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Seed ecology of the important medicinal shrub, *Barleria prionitis* L.

Alokemoy Basu[1] and Pankaj K. Pal*

Department of Botany and Centre of Advanced Studies Phase II [UGC],
The University of Burdwan, Burdwan-713104, India.
[1]Present address: Department of Botany, Acharya Brojendra Nath Seal College,
Cooch Behar-736101, India.
*Corresponding author's e-mail: pkpalbot@gmail.com

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ABSTRACT

Various aspects of seed ecology of the medicinally important shrub, *Barleria prionitis* L. belonging to family Acanthaceae have been investigated. The present study hovers on seed production, seed set percentage, seed structure, seed dispersal, seed fertility and loss of viability as well as moisture content of seeds through time under storage condition. The seed of *Barleria prionitis* is forcefully dispersed from the fruits by a jaculator, which is morphologically a modification of the funicule. Structurally, the seed is exotestal one and with dense slender hair over the surface. Fertility of freshly harvested seed is quite high (98.66%) and under storage viability is persists all through the first month of storage. Subsequently, viability falls steadily through time, resulting into 2.33% after six months of storage and finally the seeds after seven months of storage exhibit no germination. A positive correlation has been noticed between the viability and moisture content of seeds under storage.

Key-words: *Barleria prionitis,* Seed ecology, Jaculation.

INTRODUCTION

Seeds, derived from mature ovules are the final product of successful fertilization during the course of sexual reproduction of flowering plants. Not all the ovules of a multiovulate gynoecium necessarily develop into mature seeds. However, for the species having limited number of ovules in ovary, reproductive success depends on the number of seeds produced in fruits (Wiens 1984, Fenner & Thompson 2005). Therefore, the seed/ovule ratio and percentage of seed-set of a particular species is significant with respect to its reproductive ability. The embryo inside the seed gives rise to the individual of next generation. The precious embryo inside the seeds remains protected by a hard sclerenchymatous seed coat. Besides, the seed coat is often provided with a variety of structural modifications related to moisture regulation, dispersal, water absorption, germination, dormancy etc. (Werker et al. 1979, Egley 1989, Gutterman 1993, Vleeshouwers et al. 1995, Baskin & Baskin 1998). Duration of seed viability varies from species to species which is often related to the moisture content of the seed (Leopold et al. 1988, Foley 1994).

Barleria prionitis L., a moderate-sized, bushy, highly spiny shrub belonging to the family Acanthaceae is referred to as *Kuranti* and *Vajradanti* in Sanskrit and Hindi vernaculars respectively. The species is well known for its medicinal importance. The bark from the mature plant is used in whooping cough and its aerial green parts are used in toothache, leucoderma,

bronchitis, skin disease and inflammations (Kirtikar & Basu 1935, Patro et al. 2007, Palombo 2009). The leaves and stem contain iridoid glycosides like barlerin, barlerinoside, shanzhiside methyl ester, 6-O-trans-â-coumaroyl-8-O-acetylshanzhiside methyl ester, acetylbarlerin, 7-methoxydiderroside, lupulinoside and verbascoside (Ata et al. 2009, Chan et al. 1998). Barlerinoside is the most potent glutathione *S*-transferase (GST) enzyme inhibitor and free radical scavenger (Ata et al. 2009, Chan et al. 1998) and 6-O-trans-â-coumaroyl-8-O-acetylshanzhiside methyl ester together with its *cis* isomer have anti-viral activity against Respiratory Syncytial Virus (Chan et al. 1998). The plant is a native of Africa and tropical Asia including India (Ata et al. 2007). In the state of West Bengal, the plant grows wild in southern districts. For medicinal purposes *B. prionitis* is solely used from the wild source and is not known to be cultivated anywhere. The species has become almost threatened in the region because of habitat destruction and other anthropogenic ativities.

Presence of jaculator and rudimentary aril in the genus *Barleria* was mentioned earlier by Corner (1976) while he described the seed anatomy of the family Acanthaceae in general. Dispersal mechanism of seeds of some members of Acanthaceae, other than *Barleria*, has been worked out by Witztum and Schulgasser (1995). As such seed ecology of *Barleria prionitis* L. is hitherto unknown. In order to formulate suitable strategies for conservation and successful cultivation of the medicinally important plant which is going to be threatened in near future, the present study on its seed ecology has been undertaken.

MATERIAL AND METHODS

The present study is based on wild population of *Barleria prionitis* L. in Chandannagar area (22.8648° N, 88.3633° E) of Hooghly District, West Bengal together with the plants grown in experimental condition in the garden of the Department of Botany, University of Burdwan (23.2324° N, 87.8615° E) both belonging to the state of West Bengal, India. In the research plot altogether 25 plants were grown in 5 rows, each being 2 ft. apart with 5 plants in each row at 2 ft. intervals. Seed production, seed ovule ratio and dispersal mechanism were studied in plants in wild habitat as well as in the plot of experimental research through different seasons, based on randomly selected 100 fruits in each seasons. Mature fruits were collected in paired petri dishes in the months of March and April just before seed dispersal and kept in open sunlight. Seeds were automatically released by the mechanism of fruit dehiscence. After sun-drying (35 ± 2^0C for 5 hours each for 2-3 consecutive days), seeds were divided into isolated lots of 20 seeds and stored in Borosil glass containers with loosely fitted Bakelite caps under laboratory conditions. Determination of seed weight was based on 10 sets, each with 20 seeds. Morphological and anatomical details were recorded by mechanical splitting, and sectioning, followed by observations under stereo binocular microscope and bright field compound microscope. Germination experiments were performed by following the guidelines of International Seed Testing Association (1996). Seed lots (20 seeds in each lot) were allowed to germinate in distilled water on a petri dish (Borosil, 10 cm in diameter). Before sowing, seeds were rinsed with 0.1% $HgCl_2$ solution for 90 seconds for surface sterilization. Later on, it was washed thoroughly with double distilled water to remove any traces of $HgCl_2$. The moisture contents of freshly harvested and stored seeds through time were deduced from: weight of seeds before drying – weight of seeds after complete dehydration / weight of freshly harvested seeds before drying x 100.

While doing so, complete dehydration of seeds was done by keeping them in a hot air oven at 50°C for seven consecutive days. The moisture contents of stored seeds were measured at 15 days intervals since the day of harvesting. Viability of seeds under storage was studied at 30 days intervals by performing germination experiments as well as Tetrazolium test. WILD M3B Leica (Switzerland) sterio binocular microscope and Leitz Laborlux S (Germany) bright-field microscope aided with Leica DFC 295 digital camera were used for microscopic observation and photomicrography. Macroscopic photography was done by Nikon D7000 digital SLR camera. A LABARD Hot Air Oven was used for drying and a Satorious GE212 Digital balance was used for measuring the seed weight.

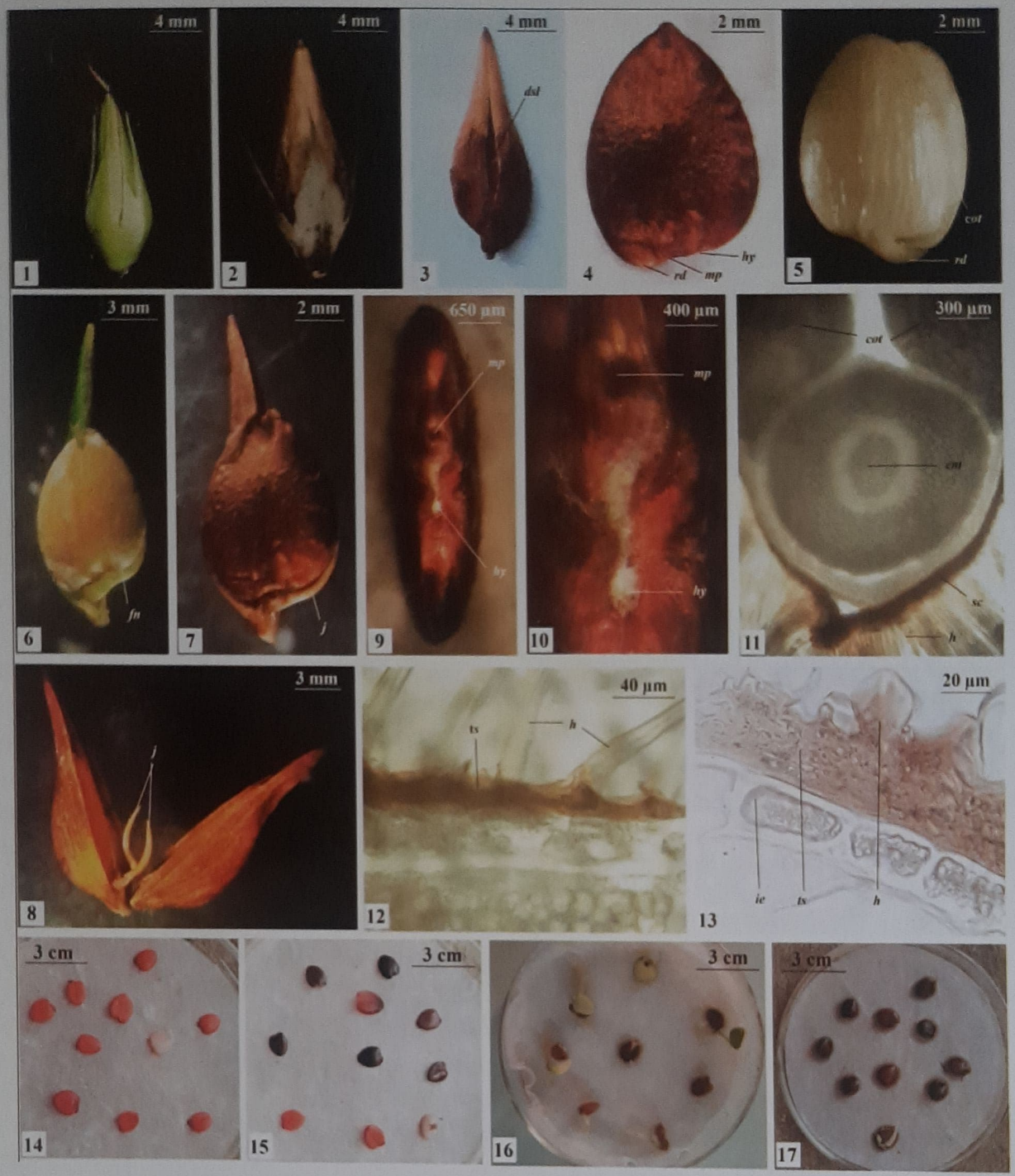


PLATE 1

1. An immature green fruit, partially covered by four green sepals with the dehydrating style attached terminally. **2.** Brown coloured mature fruit with four dried brownish gray sepals attached with it. **3.** The Mature fruit without calyx. A dehiscence slit (*dsl*) is seen along the seam of the two valves of the fruit which originate near the base and moves towards the tip of the fruit. **4.** A mature sundried fruit with glossy brown hairs all over the surface. The impression of the radical on the seed coat is visible at the left side of the base of the seed; positions of the hylum, micropyle and radical are marked. **5.** Seed without seed coat. The cotyledons are mechanically separated slightly for display. The position of radical is marked in the photograph. **6.** A single valve of a mechanically splitted young fruit. The fruit wall is partially removed to display the position of seed and its attachment with the funicle marked there in. The basal part of the funicle is thick and the upper part is fused with the seed. **7.** A single valve of a mechanically splitted mature fruit. The fruit wall is partially removed to display the seed and the funicle which is dried and modified into jaculator. The jaculator is loosely placed at the base of the seed. **8.** The valves after jaculation. The positions of the jaculators are marked. **9-10.** The basal part of the seed. **9.** shows the position of the hylum, micropyle and the depression made by the funicle during attachment. **10.** is more magnified view showing the hylum and micropyle. **11.** Part of the transverse section of a seed showing the embryo, cotyledons, seed coat and hairs. **12.** Part of the seed coat showing the hairs and brown coloured crushed testa. **13.** The magnified view of the seed coat showing the uniseriate inner epidermis, the crushed testa and the base of the hairs. **14.** Tetrazolium test result showing 87% viable seeds after ± 60 days of storage. **15.** Tetrazolium test result, showing 20% viable seeds after ± 140 days of storage. The dark coloured seeds shown in the photograph are rotten ones. **16.** 90% seed germination after ± 45 days of storage. **17.** 10% seed germination after ± 150 days of storage. [cotyledons (*cot*), dehiscence slit (*dsl*), embryo (*em*), funicle (*fn*), hairs (*h*), hylum (*hy*), inner epidermis (*ie*), jaculator (*j*), micropyle (*mp*), radical (*rd*), seed coat (*sc*) and testa (*ts*)]

RESULTS AND DISCUSSION

The fruits of *B. prionitis* are flattened, radially symmetrical, obovate capsules with pointed tip, developed from bilocular, biovuleted syncarpous ovaries. The ovules are anatropous, unitegmic, tenuinucellate and exalbuminous (Corner 1976). The young fruits are deep green in colour. They gradually turn yellow, then straw-coloured and finally become dark brown by drying upon maturity. The pericarp, mesocarp and endocarp are fused together and papery in construction, only the common wall of the locules is woody. The seeds become detached from placenta and remain in the locules. Seeds are dehisced forcefully by jaculation to a distance.

The number of seeds in a fruit is generally two, but in some cases only one seed is found in a fruit. Seed set percentage of the species is 79.36 in natural conditions. Mature seeds are dark brown in colour, completely covered by long brown hairs. The seed is moderate in size and narrowly lanceolate in outline (6.3 – 6.7 mm wide and 7.2 – 7.6 mm long), dorsi-ventrally flattened (1.4 – 1.6 mm in thickness) and lenticular in sectional view.

Weight of each seed is 448.6 – 453.3 mg (mean value 451.2 ± 4.7 mg). The mycropylar region is slightly elevated than the chalazal region. The funicle is robust, conspicuous up to 1/3rd part of the seed, after which it is merged with the seed coat and becomes non-recognizable. The robust funicle is modified into jaculator in the mature seeds (Corner 1976). A small outgrowth is present at the base of the funicle near the hylum. Corner (1976) referred this structure as rudimentary aril. In the mature seed hylum is marked by a scar at the attachment point. The micropyle is a slit inside a circular depression adjascent to the hylum. Raphe is recognizable as the depression at one edge of the flattened seed where the funicle remains attached to the seed. The hypostase is woody in nature (Corner 1976).

The seed coat of *B. prionitis* is very thin and papery. The cells of the outer epidermal layer of the testa become thick walled and are modified into long, rigid, uniseriate, unicellular, tubular hairs, while the inner layers of the testa become crushed. The inner epidermal leyer is composed of single layer of rectangular cells with relatively thick cell wall. There is no separate mechanical layer in the seed coat, only the crushed testal layer bearing hairs provides protection. The colour of the testal layer is brown which gives the characteristic colour of the *B. prionitis* seed. The seeds being tenuinucellate and exalbuminous, the connection between the embryo and the seed coat is absent in the mature state. The embryo is circular in cross-section with a slightly pointed tip at the plumular region. The radicular part is tubular, curved, adperessed with the cotyledons and partially covered by them. The plumule part is completely hidden between the cotyledons. The hypocotyle is short.

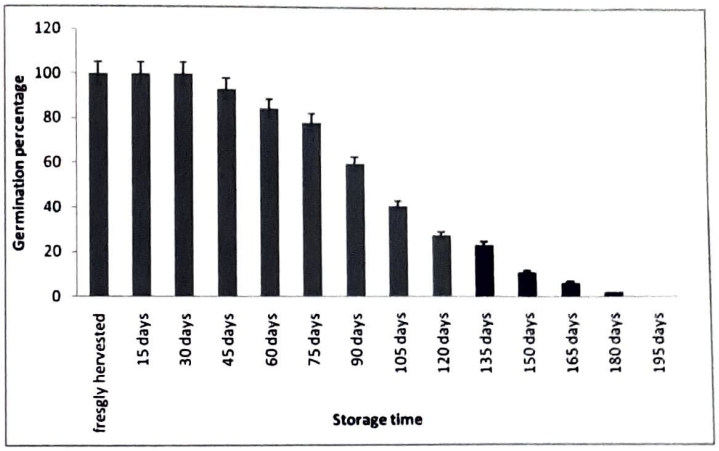
Seeds of *B. prionitis* are dispersed by septisidal jaculation. The ovules of *B. prionitis* remain in axile placentation; therefore, the funicles arise from the two opposite sides of the central axis. These two funicles when modified into jaculators, provide mechanical pressure to the opposite walls of the two valves. These valves are attached together by a seam along the central axis. The jaculation takes place with a cracking sound when the vertical walls of the cementing tissue among the seam become degenerated and the valves cannot hold the pressure of the jaculators any more. The jaculation mechanisms of seeds in Acanthaceae are either hydrostatic or xerostatic (Witztum & Schulgasser 1995). In case of *B. prionitis* it is xerostatic. When the fruits are sufficiently dried, the wall degeneration of seam tissue starts from the base to the apex. After jaculation, the seeds are discharged from the inflorescence axis. The flattened seeds of the species are light weight and aerodynamically streamlined. These features help the longer flight of the seeds in air and dispersal takes place as far as 8 – 9 ft. from the mother plant.

The plant prefers to avoid excess water in the soil. Plants are seen to grow in slightly inclined land or in the soil with good drainage of water. The felt of hairs over the surface of the seeds play several vital roles in maintaining such ecological preferences of this species. Firstly, they facilitate the fast absorption of water and thereby quick imbibation of the seeds. Secondly, the hairs help in retention of water in the seed for a longer period so that they can overcome sudden dehydration

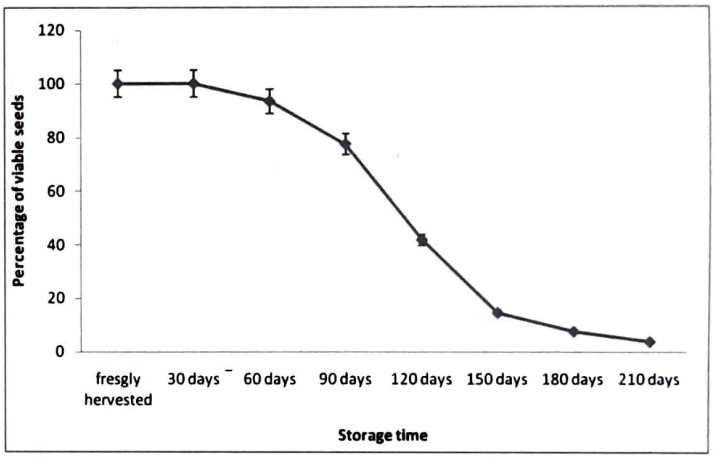
stress before germination. Thirdly, the hydrated hairs provide the seeds good anchorage in the ground soil and prevent further migration.

The seed coats of freshly harvested seeds are quite tender. After sun-drying, the seed coat becomes more papery and relatively stronger. Freshly harvested, sundried *B. prionitis* sees showed 100% germination in distilled water after imbibation for 1 – 3 days under normal laboratory conditions. It was evident that there is no dormancy period for the seeds of this species. The seeds were stored in glass vials with loosely fitted caps. It was observed that the high germinability of the seeds was maintained up to 2 months of storage having 84.66% germination, but they required longer imbibation period (8 – 10 days). The germination percentage fell up to 27.66 % with 14 – 16 days of imbibation period after 4 months of storage. After 6 months only 2.33% stored seed germinated (Table 1, Text-Figure 1, Plate 1)

The Tetrazolium test revealed that the decrease in germination was not due to the dormancy, but the viability of the seeds was lost gradually during storage. The viability of the freshly harvested seeds was 98.66%. The same of the seeds after 2 months of storage was 93.33%, which also is very high. After 4 months of storage 41 ± 6.26 % seeds remained viable and at 6 month the seed viability reached 4%. Viability of seeds was lost completely within 7 months (Text-Figure 2, Plate 1).



Text-Figure 1. Bar graph showing the gradual loss of germinability of seeds through time under storage.

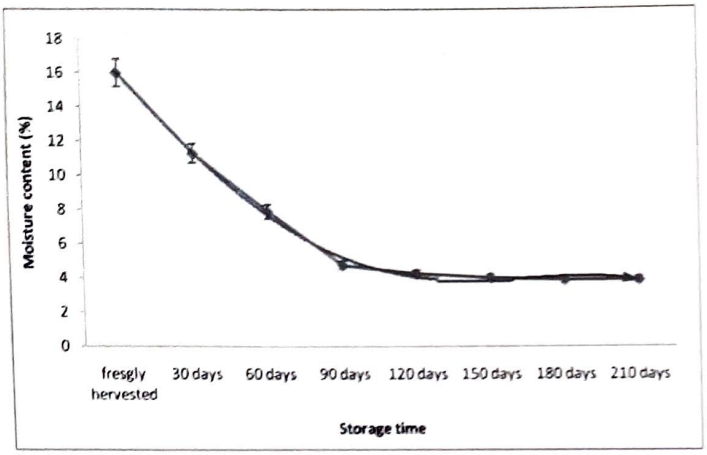


Text-Figure 2. Graph showing the gradual loss of viability of seeds through time under storage as reveiled by Tetrazolium reaction.

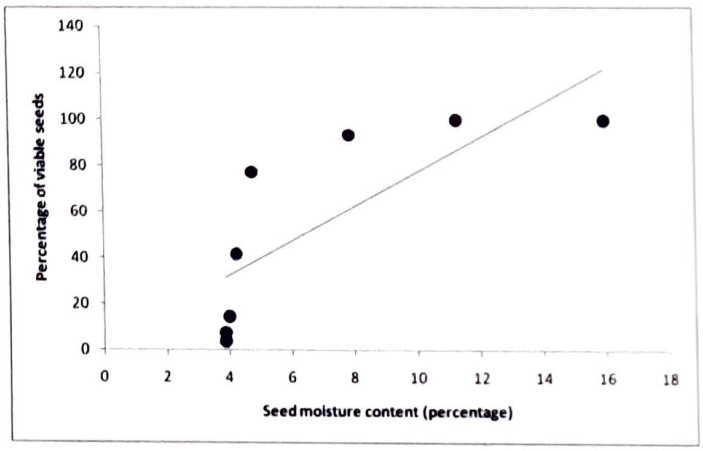
It was observed that the moisture content in the seeds was 16.01 ± 0.58 % just after harvesting. Gradually with the increase in storage duration, the moisture content decreased and after 6 months of

Table 1. Cumulative germination percentage of seeds of *Barleria prionitis* in storage, after different imbibation period.

Storage time in days	Imbibation period					
	Day 1 - 3	Day 4 - 6	Day 7 – 9	Day 8 - 10	Day 11 – 13	Day 14 – 16
1	98.66	-	-	-	-	-
15	93.33	98.66	-	-	-	-
30	64.76	86.66	98.66	-	-	-
45	34.36	66.66	80	93.33	-	-
60	26.57	46.66	60	84.66	-	-
75	0	6.25	17.66	47.66	78.33	-
90	0	0	0	12.66	60	-
105	0	0	0	6.66	29.33	41
120	0	0	0	5.33	10.66	27.66
135	0	0	0	0	5.66	24
150	0	0	0	0	0	10.66
165	0	0	0	0	0	6.66
180	0	0	0	0	0	2.33
195	0	0	0	0	0	0



Text-Figure 3. Graph showing the gradual loss of moisture content of seeds through time under storage.



Text-Figure 4. Graph showing a positive correlation between viability and moisture content of seeds through time under storage.

storage it was 3.89 ± 0.64%. Most of the seed moisture was lost within the first 3 months. In the first month 4.69% and in the second and third months 3.14% and 3.12% moisture was lost respectively. Form fourth month onwards the rate of moisture loss decreased to 0.53%, 0.24% and 0.13% in fourth, fifth and sixth months respectively. After that no further significant moisture was observed (Text Figure 3, Plate 1). A positive correlation exists between the viability and moisture content of the seeds under storage (Text Figure 4, Plate 1).

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REFERENCES

Ata A., Bosch S.A.V.D., Harwanik D.J. & Pidwinski G.E. 2007. Glutathione S-transferaseand acetylcholinesterase-inhibiting natural products from medicinally important plants. Pure and Applied Chemistry 79(12): 2269-76.

Ata A., Kalhari K. S. & Samarasekera R. 2009. Chemical constituents of *Barleria prionitis* and their enzyme inhibitory and free radical scavenging activities Phytochemistry Letters 2: 37-40.

Baskin C.C. & Baskin J.M. 1998. Seeds: ecology, biogeography and evolution of dormancy and germination. San diego, CA: Academic Press.

Chen J.L., Blanc P., Stoddart C.A., Bogan M., Rozhon E.J., Parkinson N., Ye Z., Cooper R., Balick M., Nanakorn W. & Kernan M. R. 1998. New Iridoids from the Medicinal Plant *Barleria prionitis* with Potent Activity against Respiratory Syncytial Virus, Journal of Natural Products 61: 1295-1297.

Chopra R. N., Chopra J. C., Handa K.L. & Kapur L.D. 1958. Chopra's indigenous drugs of India, 2nd ed., U.N. Dhur and Sons Private Limited, Calcutta.

Copeland L O. & McDonald M.B. 2001. Principles of seed science and technology, 4th ed., Kluwer Academic Publishers, U.S.A.

Corner E.J.H. 1976. The seeds of dicotyledons I. Cambridge: Cambridge University Press.

Egley G.H. 1989. Water-impermeable seed coverings as berries to germination. In R.B. Taylorson (Ed.), Recent Advances in the development and germination of seeds. New York: Plenum Press.

Faegri K. & Van der Pijl L. 1979. The principles of pollination ecology, 3rd ed., Pergamon Press, Oxford.

Fenner, M. & Thompson, K. 2005. The ecology of seeds. Cambridge University Press, Cambridge.

Gutterman Y. 1993. Seed germination in desert plants. Berlin: Springer-Verlag.

International Seed Testing Association 1996. International rules for seed testing. Seed Science and Technology 24: 335.

Kirtikar K.R. & Basu B.D. 1935. Indian Medicinal Plants, Vol. III, L.M. Basu, Allahabad.

Leopold C.A. & Kriedemann P.E. 1975. Plant growth and development, 2nd ed., Tata Mcgraw-Hill Publication Company, New Delhi.

Palombo E.A. 2009. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evidence based Complementary and Alternative Medicine 10: 1-15.

Patro B.K., Ravi Kumar B., Goswami A., Mathur V.P. & Nongkynrih B. 2008. Prevalence of dental caries among adults and elderly in an urban resettlement colony of New Delhi. Indian Journal of Dental Research 19(2): 95-8.

Vleeshouwers L.M., Bouwmeester H.J. & Karssen C.M. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. Journal of ecology 83:1031-1037.

Werker E., Marbach I. & Mayer A.M. 1979. Relation between the anatomy of the testa, water permeability and the presence of Phenolics in the genus *Pisum*. Annals of Botany 43:765-771.

Wiens D. 1984. Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. Oecologia 64:47-53.