover) and storage temperature (ambient temperature and 15°C) revealed that the seeds having 8% moisture packed in polythene cover and stored at 15°C recorded maximum shoot length, seedling length and seedling vigour index I of 7.57 cm, 14.70 cm and 1373, respectively. Shoot was not grown and minimum seedling length and seedling vigour index I of 3.60 cm and 172, respectively was recorded in the seeds having 6 per cent moisture, packed in butter paper bag and stored at 15°C up to 90 days of storage. Therefore, seeds kept for germination on between paper and placed at 20/30°C recorded germination. Whereas, seeds having 8% moisture, stored at 15°C packed with polythene and aluminium pouch was found better for maintaining viability and vigour up to 90 days of storage.

Key words: *Decalepis hamiltonii*, seed germination, storage, media, temperature, moisture content

INTRODUCTION

Decalepis hamiltonii Wight & Arn. is an important threatened climbing medicinal herb of the Periplocaceae family. It is locally called as "*Nannarikommulu*" or "*Barresugnadhi*" in Telugu, "*Makaliber*" in Kannada, "*Magalikizhangu*" in Tamil and "Swallow root" in English (Shahzad and Sharma, 2014). Roots of *D. hamiltonii* contain profuse quantities of 2-hydroxyl-4-methoxy benzaldehyde (37.45%), 2-hydoxybenzaldehyde (31.01%), 4-O-methylresorcy-

*Corresponding author e-mail: G.Smitha@icar.gov.in, smithagingade@gmail.com

laldehyde (9.12%) and benzyl alcohol (3.16%) which are used as hepatoprotective, antibacterial and anti-inflammatory compound (Nagarajan *et al*., 2001). Roots are used in *Ayurvedic* and folk medicines for bronchial asthama, blood purification, wound healing, fever, intrinsic haemorrhage etc. (Vijayakumar and Pullaiah, 1998). The roots can also be used for flavouring ice creams and chocolates due to the presence of vanillin (0.45%) (Shahzad and Sharma, 2014).

Decalepis hamiltonii Wight & Arn. has been listed in the IUCN red list of threatened species (Ved *et al*., 2015). It is one among the 178 plant species which are consumed in high volumes exceeding 100 MT per year. It has been included under the list of 242 plant species collected in wild/ cultivated/ imported for main use as herbal raw drugs with estimated annual trade of 100-200 MT (Ved and Goraya, 2017).

Though the plants of *D. hamiltonii* are relatively pervasive, its populations are patchy and gradually declining due to destructive unscientific harvesting of the tuberous roots (Giridhar *et al*., 2005). Hence, conservation of this threatened species is crucial. One of the ways in conservation of this species is the standardization of propagation techniques and storage protocols so as to make them available for commercial cultivation and for *ex-situ* conservation. But very meagre efforts have been made in this regard. Seed germination is a complex process where individual factors and reactions are involved. Germination takes place over a definite range of temperature which varies from species to species and also use of definite media allows good results (Gairola *et al*., 2011). Knowledge on storage behaviour of any given species is prerequisite for successful *ex-situ* conservation (Sivakumar *et al*., 2006). Storage of seeds at an optimum storage temperature, seed moisture content and use of suitable packaging material allows longer storability besides maintains the seed viability and vigour during storage.

Therefore, the present study aims at standardization of media and temperature for seed germination and effect of seed moisture content, storage temperature and packaging material on storage of *Decalepis hamiltonii* Wight and Arn. besides conservation. Large scale multiplication of this species will help in cultivation and supply of raw material to pharmaceutical industries to fulfil their growing demand.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at ICAR- Indian Institute of Horticultural Research, Bengaluru. It is situated in South-East tract of Karnataka state at 12°58 North latitude and 77°34 East longitude and at an altitude of 900 m above mean sea level.

Seed collection

The fresh follicles of *D. hamiltonii* were harvested at full maturity just before the dehiscence from the Field Gene Bank of Division of Plant Genetic Resources, ICAR- Indian Institute of Horticultural Research, Bengaluru during March, 2019. The seeds were extracted from follicles.

Experimental description

The seeds were preliminarily treated with 0.5% Sodium hypochlorite to prevent fungal infections. For germination studies, the experiment was laid out in two factorial CRD with three replications having 25 seeds each. The seeds were tested on three different types of media *i.e.* top paper method (germination paper placed on petriplates), between paper method (germination paper) and cocopeat, which were placed at three different temperature regimes *i.e.* 20/30°C at germination chamber, 20°C and 30°C at incubator. For storage studies, the experiment was laid out in three factorial CRD with three replications having 25 seeds each. Seeds having 8 per cent and 6 per cent moisture were packed in three types of packaging material *i.e.* aluminium pouch, polythene bags and butter paper bags and were stored at ambient and 15°C temperature up to 90 days.

Germination test

In germination study, count was taken at 15 days after sowing in top and between paper method and 30 days after sowing in cocopeat and the mean count was expressed in percentage. Whereas in storage study, germination test was conducted at 30 days intervals up to 90 days of storage. The seeds were tested on between paper method, first and final count were taken at 8 and 15 days after sowing, respectively. The mean germination count taken was expressed in percentage.

Growth parameters

The growth parameters such as shoot and root length was taken at final count. Shoot length was measured from the

collar region to tip of the shoot and root length was measured from the collar region to the tip of the longest root. Seedling length was taken by adding shoot and root length. Mean shoot, root and seedling length was expressed in centimetre.

Seedling vigour index

The seedling vigour was calculated based on the following formula given by Bewley and Black (1982) as shown below.

Seedling vigour index I = Germination percentage × [Shoot length (cm) + Root length (cm)]

Seedling vigour index II = Germination percentage × Dry weight of seedling

Biomass parameters

The fresh weight of seedling was recorded. The seedlings were packed in butter paper bag and dried in hot air oven at 60°C for 24 hours. The mean fresh and dry weight of seedling was expressed in milligrams.

Moisture content

The initial moisture content of the seeds was recorded and moisture regimes of the seeds were reduced to 6 and 8 per cent by packing the seeds in butter paper bag and placing them in zeolite granules. The moisture content was measured at 30 days interval up to 90 days using moisture meter.

Statistical analysis

The experimental data were analysed using Factorial Completely Randomised Design (FCRD) with three replications. The statistical software, WASP (Web based Agricultural Statistical Software) was used for statistical analysis of data and the results were interpreted at 1% probability level.

RESULTS AND DISCUSSION

Germination study

Seed germination is influenced by media and temperature as revealed in the present study. Seeds placed at temperature of 20/30°C tested on between paper (A_1B_2) (Table 1) has recorded maximum germination percentage (96.00 %), root length (6.48 cm) and seedling vigour index (955.77) which are *on par* with seeds placed at 20/30°C tested on top paper method (A_1B_1) for germination percentage (94.66 %) (Figure 1). Seed germination and growth are improved when the seeds are provided with fluctuating day/night temperatures (Hartman and Kester, 2013). Roberts (1988) reported that alternate temperature shows a positive linear relation between the base temperature (at and below which the rate is zero) and the optimum temperature (at which the rate is maximal). The results can be correlated with the findings of Emanoela *et al*. (2016) in *Salvia hispanica*. Seeds are placed intact in between paper method which also provides sufficient moisture and oxygen for the developing seeds and is proved best for medium sized seeds (Nawabahar, 2008). These results support the findings of Venudevan *et al*. (2013) in *Aegle marmelos*. In top paper method as seeds are enclosed in petri plates, it maintains humidity and provides the seeds with congenial environment and sufficient moisture to germinate. Similar results were obtained by Nawabahar, 2008 in *Cupaniopsis anacardioides*.

The parameters such as shoot length (10.40 cm), seedling length (16.14 cm), fresh (91.16 mg) and dry weight (8.76 mg) was maximum in the seeds placed at 30°C temperature sown in cocopeat (A_3B_3) (Table 1) (Figure 1). The optimum temperature for germination in tropical plants is between 15°C to 30°C (Larcher, 1975). As *Decalepis hamiltonii* is a tropical plant species, germination is more at higher temperatures of 30°C which activates the embryo resulting in faster growth of the seedling which in turn increases the biomass. The results are in line with the findings of Ashish *et al*. (2015) in *Artemisia pallens* and *Sabja Ocimum pallens*. The cocopeat provides sufficient moisture, holds the seeds intact and exposes the shoot to the surrounding which paves way for increasing shoot length in turn increasing the biomass. Similar results were obtained by Pramod and Minal (2017) in sarpagandha.

No germination was noticed when the seeds were placed at 20°C tested on cocopeat (A_2B_3). The process of thermos inhibition exhibits lower germination in some seed species where seeds fail to germinate at higher or lower temperatures (Hartman and Kester, 2013). Results are in close proximity with the studies conducted by Punit *et al*. (2013) in *Ashwagandha* (*Withania somnifera*). Seeds take more time to emerge from the cocopeat even though the final count day was extended by a week. The presence of excess moisture in cocopeat might have led to the development of mould resulting in lower germination percentage and vigour index.

Storage study

Seed moisture content, storage temperature and packaging material are among the factors which influence seed viability

Table 1: Effect of media and temperature on germination percentage, shoot length, root length, seedling length, seedling vigour index, fresh and dry weight of *Decalepis hamiltonii*

Treatments	Germination percentage	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Seedling vigour index I	Fresh weight of seedling (mg)	Dry weight of seedling (mg)
Temperature (A)							
A_1	92.88(76.69)	3.00(1.86)	4.59(2.23)	7.59(2.83)	708(26)	54.01(7.36)	6.14(2.57)
A_2	43.11(35.95)	0.08(0.76)	0.71(1.05)	0.80(1.08)	57(5)	6.81(2.36)	0.83(1.10)
A_3	39.11(37.70)	8.56(2.99)	5.13(2.35)	13.70(3.75)	499(21)	66.43(8.02)	6.61(2.63)
S.Em±	1.973(1.970)	0.154(0.027)	0.110(0.023)	0.233(0.033)	27.679(0.600)	1.802(0.112)	0.189(0.038)
CD @ 1%	8.149(8.138)	0.639(0.115)	0.456(0.097)	0.962(0.137)	114.322(2.480)	7.443(0.462)	0.781(0.156)
Media (B)							
B_1	70.22(59.41)	3.48(1.76)	2.57(1.67)	6.06(2.33)	442(18)	27.95(5.01)	3.28(1.86)
B_2	71.55(60.63)	3.52(1.85)	4.82(2.24)	8.34(2.83)	556(22)	49.87(6.78)	5.26(2.32)
B_3	33.33(30.30)	4.65(2.00)	3.03(1.72)	7.68(2.50)	267(13)	49.42(5.95)	5.03(2.12)
S.Em±	1.973(1.970)	0.154(0.027)	0.110(0.023)	0.233(0.033)	27.679(0.600)	1.802(0.112)	0.189(0.038)
CD @ 1%	8.149(8.138)	0.639(0.115)	0.456(0.097)	0.962(0.137)	114.322(2.480)	7.443(0.462)	0.781(0.156)
Temperature × Media (A×B)							
A_1B_1	94.66(78.95)	1.98(1.57)	3.94(2.10)	5.92(2.53)	561(23)	43.63(6.63)	5.00(2.34)
A_1B_2	96.00(80.58)	3.47(1.99)	6.48(2.64)	9.95(3.23)	955(30)	61.30(7.86)	7.10(2.75)
A_1B_3	88.00(70.54)	3.54(2.01)	3.36(1.96)	6.91(2.72)	608(24)	57.10(7.58)	6.33(2.61)
A_2B_1	53.33(46.92)	0.00(0.70)	0.43(0.96)	0.43(0.96)	23(4)	5.60(2.46)	0.66(1.07)
A_2B_2	76.00(60.66)	0.26(0.87)	1.70(1.48)	1.96(1.57)	149(12)	14.83(3.90)	1.83(1.52)
A_2B_3	0.00(0.28)	0.00(0.70)	0.00(0.70)	0.00(0.70)	0(0.70)	0.00(0.70)	0.00(0.70)
A_3B_1	62.66(52.36)	8.47(2.99)	3.35(1.96)	11.82(3.51)	741(27)	34.63(5.92)	4.20(2.16)
A_3B_2	42.66(40.64)	6.82(2.70)	6.30(2.60)	13.12(3.68)	562(23)	73.50(8.57)	6.86(2.70)
A_3B_3	12.00(20.09)	10.40(3.30)	5.74(2.49)	16.14(4.08)	193(13)	91.16(9.56)	8.76(3.04)
S.Em±	3.417(3.412)	0.268(0.048)	0.191(0.040)	0.403(0.057)	47.941(1.040)	3.121(0.194)	0.327(0.065)
CD @ 1%	14.115(14.095)	1.108(0.199)	0.790(0.168)	1.667(0.238)	198.0126(4.29)	12.891(0.801)	1.353(0.271)
CV %	10.14(11.79)	11.96(4.54)	9.53(3.61)	9.49(3.85)	19.68(10.03)	12.74(5.68)	12.53(5.41)

A-Temperature, $\mathbf{A_1}$- 20/30 C, $\mathbf{A_2}$- 20°C, $\mathbf{A_3}$- 30°C, **B**- Media, $\mathbf{B_1}$- Top paper, $\mathbf{B_2}$- Between paper, $\mathbf{B_3}$- Cocopeat (Values in the parenthesis are square root transformed values and percentage data is arc sin transformed)

during storage and appropriate use of these factors results in maintenance of seed viability in storage. Results of the parameters taken before storage revealed initial moisture content (14.31%), first count (80%), germination percentage (96%), shoot length (8.32 cm), root length (6.59 cm), seedling length (14.91 cm), seedling vigour index I (1431), dry weight (7.56 mg) and seedling vigour index II (725). The above results help in comparing the seed viability, germination and growth after storage period.

The germination, growth, vigour index and biomass of the seeds had decreased with the increase in storage duration up to 90 days. The effect of moisture content, storage temperature and packaging material revealed non-significant effect on first count, germination percentage, root length, dry weight and seedling vigour index II in the seeds stored up to 90 days (Table 2 and 3). The shoot length and seedling length was non-significant in seeds stored for first 30 days and 60 days of storage and was significant in seeds stored for 90 days. Maximum shoot length of 7.57 cm was recorded in the seeds having 8% moisture packed with polythene stored at 15 C temperature ($M_1P_2T_2$) which was *on par* with $M_1P_1T_1$ (7.00 cm), $M_1P_1T_2$ (7.03 cm), $M_1P_2T_1$ (7.37 cm), $M_2P_1T_1$ (6.67 cm), $M_2P_1T_2$ (5.77 cm), $M_2P_2T_1$ (6.97 cm), $M_2P_2T_2$ (6.90 cm), $M_2P_3T_1$ (6.27 cm). Maximum seedling

Figure 1: *Decalepis hamiltonii* plant and seedlings

a. Plant; **b.** Mature follicle; **c.** Seeds; **d. e.** and **f.** Seed germination as influenced by different media and temperature ($\mathbf{A_1}$- 20/ 30°C, $\mathbf{A_2}$- 20°C, $\mathbf{A_3}$- 30°C, **B**- Media, $\mathbf{B_1}$- Top paper, $\mathbf{B_2}$- Between paper, $\mathbf{B_3}$- Cocopeat)

Table 2: Effect of seed moisture content, packaging material and storage temperature on first count, germination percentage, shoot and root length of *Decalepis hamiltonii* 90 days after storage

Factors	First count (%)			Germination (%)			Shoot length (cm)			Root length (cm)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Moisture content (M)												
M_1	80.67	72.00	64.45	95.78	88.22	82.89	8.31	8.22	6.56	5.86	6.37	6.26
M_2	74.22	68.00	50.00	84.89	83.11	63.33	7.03	6.88	5.43	5.78	5.52	5.84
S.Em±	0.963	0.868	1.260	0.967	1.076	1.437	0.226	0.168	0.214	0.186	0.134	0.150
CD @ 1%	3.839	3.463	5.023	3.858	4.291	5.730	0.900	0.669	0.855	NS	0.532	0.600
Packaging material (P)												
P_1	75.33	76.66	68.00	89.00	94.67	82.33	7.97	7.73	6.62	6.36	6.08	6.71
P_2	79.00	72.33	69.33	90.33	89.33	84.33	7.74	7.48	7.20	5.97	6.17	7.05
P_3	78.00	61.00	34.33	91.67	73.00	52.67	7.29	7.45	4.16	5.13	5.58	4.38
S.Em±	1.179	1.064	1.543	1.185	1.318	1.760	0.276	0.206	0.263	0.228	0.164	0.184
CD @ 1%	NS	4.241	6.152	NS	5.256	7.018	NS	NS	1.047	0.911	NS	0.734
Storage temperature (T)												
T_1	76.88	68.22	50.44	91.33	84.44	65.78	7.66	7.49	6.54	5.50	5.89	5.89
T_2	78.00	71.77	64.00	89.33	86.89	80.44	7.68	7.61	5.44	6.13	5.99	6.20
S.Em±	0.963	0.86	1.260	0.967	1.076	1.437	0.226	0.168	0.214	0.186	0.134	0.150
CD @ 1%	NS	3.463	5.023	NS	NS	5.730	NS	NS	0.855	NS	NS	NS
Moisture content × Packaging material (M×P)												
M_1P_1	80.00	79.33	72.00	97.33	98.00	89.33	8.68	8.77	7.02	6.72	6.23	6.70
M_1P_2	82.00	72.00	73.33	96.00	91.33	90.67	8.02	7.93	7.47	5.73	6.62	7.18
M_1P_3	80.00	64.66	48.00	94.00	75.33	68.67	8.22	7.97	5.18	5.12	6.25	4.88
M_2P_1	70.66	74.00	64.00	80.67	91.33	75.33	7.25	6.68	6.22	6.00	5.93	6.72
M_2P_2	76.00	72.66	65.33	84.67	87.33	78.00	7.47	7.02	6.93	6.20	5.72	6.92
M_2P_3	76.00	57.33	20.66	89.33	70.67	36.67	6.37	6.93	3.13	5.13	4.92	3.88
S.Em±	1.668	1.504	2.182	1.676	1.864	2.489	0.391	0.291	0.371	0.323	0.231	0.260
CD @ 1%	NS	NS	8.701	6.682	NS	9.925	NS	NS	NS	NS	NS	NS
Moisture content × Storage temperature (M×T)												
M_1T_1	79.11	70.22	56.44	95.11	86.67	74.22	8.21	8.26	6.46	5.51	6.33	6.12
M_1T_2	82.22	73.77	72.44	96.44	89.78	91.56	8.40	8.19	6.66	6.20	6.40	6.39
M_2T_1	74.66	66.22	44.44	87.56	82.22	57.33	7.10	6.73	6.63	5.49	5.46	5.67
M_2T_2	73.77	69.77	55.55	82.22	84.00	69.33	6.96	7.02	4.22	6.07	5.59	6.01
SEm±	1.362	1.228	1.782	1.368	1.522	2.032	0.319	0.237	0.303	0.264	0.189	0.213
CD @ 1%	NS	NS	NS	NS	NS	NS	NS	NS	1.209	NS	NS	NS
Packaging material × storage temperature (P×T)												
P_1T_1	74.66	77.33	64.66	89.33	96.67	79.33	8.03	7.73	6.83	6.00	6.05	6.33
P_1T_2	76.00	76.00	71.33	88.67	92.67	85.33	7.90	7.72	6.40	6.72	6.12	7.08
P_2T_1	80.00	73.33	67.33	92.67	90.67	80.67	7.75	7.47	7.17	5.37	6.28	6.95
P_2T_2	78.00	71.33	71.33	88.00	88.00	88.00	7.73	7.48	7.23	6.57	6.05	7.15
P_3T_1	76.00	54.00	19.33	92.00	66.00	37.33	7.18	7.28	5.63	5.13	5.35	4.40
P_3T_2	80.00	68.00	49.33	91.33	80.00	68.00	7.40	7.62	2.68	5.12	5.82	4.37
S.Em±	1.668	1.504	2.182	1.676	1.864	2.489	0.391	0.291	0.371	0.323	0.231	0.260
CD @ 1%	NS	5.998	8.701	NS	7.433	9.925	NS	NS	1.481	NS	NS	NS

Table 2 contd.....

Factors	First count (%)			Germination (%)			Shoot length (cm)			Root length (cm)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Moisture content × Packaging material × storage temperature (M×P×T)												
$M_1P_1T_1$	77.33	77.33	69.33	94.67	98.67	85.33	8.57	8.83	7.00	6.27	6.30	6.50
$M_1P_1T_2$	82.66	81.33	74.66	100.00	97.33	93.33	8.80	8.70	7.03	7.17	6.17	6.90
$M_1P_2T_1$	84.00	72.00	72.00	98.67	90.67	88.00	8.03	7.63	7.37	5.40	6.67	7.23
$M_1P_2T_2$	80.00	72.00	74.66	93.33	92.00	93.33	8.00	8.23	7.57	6.07	6.57	7.13
$M_1P_3T_1$	76.00	61.33	28.00	92.00	70.67	49.33	8.03	8.30	5.00	4.87	6.03	4.63
$M_1P_3T_2$	84.00	68.00	68.00	96.00	80.00	88.00	8.40	7.63	5.37	5.37	6.47	5.13
$M_2P_1T_1$	72.00	77.33	60.00	84.00	94.67	73.33	7.50	6.63	6.67	5.73	5.80	6.17
$M_2P_1T_2$	69.33	70.66	68.00	77.33	88.00	77.33	7.00	6.73	5.77	6.27	6.07	7.27
$M_2P_2T_1$	76.00	74.66	62.66	86.67	90.67	73.33	7.47	7.30	6.97	5.33	5.90	6.67
$M_2P_2T_2$	76.00	70.66	68.00	82.67	84.00	82.67	7.47	6.73	6.90	7.07	5.53	7.17
$M_2P_3T_1$	76.00	46.66	10.66	92.00	61.33	25.33	6.33	6.27	6.27	5.40	4.67	4.17
$M_2P_3T_2$	76.00	68.00	30.66	86.67	80.00	48.00	6.40	7.60	0.00	4.87	5.17	3.60
S.Em±	2.358	2.127	3.086	2.370	2.636	3.520	0.553	0.411	0.525	0.457	0.327	0.368
CD @ 1%	NS	8.482	NS	NS	NS	NS	NS	NS	2.095	NS	NS	NS
CV%	5.275	5.264	9.340	4.544	5.330	8.339	12.484	9.430	15.191	13.597	9.534	10.547

M-Moisture content, $\mathbf{M_1}$- 8 % moisture, $\mathbf{M_2}$- 6 % moisture, **P**- Packaging material, $\mathbf{P_1}$- Aluminium pouch, $\mathbf{P_2}$- Polythene cover, $\mathbf{P_3}$- Butter paper cover, **T**- Storage temperature, $\mathbf{T_1}$- Ambient temperature, $\mathbf{T_2}$- 15°C temperature, **DAS**- Days after storage

length (14.70 cm) was recorded in seeds having 8 per cent moisture packed with polythene stored at 15°C temperature ($M_1P_2T_2$) which was *on par* with $M_1P_1T_1$ (13.50 cm), $M_1P_1T_2$ (13.93 cm), 14.60 cm ($M_1P_2T_1$), $M_2P_1T_1$ (12.83 cm), $M_2P_1T_2$ (13.03 cm), $M_2P_2T_1$ (13.63 cm), $M_2P_2T_2$ (14.07 cm). Seedling vigour index I was non-significant in the seeds stored for 30 days (Table 5). Whereas, seeds stored for 60 days and 90 days has recorded significant differences. Maximum seedling vigour index I of 1494 was recorded in the seeds having 8 per cent moisture packed with aluminium pouch stored at ambient temperature ($M_1P_1T_1$) for 60 days which was *on par* with $M_1P_1T_2$ (1446), $M_1P_2T_1$ (1296), $M_1P_2T_2$ (1361). The same trend was followed in the seeds stored for 90 days where the seedling vigour index decreased with the increase in storage duration.

Seed moisture content is most important factor in maintaining viability during storage, it is primary for control of all the activities. Metabolic activities can be reduced by keeping the seeds in dry state (Bonner, 2008). The seeds of *Decalepis hamiltonii* Wight and Arn. are orthodox as the higher germination percentage and growth were obtained at moisture content of 8%. However, 6% moisture content was very less to maintain the metabolic activity of the seeds hence, germination percentage was less. The results are similar to findings of Pu *et al*. (2015) in *Sophora tonkinensis*. The superior values were obtained in the seeds packed with aluminium pouch and polythene cover stored for 90 days. Aluminium pouch and polythene cover acts as the vapour proof barriers in maintaining moisture content of seeds (Figure 2). Moisture proof or moisture resistant package is more valuable in sustaining vigour and germination frequency (Harrington, 1973). This is probably due to retaining of proper moisture content and exchange of gases in the seeds which also helps in maintaining proper level of sugar and starch which are required for good germination of seeds. Similar results were obtained by (Narender *et al*., 2009). Storage of seeds at ambient temperature probably favoured the increase of those enzymes, which positively affected germination. Results agree with the findings obtained by Dadjo *et al*. (2019) in *Garcinia kola*. Whereas, in the seeds stored at 15°C due the maintenance of moisture content, temperature and humidity would have contributed to the higher germination (Figure 2). Low temperature also prevents the deterioration of seeds due to the reduced metabolic activity. The results were similar to that obtained by Genes and Nymora (2018) in *Escoecaria bussei*.

Table 3: Effect of seed moisture content, packaging material and storage temperature on seedling length and vigour index I of *Decalepis hamiltonii* at 90 days after storage

Factors	Seedling length (cm)			Seedling vigour index I			Dry weight (mg)			Seedling vigour index II		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Moisture content (M)												
M_1	14.16	14.59	12.81	1357	1290	1086	6.76	6.47	4.67	647	572	396
M_2	12.81	12.40	11.27	1084.98	1036	754	5.69	4.93	4.09	482	413	265
SEm±	0.244	0.223	0.258	24.693	21.142	26.438	0.199	0.157	0.256	18.136	16.023	20.283
CD @ 1%	0.971	0.890	1.030	98.462	84.302	105.419	0.794	0.628	NS	72.317	63.892	80.876
Packaging material (P)												
P_1	14.33	13.81	13.33	1285	1310	1100	6.44	5.83	4.85	579	555	403
P_2	13.71	13.64	14.25	1236	1220	1205	6.30	5.60	5.00	568	503	421
P_3	12.42	13.03	8.54	1142	958	455	5.94	5.67	3.29	547.36	420	167
SEm±	0.298	0.273	0.316	30.242	25.893	32.379	0.244	0.193	0.314	22.212	19.624	24.841
CD @ 1%	1.190	NS	1.262	120.590	103.248	129.112	0.973	NS	1.250	NS	78.251	99.053
Storage temperature (T)												
T_1	13.16	13.39	12.44	1203	1141	851	6.14	5.53	4.63	561	473	311
T_2	13.81	13.60	11.64	1239	1185	989	6.31	5.87	4.13	568	512	350
SEm±	0.244	0.223	0.258	24.693	21.142	26.438	0.199	0.157	0.256	18.136	16.023	20.283
CD @ 1%	NS	NS	NS	NS	NS	105.419	NS	NS	NS	NS	NS	NS
Moisture content × Packaging material (M×P)												
M_1P_1	15.40	15.00	13.72	1501	1470	1226	7.00	6.75	5.35	682	661	481
M_1P_2	13.75	14.55	14.65	1317	1328	1333	6.60	6.25	5.08	631	571	463
M_1P_3	13.33	14.22	10.07	1253	1071	699	6.68	6.42	3.57	627	484	245
M_2P_1	13.25	12.62	12.93	1069	1151	974	5.88	4.92	4.35	475	450	325
M_2P_2	13.67	12.73	13.85	1154	1111	1078	6.00	4.95	4.92	506	434	379
M_2P_3	11.50	11.85	7.02	1031	845	211	5.20	4.92	3.02	466	356	89
SEm±	0.422	0.386	0.448	42.769	36.618	45.791	0.345	0.273	0.443	31.412	27.753	35.130
CD @ 1%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Moisture content × Storage temperature (M×T)												
M_1T_1	13.72	14.59	12.58	1305	1268	973	6.56	6.31	4.53	622	548	351
M_1T_2	14.60	14.59	13.04	1409	1311	1199	6.97	6.63	4.80	671	596	441
M_2T_1	12.59	12.19	12.30	1101	1013	728	5.73	4.76	4.73	500	398	272
M_2T_2	13.02	12.61	10.23	1068	1058	780	5.66	5.10	3.46	465	429	258
SEm±	0.344	0.316	0.365	34.921	29.899	37.388	0.282	0.223	0.362	25.648	22.660	28.684
CD @ 1%	NS	NS	1.457	NS	NS	NS	NS	NS	NS	NS	NS	NS
Packaging material × storage temperature (P×T)												
P_1T_1	14.03	13.78	13.17	1258	1335	1047	6.38	5.70	4.95	573	553	394
P_1T_2	14.62	13.83	13.48	1311	1286	1153	6.50	5.97	4.75	585	557	412
P_2T_1	13.12	13.75	14.12	1217	1245	1144	6.13	5.67	5.00	568	515	404
P_2T_2	14.30	13.53	14.38	1254	1194	1267	6.47	5.53	5.00	568	490	439
P_3T_1	12.32	12.63	10.03	1133	842	362	5.92	5.23	3.95	544	351	136
P_3T_2	12.52	13.43	7.05	1150	1074	548	5.97	6.10	2.63	550	489	198
SEm±	0.422	0.386	0.448	42.769	36.618	45.791	0.345	0.273	0.443	31.412	27.753	35.130
CD @ 1%	NS	NS	1.785	NS	146.015	NS	NS	NS	NS	NS	NS	NS

Table 3 contd.....

Factors	Seedling length (cm)			Seedling vigour index I			Dry weight (mg)			Seedling vigour index II		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Moisture content × Packaging material × storage temperature (M×P×T)												
$M_1P_1T_1$	14.83	15.13	13.50	1406	1494	1153	6.77	6.70	5.07	642	660	436
$M_1P_1T_2$	15.97	14.87	13.93	1596	1446	1299	7.23	6.80	5.63	723	661	526
$M_1P_2T_1$	13.43	14.30	14.60	1325	1296	1292	6.37	5.87	5.03	627	534	447
$M_1P_2T_2$	14.07	14.80	14.70	1310	1361	1373	6.83	6.63	5.13	634	609	480
$M_1P_3T_1$	12.90	14.33	9.63	1185	1014	474	6.53	6.37	3.50	599	451	172
$M_1P_3T_2$	13.77	14.10	10.50	1321	1128	924	6.83	6.47	3.63	656	517	318
$M_2P_1T_1$	13.23	12.43	12.83	1111	1177	941	6.00	4.70	4.83	504	446	353
$M_2P_1T_2$	13.27	12.80	13.03	1026	1126	1007	5.77	5.13	3.87	447	454	298
$M_2P_2T_1$	12.80	13.20	13.63	1110.13	1194	995	5.90	5.47	4.97	509	496	361
$M_2P_2T_2$	14.53	12.27	14.07	1199.33	1028	1161	6.10	4.43	4.87	502	372	398
$M_2P_3T_1$	11.73	10.93	10.43	1082.00	670	250	5.30	4.10	4.40	488	251	101
$M_2P_3T_2$	11.27	12.77	3.60	980.13	1020	172	5.10	5.73	1.63	445	460	78
SEm±	0.597	0.547	0.633	60.484	51.786	64.759	0.488	0.386	0.627	44.424	39.248	49.682
CD @ 1%	NS	NS	2.524	NS	206.496	258.223	NS	NS	NS	NS	NS	NS
CV%	7.663	7.013	9.107	8.578	7.712	12.184	13.572	11.722	24.802	13.617	13.789	25.995

M-Moisture content, $\mathbf{M_1}$- 8 % moisture, $\mathbf{M_2}$- 6 % moisture, **P**- Packaging material, $\mathbf{P_1}$- Aluminium pouch, $\mathbf{P_2}$- Polythene cover, $\mathbf{P_3}$- Butter paper cover, **T**- Storage temperature, $\mathbf{T_1}$- Ambient temperature, $\mathbf{T_2}$- 15°C temperature, **DAS**- Days after storage

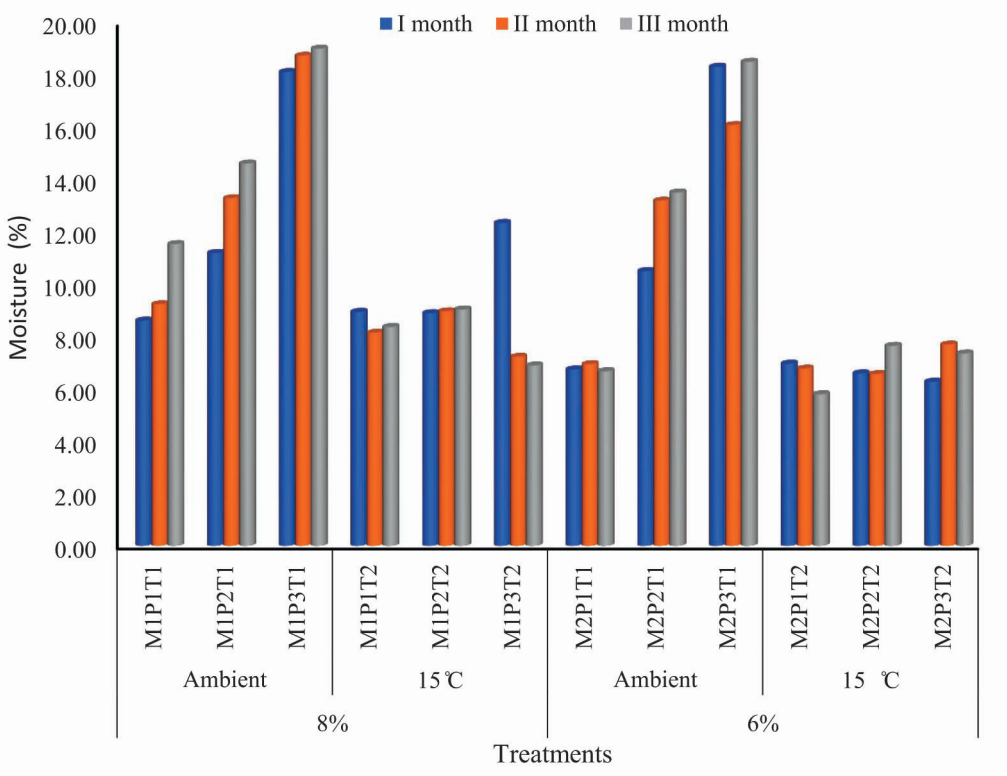

Figure 2: Seed moisture content of *Decalepis hamiltonii* Wight and Arn. as influenced by seed moisture content, storage temperature and packaging material recorded during storage
M-Moisture content, $\mathbf{M_1}$- 8 % moisture, $\mathbf{M_2}$- 6 % moisture, **P**- Packaging material, $\mathbf{P_1}$- Aluminium pouch, $\mathbf{P_2}$- Polythene cover, $\mathbf{P_3}$- Butter paper cover, **T**- Storage temperature, $\mathbf{T_1}$- Ambient temperature, $\mathbf{T_2}$- 15°C temperature

Shoot length (Nil), seedling length (3.60 cm) and seedling vigour index I (172 cm) was minimum in the seeds having 6 per cent moisture packed with butter paper cover stored at 15°C ($M_2P_3T_2$) which was *on par* with $M_2P_3T_1$ (250) for seedling vigour index I (Table 2 and 3). Butter paper cover is moisture pervious and storing it at lower temperature of 15°C results in wide variation in the moisture content and at ambient temperature causes development of mould and fungus both resulting in poor germination and growth. The results relate to the findings of Tiwari and Das (2014) in seven medicinal plants.

CONCLUSION

From the present investigation, it was found that the seeds of *D. hamiltonii* are orthodox as they are desiccation tolerant up to 8% moisture. Placing seeds on between paper method, placed at alternate temperature of 20/30°C results in higher germination percentage and vigour index. Whereas, for the seeds sown in cocopeat placed at 30°C has recorded maximum growth and biomass. The 90 days storage study revealed that shoot length, seedling length and vigour index was maximum in seeds having 8% moisture packed with polythene stored at 15°C temperature. Hence, from this study it can be concluded that, *D. hamiltonii* requires higher alternate temperature of 20/30 C or 30°C rather than low temperature for germination as it is a tropical species and use of between paper method for seed testing ensures better results. Storage of seeds at lower temperature of 15°C, having at least 8% moisture content and use of moisture proof packaging material results in maintenance of seed viability during storage thereby extending the seed longevity.

ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru and College of Horticulture, UHS Campus, GKVK Post, Bengaluru for providing facilities for this research.

REFRENCES

Ashish L, Rajesh D, Rajesh G, Sanjiv C and Prachi L (2015). Study on seed dormancy and seed germination procedures in some medicinal plant species. *Int. J. Res. Biosci. Agric. Technol*., 6: 208-210.

Bewley JD and Black BM (1982). Physiology and biochemistry of seed germination, Part- 2, *Springer Verlag*, New York, pp 32-34.

Bonner FT (2008). Storage of seeds Woody plant seed manual. National seed laboratory USA, 89- 90.

Dadjo C, Nyende AB, Kahia J and Assogbadjo AE (2019). Effect of storage temperature and dormancy breaking pre-treatments on germination and early seedling growth of *Garcinia kola* (Heckel): a threatened medicinal fruit tree in Benin. *Forest Sci. Tech*., pp 1-7.

Emanoela PDP, Salvador BT, Francisco VDSS, Narjara WN, Rômulo MODF and Moadir DSL (2016). Light regime and temperature on seed germination in *Salvia hispanica* L. *Acta Scientiarum*, 38(4): 513-519.

Gairola KC, Nautiyal AR and Dwivedi AK (2011). Effect of temperatures and germination media on seed germination of *Jatropha curcas* Linn. *Adv. Biores*., 2(2): 66-71.

Genes F and Nyomora AMS (2018). Effect of storage time and temperature on germination ability of *Escoecaria bussei*. *Tanz. J. Sci*., 44(1): 123-133.

Giridhar P, Gururaj B and Ravishankar GA (2005). *In vitro* shoot multiplication through shoot tip cultures of *Decalepis hamiltonii* Wight & Arn., a threatened plant endemic to southern India. *In Vitro cell Biol. Plant*, 41: 77-80.

Harrington JF (1973). Biochemical basis of seed longevity. *Seed. Sci. Tech*., 1: 453-461.

Hartman HT, Kester DE, Dale E, Davies FT and Geneve R (2013). Plant Propagation: Principles and practices, *8th Edition, Pearson education Inc*, New York. pp 146-149.

Larcher W (1975). Physiological plant ecology. *Ecology springer*, Berlin.

Nagarajan SL, Rao JM and Gurudatta KN (2001). Chemical composition of the volatiles of *Decalepis hamiltonii* Wight &Arn., *Flavour. Frag. J*., 16: 27-29.

Narender KV, Anil KV and Dharminder K (2009). Effect of storage containers on seed germination and viability in *Aconitum heterophyllum* and *Podophyllum hexandrum*- endangered medicinal plants species of Himalayan region. *Bio. forum*., 1(1): 8-11.

Nawabahar (2008). Effect of media on seed germination of *Cupaniopsis anacardioides* (A. Rich.) Radlk. *Indian J. For*., 31(1):137-139.

Pramod K and Minal K (2017). Propagation of *Rauvolfia serpentina*- a small approach towards creating awareness for its conservation and propagation. *Int. Res. J. Pharm*., 8 (1): 70-73.

Pu X, Huang YF, Pan CL, Yao L, Ai XR and Deng ZJ (2015). Effects of temperature, light, desiccation and cold storage on germination of *Sophora tonkinensis* (Leguminosae) seeds. *Afr. J. Biotechnol*., 14(12): 1015-1019.

Punit KK, Arun K, Ratna C and Verma V (2013). Germination behaviour of seeds of *Withania somnifera* (L.) Dunal: a high value medicinal plant. *Physiol. Mol. Biol. Plants*, 19(3): 449-454.

Roberts, E. H (1988). Temperature and seed germination. *Symp. Soc. Exp. Biol*., 42: 109-132.

Shahzad A and Sharma S (2014). An over review of *Decalepis*- a genus of woody medicinal climbers. *J. Plant Sci. Res*., 1(1) 104.

Sivakumar V, Warrier RR, Anandalakshmi R, Tigabu R, Oden PC, Vijayachandran SN, Geetha S and Singh BG (2006). Germination requirements and storage behaviour of *Myristica dactyloides* Gaertn. Seeds. *Seed Sci. & Technol*., 34: 729-733.

Tiwari RKS and Das K (2014). Impact of differential storage conditions on seed germination and viability of some medicinal plants. *Afr. J. Agric. Res.*, 9(20): 1578-1585.

Ved D, Saha D, Ravikumar K and Haridasan K (2015). Decalepis hamiltonii. The IUCN Red list of threatened species.

Ved DK and Goraya GS (2017). Medicinal plants in India: an assessment of their demand and supply, National medicinal plants board.

Venudevan B, Srimathi P, Natarajan N and Vijayakumar RM (2013). Standardization of germination media for the endangered medicinal tree, bael (*Aegle marmelos*), *Afr. J. Agric. Res.*, 8(24): 3172-3176.

Vijayakumar R and Pullaiah T (1998). An ethnomedico-botanical study of Prakasam district, Andhra Pradesh, India. *Fitoterapia*, 69: 483-489.