Journal of Research in Biology

An International Scientific Research Journal

ISSN No: Print: 2231 – 6280; Online: 2231 - 6299.

Original Research

High adaptability of *Blepharis sindica* T. Anders seeds towards moisture scarcity: A possible reason for the vulnerability of this medicinal plant from the Indian Thar desert

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ABSTRACT:

The seeds of *Blepharis sindica* T. Anders (Acanthaceae) are the official part of the plant for its medicinal values and also as the promise of its future. Dunes of the Thar desert with high percolation capabilities are the most preferred habitat of this vulnerable medicinal plant. It produces 1337.26 seeds/plant as an average and shows high viability and germination percentage under *in-vitro* conditions, but efficiency of seedling establishment was observed poor at natural sites. Occurrence of seed coat layers as sheath of hygroscopic hairs is a sign of its extreme capabilities to initiate life under lesser soil moisture availabilities in desert. Seeds with 0.5 to 1.0 ml distilled water were observed most suitable for the production of maximum shoot and root lengths under controlled conditions. Maximum biomass of shoot and root modules were observed in 0.5 ml distilled water. Maximum amount of non-soluble sugar was found in intact seeds devoid of any imbibition. Seeds with 0.5 ml distilled water produced maximum amount of shoot biomass and soluble sugar, while seedlings with 1.0 ml had maximum root biomass. Seedlings treated with >1.5 ml of distilled water showed a decreasing trend in all parameters. Excessive water always found to cause seedling collapse and failure of its establishment.

Keywords:
Thar desert, medicinal plant, vulnerable, hygroscopic hairs, moisture, seedling collapse.

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Article Citation:
Purushottam Lal, Sher Mohammed and Pawan K. Kasera.
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Journal of Research in Biology (2014) 4(2): 1293-1300

Dates:
Received: 02 Jan 2014 **Accepted:** 04 Feb 2014 **Published:** 22 May 2014

Journal of Research in Biology

INTRODUCTION:

Indian Thar desert is characterised by scanty rainfall and long dry periods throughout the year, which pushes the typical scrub vegetation to firm adopt specific life sustaining adaptabilities (Sen, 1982). In desert ecosystems, long dry periods and scanty rainfall impose severe water deficit in natural vegetation (Sen, 1982; Raghav and Kasera, 2012). Biodiversity of desert areas is a better reflection of highly synchronised life patterns of living beings against the environmental entities which always restrict the life to express beyond their biotic potentials. The Indian Thar desert has a unique vegetation cover as compared to other deserts around the world. Besides harsh climatic conditions and much constrains on growth potentials, the plant species of arid zone synthesise and accumulate a variety of bioactive compounds which have different values to serve mankind. Due to their medicinal as well as economic importance, the medicinal plants and their different parts are being exploited largely from natural habitats. Habitat destruction, unscientific collection, ecological limitations, etc. are crucial factors to push valuable medicinal plants under verge of extinction. UNDP (2010) have published Red List Categories for 39 medicinal plants of Rajasthan State, of which *Blepharis sindica* is considered as "Vulnerable". Thus, it is quite important to know its adaptability to conserve in natural habitat.

B. sindica is a lignified annual plant with characteristically dichotomously branching habit. It is locally known as Billi khojio, Bhangara and Unt-katalo (Bhandari, 1990). It grows on loose soils, along the crop fencings and much especially on dune slopes. Sandy soil with heavy percolation is much preferred by this plant. After a successful completion of life cycle (July to December), capsules loaded spikes remain attached to the dried plant and provide a special distinguishable appearance to the species. Seeds within capsules remain open to face the extreme of winter and summer temperatures till their first imbibition. Habitat limitation plays an excellent role for this species as sand shifting and eolian deposition cause to bury the spikes which trigger microbial decomposition of lignified bracts. The plant emerge through seeds after first rain as soon as fruit wall split explosively from distal tapered end and release seeds to imbibe (Fig. 1).

Compressed seeds with densely clothed hygroscopic hairs are used in the preparation of herbal medicines and it is used as aphrodisiac (Shekhawat, 1986; Singh *et al*., 1996; Mathur, 2012). Its roots are used for urinary discharge and dysmenorrhoea. Powdered plant is applied locally on the infections of genitals and on the burns (Khare, 2007). Seeds contain flavonoides (apigenin, blepharin, prunine-6″-O-coumarate, and terniflorin), steroid (β-sitosterol) and triterpinoide-oleanolic acid (Ahmad *et al*., 1984).

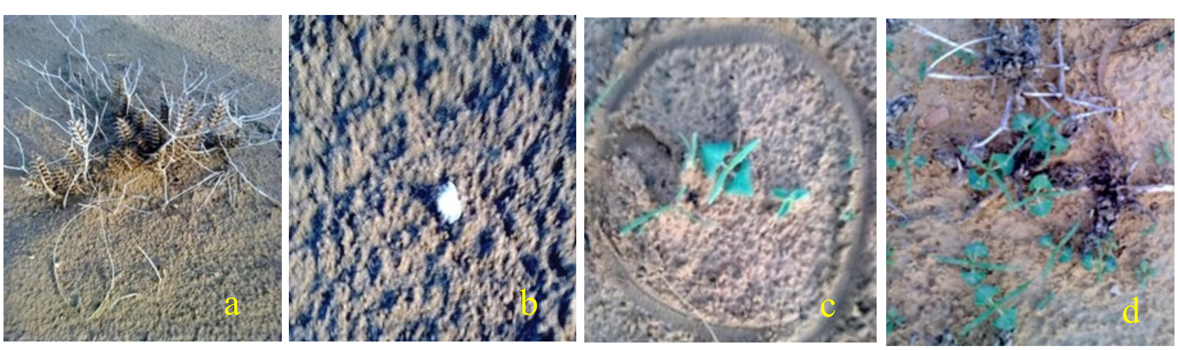

Fig. 1: *Blepharis sindica*: One-year-old plant after first rains, showing spreading of seeds to initiate germination (a), freshly fallen seed after moisture uptake by hygroscopic hairs at sandy surface of dune (b), single young seedling (c), and seedlings in association (d).

Hence in the present study, an attempt has been made to identify a correlation between availed moisture and seedling establishment in *B. sindica* germplasm collected from different localities of the Churu district, a part of Indian Thar desert.

MATERIALS AND METHODS:

The germplasm of this species was collected during 2011-2012 from two different sites, *viz*., Shyampura village (Site-I; 12 km away towards west-south direction from the College Campus) and Buntia village (Site-II; 10 km towards north-east), a part of the Indian Thar desert. The seed size was measured with the help of vernier caliper and graph paper. Seed volume and density estimations were based on water displacement method (Misra, 1968). Values were calculated for 100 seeds in triplicate and confirmed twice. Arithmetic mean and standard deviation were computed for each parameter. Seed viability was tested by T.T.C. method (Porter *et al*., 1947). The seed germination experiments were performed in seed germinator at 28°C. Seeds were placed in the sterilized petri dishes lined with single layer of filter paper to evaluate germination behaviour. To evaluate moisture response, the filter paper in each experiment was moistened with 0.5, 1.0, 1.5, 2.0, 5.0 and 10.0 ml volume of distilled water separately. Each petri dish containing 10 seeds in triplicate was used and experiment was repeated for two times for the confirmation of results. After one week of setting the experiments, germination percentage (%) and root & shoot lengths of seedlings were measured with the help of a graph paper. Shoot and root biomass values of seedlings against different moisture regimes were estimated by oven-dried weight basis. Amount of sugars in seedlings after varied doses of distilled water was estimated by using anthrone reagent method (Plummer, 1971). Differences in biomass & sugar contents of seedlings from various moisture regimes were compared with the values for intact seeds and measured in percentage basis. The relation between total biomass % and total sugars % in comparison to intact seeds were expressed as metabolic efficiencies of seedlings at particular moisture regime. The pooled data of entire season were analyzed statistically as per the methods of Gomez and Gomez (1984), presented in tabular and figure forms.

RESULTS:

The data on various morphological parameters, *viz*. weight, size, volume, density and viability of seeds collected from different sites are given in Table 1. Morphological variations provide understanding about germplasm variability, which is an important adaptation skill of desert plants. Seed length and density values were observed higher at site-I, whereas other parameters at site-II.

Morphological parameters revealed that higher (5.73 x 4.13 x 0.10 mm) values of seed size were observed at site-II, while lower (5.75 x 4.11 x 0.07 mm) at site-I. Weight of 100 seeds was greater (1.33 g) at site-II than site-I (1.16 g). Volume of 100 seeds was more (1.57 ml) at site-II, whereas less (1.13 ml) at site-I.

Table 1. Variation in morphological parameters of *B. sindica* seeds collected from sites- I & II.

Sites	Weight of 100 seeds (g)	Seed size (mm) Length	Breadth	Thickness	Volume of 100 seeds (ml)	Density (g ml^{-1})	Viability (%)
I	1.16±0.015	5.75±0.010	4.11±0.006	0.07±0.0004	1.13±0.028	1.02±0.057	100.00±0.00
II	1.33±0.022	5.73±0.010	4.13±0.006	0.10±0.0004	1.57±0.028	0.85±0.021	100.00±0.00

± = Standard deviation

Freshly collected seeds from both sites exhibited cent percent viability.

To evaluate the significance of moisture regimes on germination process, 0.5 ml to 10.0 ml range of distilled water was provided to seeds. Under controlled laboratory conditions, cent percent germination was observed in 0.5, 1.0, 1.5 and 2.0 ml moisture regimes for both sites. 5.0 and 10.0 ml moisture regimes caused deterioration for seed germination.

Shoot length parameter was found to have increasing trend from 0.5 ml to 2.0 ml range, afterwards it gets decreased (Table 2). Maximum shoot length (10.47 mm) was observed at 2.0 ml moisture for site-II, while at 0.5 ml moisture slight expansion in cotyledons was occured without shoot development for both sites. Higher values of root length were observed at 1.0 ml moisture for both sites, being maximum (61.97 mm) for site-I.

At 0.5 ml level, only radicle emerged out without any shoot elongation; whereas at 5.0 & 10.0 ml levels shoot and root axies collapsed after a short growth (Fig. 2). The expression of comparative relation between shoot and root lengths as R/S ratio was found significant at 1.0, 1.5 & 2.0 ml regimes. It was observed maximum (45.23) at 1.0 ml moisture for both sites, while minimum (0.82) at 10.0 ml for site-I. Seedlings from site-I showed a rapid decline in R/S ratio along with increasing moisture levels as compared to site-II.

Anabolic efficacy of germinating seeds was measured in the form of over-dried biomass of seedlings. Shoot biomass was found more as compared to root ones. Maximum (0.28 g d. wt.) shoot biomass was estimated at 0.5 ml moisture for site-II, while minimum (0.09 g d. wt.) at 10.0 ml for site-II. Maximum extension of root axis was observed at 1.0 ml levels, while maximum (0.05 g d. wt.) root biomass were found at 1.0, 1.5 & 2.0 ml levels for site-II. Total biomass was increased after seeds were permitted to imbibing and found maximum (0.31 g d. wt.) with 0.5 ml and 1.0 ml moisture for site-II. Total biomass values exhibited declining trend along with increasing moisture regimes (Fig. 3).

Table 2. Effect of different amount of distilled water on seed germination (%), seedling growth (mm), seedling biomass (g) and sugar contents (mg g^{-1} d. wt.) during seedling establishment in *B. sindica* seeds under laboratory conditions at sites- I & II (Observations taken after 7 days).

	Amount of distilled water provided (moisture regime)							
Site-I	**Seed**	**0.5 ml**	**1.0 ml**	**1.5 ml**	**2.0 ml**	**5.0 ml**	**10.0 ml**	**CD**
Germination	-	100.00	100.00	100.00	100.00	36.67	6.67	1.4684 ns
Shoot length	-	0.00	1.37	4.60	8.20	2.53	1.67	0.1457ns
Root length	-	8.73	61.97	44.53	50.73	5.90	1.37	0.7204*
R/S ratio	-	#	45.23	9.68	6.19	2.33	0.82	1.3604ns
Shoot biomass	-	0.26	0.23	0.23	0.22	0.13	0.11	0.0071ns
Root biomass	-	0.02	0.04	0.04	0.04	0.01	0.01	0.0021ns
Total biomass	0.12	0.28	0.27	0.27	0.26	0.14	0.12	0.0047ns
Soluble sugar	28.87	29.12	28.87	28.25	27.08	18.61	5.62	0.6669*
Non-soluble sugar	2.34	1.91	1.92	1.78	1.91	1.59	1.21	0.1132*
Site-II								
Germination	-	100.00	100.00	100.00	100.00	50.00	50.00	1.5604ns
Shoot length	-	0.00	1.50	8.77	10.47	7.53	6.27	0.0092ns
Root length	-	13.77	51.03	50.93	46.60	11.63	8.60	0.4439 ns
R/S ratio	-	#	34.02	5.81	4.45	1.54	1.37	1.2667ns
Shoot biomass	-	0.28	0.26	0.25	0.25	0.17	0.09	0.0054ns
Root biomass	-	0.03	0.05	0.05	0.05	0.02	0.01	0.0026ns
Total biomass	0.13	0.31	0.31	0.30	0.30	0.19	0.10	0.0065ns
Soluble sugar	29.12	29.75	29.27	28.42	26.42	17.87	7.87	0.1553ns
Non-soluble sugar	2.41	2.03	2.02	1.81	2.06	1.81	1.38	0.1307ns

- = Values are not applicable, # = Values are infinitive, *= Significant at ($P < 0.05$) level, and ns = non-significant

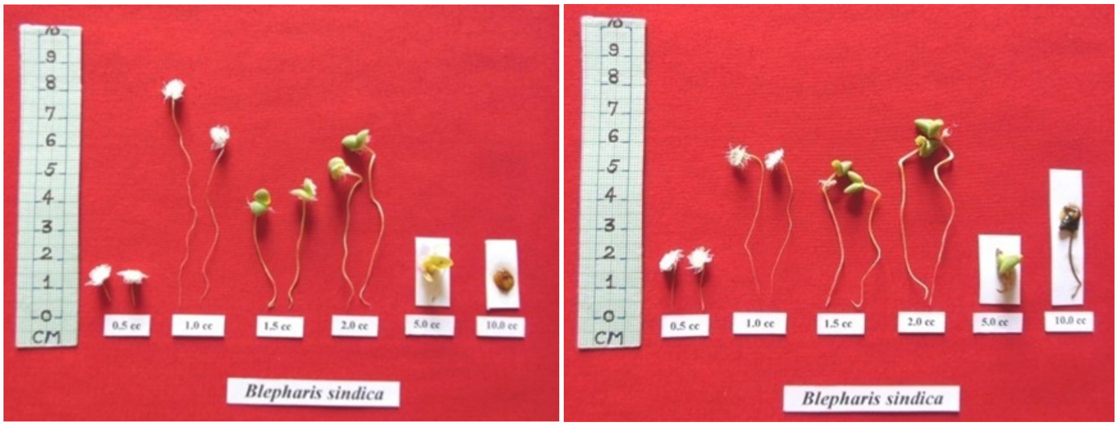

Fig.2: ***In-vitro*** **seedlings of** ***B. sindica*** **after 07 days response against varied amount of moisture regimes (0.5 to 10.0 ml distilled water per petridish) from site-I (a) and site-II (b). Fully expand hygroscopic hairs at 0.5 & 1.0 ml and collapsed seedlings at 5.0 & 10.0 ml.**

Amounts of soluble and non-soluble sugars were estimated in oven-dried seedlings obtained after response of varied moisture regimes. Soluble sugar was maximum (29.75 mg g^{-1} d. wt.) at 0.5 ml moisture level for site-II, while minimum (5.62 mg g^{-1} d. wt.) at 10.0 ml for site-I. Amount of non-soluble sugar was more in intact seeds as compared to seedlings. Its maximum (2.41 mg g^{-1} d. wt.) value was estimated in seeds from site-II. Seedlings with 10.0 ml moisture exhibited minimum values for site-I. In this species, intact seeds were found to have maximum amount of total sugars (soluble & non-soluble) and showed a decreasing trend with increasing moisture regime. On using intact seeds as reference, the total sugars loss occurred on different moisture regimes are expressed on percentage basis (Fig. 3). As compared to site-I, seedlings from site-II exhibited more sugar loss percentage at all moisture regimes, except in 10.0 ml. Maximum (78.12 %) sugar loss was occurred at 0.5 ml moisture for site-II, whereas minimum (0.58 %) at 10.0 ml for site-I. Production of total biomass (g d. wt.) in relation to total sugars loss (% mg g^{-1} d. wt.) can be used to express the metabolic efficiency (% d. wt. / % mg g^{-1} d. wt.) of seedling establishment (Fig. 4). Highest (229) value for metabolic efficiency of germinating seeds were observed at 0.5 ml moisture level for site-I, whereas minimum (-0.32) at 10.0 ml for site-II. A decline in metabolic efficiency was observed on increasing moisture regimes during seed germination. Metabolic fluctuations (percentage sugar loss & percentage biomass growth in comparison to intact seeds) and metabolic efficiency values against various moisture regimes were found non-significant ($P > 0.05$) for both sites.

DISCUSSION:

Seed germination is a crucial step of life cycle in higher plants as it determines the future of the species as well as it offers the availability of plant resources for all living beings. Most of arid plants produce seeds with hard seed coats that enable the species to cope drought constrains (Sen *et al*., 1988). In this species, seeds completely lacking of hard coverings and embryos found directly encapsulated within hygroscopic membrane which further extends in hygroscopic hairs. The seeds collected from both sites showed morphological variability, which influenced the response of seeds against different moisture regimes during *in-vitro* germination. Freshly collected seeds exhibited cent percent viability without any dormancy barrier.

Germplasm tolerance against extreme aridity of the area is solely paid by its hard capsule (fruit) coverings whereas the hygroscopic sheath (seed coat layer) has the most prominent contribution for rapid uptake of soil moisture and subsequent imbibitions. The present investigation reveals that this part,

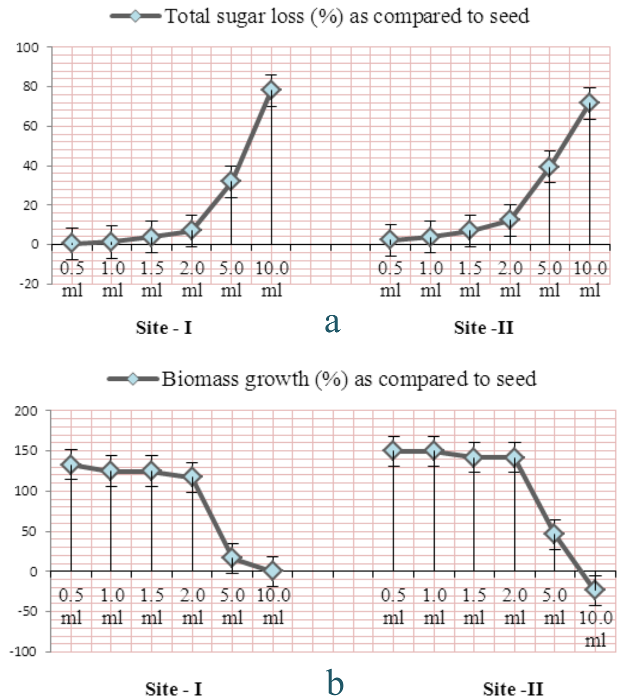

Fig. 3: Total sugar loss (a) and total biomass growth (b) in seedlings against varied amount of moisture regimes as compared to intact seeds for sites- I & II.

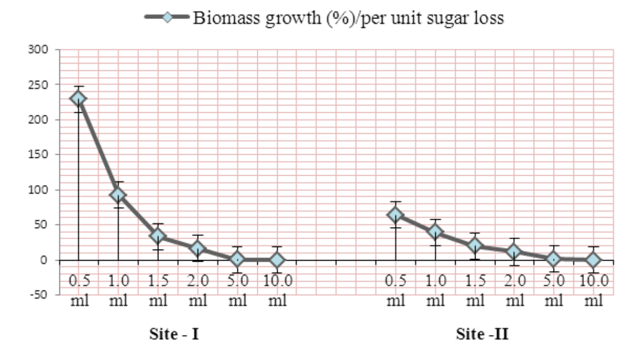

Fig. 4: Metabolic efficiency of germinating seeds under varied amount of availed moisture levels (% d. wt. total biomass / % total sugar loss) from sites- I & II (Data are average of three replicates).

i.e. hygroscopic sheath has some short of limitations in sense of its carrying capacity of soil moisture contents.

Field study of the area revealed that in spite of having cent percent viability and germination percentage, a limited number of seedlings develop *in-vivo* at its preferred sand dune surfaces during early monsoon period. Observations are in the record that mucilaginous sheathing on seeds and its other parts which provide adequate water, leds to improved germination in Cactus (Bregman and Graven, 1997; Gorai *et al.*, 2014). Excessive moisture was found to inhibit seed germination in *B. sindica,* as observed by Mathur (2012) but the present investigations point out that at particular stage of early seed germination physiology, water amount works as a master factor but interestingly it is positive for a very short range, *i.e.* 0.5 to 1.0 ml. The amount of first rain fall over detached seeds and the rate by which rain water get percolated through inter-particle spaces, which determine the value of availed moisture for seeds to imbibe. For better understanding the role of moisture amount in seed germination process; this unique experiment was designed and the results illustrate the comparative effect of different levels of moisture in sense of seedling growth, biomass production, consumption rate of reserve food contents and comparative efficiency of seedling establishment.

Higher values of seedling length and biomass production (shoot & root modules) were observed in 0.5 to 2.0 ml moisture regimes. Seed germination percentage as well as seedling vigour (length & biomass) values showed a clear decline on excessive moisture contents (5.0 & 10.0 ml). The values for biomass growth (%) in comparison to dry weight of intact seeds were found highest at minimum moisture level, *i.e.* 0.5 ml. Amount of soluble sugars, a part of nourishment ready to consume during germinating seedlings; also estimated maximum at 0.5 ml moisture level. Metabolic efficiency of germinating seeds (dry weight increase per unit reserve food loss) was also estimated highest at minimum moisture regimes, while its negative value was estimated at highest regimes of the performed experiment.

CONCLUSIONS:

Seeds of *B. sindica* are highly adjusted structures toward moisture limitations in arid habitat. The seeds exhibited absolute requirement of 0.5 ml moisture level for the better establishment of *in-vitro* seedlings. Primarily, the species has high biotic potential (1337.26 seeds / plant with 100 percent viability and germination efficiencies) and secondly the species has absolutely free from any type of grazing & fruit collection pressures. In spite of this, the number of well established seedlings and consequent mature plants were found restricted at both sites. This condition marks a clear threat at the point when its life moulds from seed to seedling phase. Metabolic diagnosis of germinating seeds, *i.e.* total sugar loss (%), total biomass growth (%) and the rate of metabolic efficiency (% d. wt. total biomass /% total sugars loss) provides ample insight into compensation efficacy of germinating seeds against a particular moisture level. Seedling collapsing at 5.0 & 10.0 ml regimes indicates the seed tissue incompatibility at excessive moisture regimes. Our results could make an excellent way to define this natural problem with this species and assessment of threat in the arid habitats of Indian desert. The entire cascade of this pioneer work justifies the esteem love of *B. sindica* seeds with that of Thar desert aridity. Such type of findings may also be helpful for conservation strategies related to different plant species of the area.

ACKNOWLEDGEMENTS:

Financial assistance received from CSIR, New Delhi in the form of SRF-NET (File No.: 08/544 (0001)/2009-EMR-I, 27.06.2009) to first author is gratefully acknowledged. Thanks are due to the Principal, Govt. Lohia PG College, Churu for providing necessary facilities. The authors are also thankful to Dr. David N. Sen (Retd. Professor & Head), Department of Botany, J.N.V. University, Jodhpur for valuable suggestions in improvement of this paper.

REFERENCES:

Ahmad VU, Burki AM, Mahmood I and Smith DL. 1984. Chemical constituents of *Blepharis sindica* seeds. Chem. Soc. Pak., 6(4): 217-223.

Bhandari MM. 1990. Flora of the Indian Desert. MPS REPROS, Jodhpur, p. 435.

Bregman R and Graven P. 1997. Subcuticular secretion by cactus seeds improves germination by means of rapid uptake and distribution of water. Annals of Botany. 80 (4): 525-531.

Gomez KA and Gomez AA. 1984. Statistical Procedures for Agricultural Research, 2nd ed. John Wiley & Sons, New York, p. 294.

Gorai M, El Aloui W, Yang X and Neffati M. 2014. Toward understanding the ecological role of mucilage in seed germination of desert shrub *Henophyton deserti*: interactive effects of temperature, salinity and osmotic stress. Plant Soil 374 (1-2): 727-738.

Khare CP. 2007. Indian Medicinal Plants - An Illustrated Dictionary. Springer-Verlag, Berlin, New York, USA, p. 812.

Mathur M. 2012. Phytosterol composition in seeds of *Blepharis sindica* and its relation with bottom up, top down and plant metabolites factors. Medicinal plants - International Journal of Phytomedicines and Related Industries 4(3): 126-132.

Mathur M and Sundaramoorthy S. 2012. Studies on distribution patterns for an endangered semi-arid plant- *Blepharis sindica*. Vegetos 25(2): 66-75.

Misra R. 1968. Ecology Work Book. IBH Publishing Company, Oxford, New Delhi, p. 242.

Plummer DT. 1971. An Introduction to Practical Biochemistry. Tata McGraw Hill Publishing Co Ltd, New Delhi, p. 369.

Porter RH, Durrell M and Romm HJ. 1947. The use of 2, 3, 5-triphenyl tetrazolium chloride as a measure of seed germinability. Plant Physiol., 22(2): 149-159.

Raghav A and Kasera PK. 2012. Seed germination behaviour of *Asparagus racemosus* (Shatavari) under *in-vivo* and *in-vitro* conditions. Asian Journal of Plant Science & Research. 2 (4): 409-413.

Sen DN. 1982. Environment and Plant Life in Indian Desert. Geobios International, Jodhpur, p. 249.

Sen DN, Mohammed S and Kasera PK. 1988. Report on weed seed longevity in arid zone-Test of seed bank. Geobios new Reports. **7:** 98-100.

Shekhawat GS. 1986. Ethnobotanical survey of desert area of Rajasthan. Ph D Thesis, University of Jodhpur, Jodhpur, India.

Singh U, Wadhwani AM and Johri BM. 1996. Dictionary of Economic Plants in India. ICAR, New Delhi, p. 288.

UNDP 2010. Rajasthan Red Listed Medicinal Plants, http://envis.frlht.org/documents/rajasthan-medicinal-plants-conservation.pdf