J. Indian bot. Soc. ISSN - 0019 - 4468 Vol. 93 (1 & 2) 2014 : 94-99

ECOLOGICAL EVALUATION OF SOME PHYSIOCHEMICAL PARAMETERS IN *BLEPHARIS SINDICA* T. ANDERS- A VULNERABLE MEDICINAL PLANT OF THE ARID REGION

PURUSHOTTAM LAL[1], SHER MOHAMMED[1] AND PAWAN K. KASERA[2]
Department of Botany, Govt. Lohia PG College, Churu-331001 (Rajasthan), India
Department of Botany, J. N. V. University, Jodhpur-342 033 (Rajasthan), India
E-Mail: smdchuru@gmail.com

The present paper deals with physiochemical responses in *Blepharis sindica*, a vulnerable medicinal plant of the Indian Thar desert. Different physiochemical parameters such as leaf moisture percentage, osmotic potential, leaf pigments, proline, total sugars, crude protein and phosphorus contents varied significantly according to different growth phases of the plant. Maximum amount of soil moisture percentage was reported in deeper (20-25cm) layers, while leaf moisture and crude protein in July. Higher values for proline and total sugars were found in late season, *i.e.* November and December, whereas total chlorophylls and phosphorus during middle of season, *i.e.* August to October.

Key words: *Blepharis sindica*, medicinal plant, physiochemical, Thar desert, vulnerable.

Deserts are well known for their poor vegetation cover. The Indian Thar desert harbors a unique composition of floristic diversity in a fine adjustment with climatic peculiarities of the area. In arid habitats, water demand by vegetation and crops is high but availability is restricted by scanty rainfall and long dry periods throughout the year (Raghav and Kasera 2012). Besides harsh conditions and much constraints on growth potentials, the plant species of arid zone synthesise and accumulate a variety of bioactive compounds which play a vital role in treatment of different health problems worldwide. Due to their medicinal as well as economic importance, the medicinal plants and their different parts are being exploited excessively 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, out of which 19 are listed as vulnerable, 12 as endangered, 6 as critically endangered and one each of near threatened and data deficient, respectively. Out of these, *B. sindica* is considered as "Vulnerable". Thus, it is quite important to conserve and know the adaptability of this plant.

Blepharis sindica T. Anders (Family: Acanthaceae) is an annual, dichotomously branched herbaceous plant. It is locally known as Billi Khojio, Bhangara, Unt-Katalo (Bhandari 1990). Plant grows on loose soils, along crop fencings and much especially on dune slopes. Sandy soil with heavy percolation is much preferred by plant. Seed loaded capsules attached on lignified dried stem within spiny cones are the part of life cycle which interacts with extremes of winter and successive summer. The plants emerge through seeds after first rain as soon as fruit wall splits explosively from distal tapered end and release seeds to imbibe. *In-vitro* seed germination studies suggest that during seed imbibitions excess water always causes failure of seedling establishment. The plant completes its life cycle within six months (July to December). Compressed seeds with densely clothed hygroscopic hairs are used in preparation of different herbal medicines and also as aphrodisiac (Bhandari 1990, Shekhawat 1986, Singh *et al*. 1996). Its roots are used for urinary discharge and dysmenorrhoea. Powdered plant is applied locally on infections of the genitals and on burns (Khare 2001). Seeds contain flavonoid (apigenin, blepharin, prunine-6'-O-coumarate, and terniflorin), steroid (β-sitosterol) and triterpenoid-oleanolic acid (Ahmad *et al*. 1984).

In the present investigation an attempt has been made to assess the physiochemical responses of this species under prevailing conditions of desert. The fluctuations in leaf moisture percentage, osmotic potential, leaf pigments, proline, total sugars, crude protein and phosphorus during successive months of growth sea-

Received on November 22, 2012 Accepted on February 1, 2014

son constitute main aspects of the present study.

MATERIALS AND METHODS

Plant samples were collected during July to December, 2011 from the open area of two different sites, *viz*. Shyampura (Site-I; 12 km toward west-south direction from the College Campus) and Buntia (Site-II; 10 km towards north-east direction from the College Campus) of Indian desert. The climatic conditions of both experimental sites are arid and with more or less similar precipitations. The leaf samples were collected as far as possible from similar nodes of different plants to minimize the variability in sample selection. Fully mature fresh leaves were collected in morning hours and estimation of leaf moisture, osmotic potential (OP), proline and pigments were carried out after randomly mixing the leaf samples in triplicate. Oven dried leaf samples (80^0 C; 48 hrs.), after grinding were used for estimation of sugars, crude protein and phosphorus. Leaf moisture percentage was calculated by simple oven dried weight basis. OP was estimated as per method suggested by Janardhan *et al*. (1975). Total pigments were estimated using Arnon's method (Arnon 1949), sugars using Anthrone reagent method (Plummer 1971), protein by micro-kjeldahl method (Peach and Tracey 1955), phosphorus by Allen *et al*. (1976) and proline as suggested by Bates *et al.* (1973). Soil samples at surface (0-5 cm) and depth (20-25 cm) levels were collected from beneath the plants and available moisture at both levels were calculated in % basis. All the analyses were conducted in triplicate and repeated twice for confirmation. The pooled data of entire season were analyzed statistically as per the methods of Gomez and Gomez (1984) and are presented in graphical and tabular forms.

RESULTS AND DISCUSSION

Soil samples were collected monthly beneath the canopy of plants at both sites and the moisture contents at surface (0-5 cm) and depth (20-25 cm) levels were expressed in percentage basis (Fig.1). Estimated values of different eco-physiological parameters, *viz.*

Table 1. Physiochemical parameters of *B. sindica* during different months at site I.

			Months			
Parameters	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Leaf moisture (%)	85.6	82.2	81.2	77.6	70.1	62.7
Osmotic Potential (-MPa)	0.76	1.08	1.28	1.35	0.99	2.40
Proline (µg g^{-1} f. wt.)	0.012	0.012	0.820	1.889	9.398	8.003
Total chlorophylls (mg g^{-1} f. wt.)	1.550	1.767	1.932	1.277	1.304	1.139
Total sugars (mg g^{-1} d. wt.)	29.58	27.78	28.49	29.54	30.55	23.87
Crude protein (% d. wt.)	7.083	4.583	4.922	4.349	4.010	2.266
Phosphorus (% d. wt.)	0.293	0.311	0.182	0.158	0.176	0.137

Table 2. Physiochemical parameters of *B. sindica* during different months at site II.

			Months			
Parameters	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Leaf moisture (%)	84.8	81.6	80.6	76.8	71.2	67.6
Osmotic Potential (-MPa)	0.82	1.04	1.21	1.58	1.46	2.11
Proline (µg g^{-1} f. wt.)	0.012	0.012	0.012	0.280	4.785	5.589
Total chlorophylls (mg g^{-1} f. wt.)	1.566	1.782	1.775	2.082	1.983	1.296
Total sugars (mg g^{-1} d. wt.)	28.76	23.72	26.77	29.33	34.37	28.72
Crude protein (% d. wt.)	8.203	5.130	5.417	4.818	5.260	2.630
Phosphorus (% d. wt.)	0.316	0.353	0.338	0.339	0.261	0.300

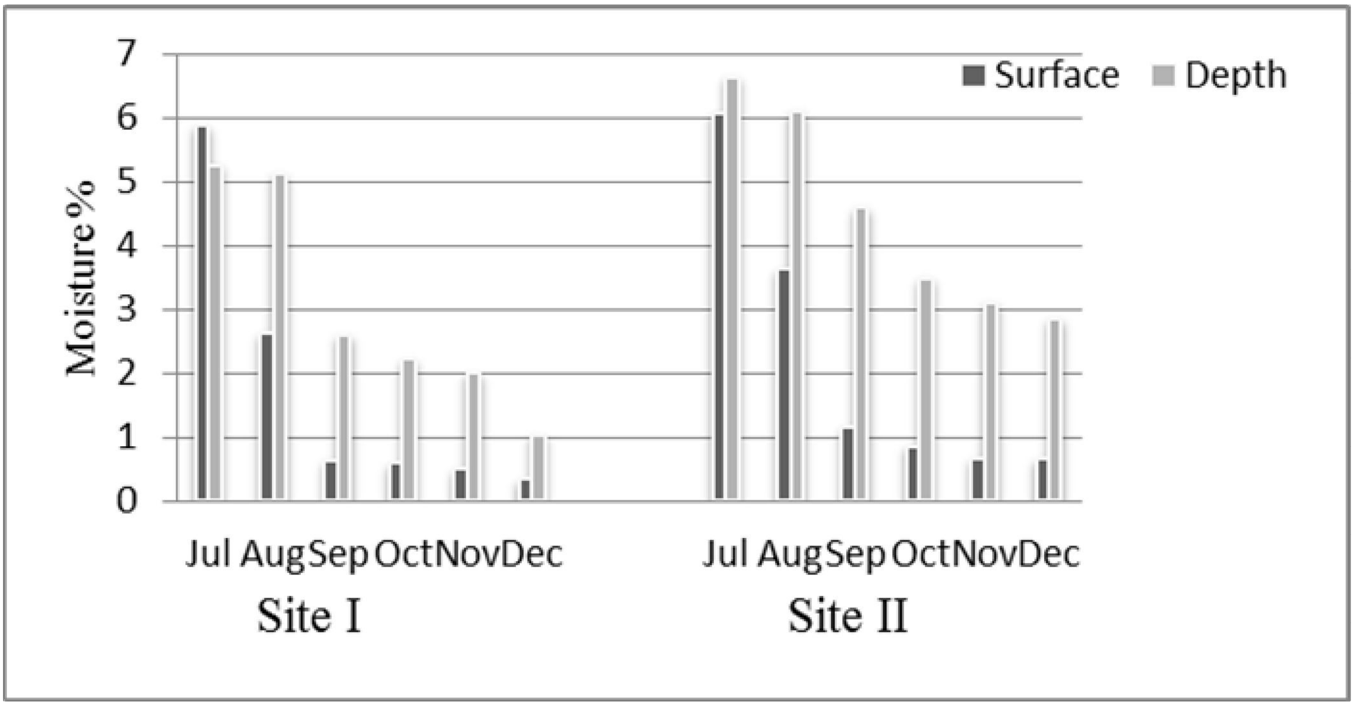

Figure.1. Monthly variations in soil moisture (%) at surface (0-5 cm) and depth (20-25 cm) under the canopy of *B. sindica* plants growing at sites - I and II.

leaf moisture, osmotic potential, leaf pigments, proline, total sugars, crude protein and phosphorus at both sites are presented in Tables 1 and 2. Soil moisture (%) was observed maximum in July at both sites being maximum (6.07 %) at surface for site-II. With a regular decreasing trend, the minimum values (0.33 %) found at site-I in December. At deeper layer (20-25 cm) it was observed maximum (6.64 %) at site-II in July whereas minimum (1.04 %) at site-I in December (Fig.1). The data were significant at 1% probability level for both surface and depth.

Moisture (%) in leaves also showed a similar gradient according to that of soil moisture. Leaf moisture (%) values were found higher at site-I when compared to plants at site-II. Maximum (85.6 %) and minimum (62.7 %) values of leaf moisture were observed at site -I in July and December, respectively. The data were significant at 1% probability level during the season at both sites.

Osmotic adjustment is recognized as an effective component of biotic resistance in several plants. Plant species reveal many differences in sensitivity and reaction to water deficit that results from drought, frost or salinity (Vahid *et al*. 2011). Plants acclimatize to the effect of low OP by accumulating compatible solutes or osmoprotectants such as proline to maintain their cellular homeostasis (Rontein *et al*. 2002). Mohammed *et al*. (1998) reported that OP in *Cassia italica* always remained higher during dry periods and lower in moist period. During growth season (July-December), a positive relationship between proline accumulation and OP was observed in this plant by Mohammed *et al.* (2012). In the present study, the OP values were observed lower in July, *i.e.* most humid month during study period, whereas highest values were in late season when soil and leaf moisture (%) were minimum. An increasing trend in OP was observed during growth season (July-December) except November, when a decrease was observed. Maximum (-2.40MPa) value of OP was estimated in December whereas minimum (-0.76 MPa) in July at site-I. The data were found significant at 5% probability level during different months.

Proline accumulates as an endogenous osmoprotectant in the cytosol as a second line of defence in plants for maintaining the water potential during stress situation and also for maintaining the integrity of cellular organelles and functions in addition to its involvement in many physiological processes. Different biotic and abiotic stresses induce a rapid accumulation of free proline in plants (Bhamburdekar and Chavan 2011). In higher plants, there is a strong correlation between increased cellular proline level and the capacity to survive under water deficit (Patel *et al*. 2005). The increased level of proline during stress period could be due to *de novo* synthesis or protein hydrolysis as reported by Nath *et al.* (2005). In the present study, proline accumulation was found at minimum level with the onset of growth season in July. Minimum (0.012 µg g^{-1} f. wt.) value of proline was observed in July at both sites and reached at maximum levels in the end of growing season. Maximum (9.398 µg g^{-1} f. wt.) value for accumulated proline was observed at site-I in November. Higher proline as well as lower protein contents in late growth stages clearly indicated proteolytic adjustment toward late phases of plant life.

Total chlorophyll values were observed to be lower during seedling and late senescence stages while higher in middle of growth period (September-October). Total chlorophylls were observed to be minimum (1.139 mg g^{-1} f. wt.) in December at site-I and maximum (2.082 mg g^{-1} f. wt.) in October at site-II. Khatun *et al.* (2003) documented that chlorophyll contents in *Moringa oleifera* increased remarkably upto maturety stage and thereafter decreased drastically at the senescence stage. Kedia *et al.* (2009) also reported higher values of total chlorophyll during flowering stage in *Peganum harmala* as compared to seedling and senescence stages. The data obtained during different months were highly significant at 5% level.

Total sugars were higher at the onset of seedling stage in July. It decreased in August, afterwards it showed a regular increasing pattern till November, *i.e.* prior to commencement of senescence. Total sugars were found to be minimum (23.72 mg g^{-1} d. wt.) in August and maximum (34.37 mg g^{-1} d. wt.) in November at site-II. The data were found significant at 5% level. Higher amount of sugars were found in early stages of plant growth in *Leucaena leucocephala* (Mishra and Bhatt 2004). Kedia *et al*. (2008) also observed higher amount of sugars in seedling stage of *Phyllanthus fraternus*. The decline in carbohydrate content during late season may result from an imbalance between carbon assimilation during photosynthesis and consumption in respiration as stated by Liu and Huang (2001). During stress periods photosynthetic apparatus provide signals about photochemical, metabolic and molecular rearrangement for stress adaptations (Biswal *et al.* 2011). Higher values of total chlorophylls during mid season also favor the increased values of total sugars in late growth stage in this species.

Protein content of various plant tissues decline under drought and saline conditions, because of increased proteolysis and decreased protein synthesis (Mohammed and Sen 1994). Mathur and Sundaramoorthy (2006) reported maximum protein contents in root and stem parts of *Blepharis sindica* during winter season. During present study the highest crude protein values were observed in seedling stage whereas lowest was observed in late season when proline contents were found to be maximum. Maximum (8.203 % d. wt.) amount of crude protein was reported in July at site-II, whereas minimum (2.266 % d. wt.) in December at site-I. The data were highly significant at 1% probability level.

The maximum values of phosphorus were recorded in growing stage in August (Tables 1 and 2). Bawa (1992) found maximum average phosphorus in the Indian desert grasses during rainy season and minimum in winter season. The concentration of phosphorus was more at the time of new foliage formation followed by a gradual decrease with advancement of growing season upto leaf fall in *Terminalia arjuna* (Naidu and Swami 1994). Phosphorus contents were found more at site-II. Maximum (0.353 % d. wt.) value of phosphors was observed in August at site-II and minimum (0.137 % d. wt.) in December at site-I. The data were found significant at 1% level.

Thus, present study reveals that different physio-chemical parameters such as leaf moisture percentage, osmotic potential, leaf pigments, proline, total sugars, crude protein and phosphorus contents varied significantly according to different growth phases of the plant. Maximum amount of soil moisture percentage was reported in deeper (20-25cm) layers, while leaf moisture and crude protein in July. Higher values for proline and total sugars were found in late season, *i.e.* November and December, whereas total chlorophylls and phosphorus during middle of season, *i.e.* August to October. Before the metabolic efficiencies slow down, the plant shows adjustment toward stress. Osmotic adjustment and flexibility within the interdependency of different biochemical pathways help the species to under go reproductive development for ensuring next generation. Ecologically the plant species faces greater constraints in its life cycle. Minimum habitat disturbances and anthropogenic activities will surely help the species to overcome pressure from its genetic makeup.

Financial assistance received from CSIR, New Delhi in the form of SRF-NET File No. 08/544(0001)/2009-EMR-I 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 Professor Dr. David N. Sen (Retd. Head), Department of Botany, J.N.V. University, Jodhpur for valuable suggestions and guidance 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.

Allen SE, Grimshaw HM, Parkinson JA, Quarmby C and Roberts JD 1976 Chemical analysis. In: *Methods in Plant Ecology*, (ed.) Chapman SB. Blackwell Scientific Publications, Oxford.

Arnon DI 1949 Copper enzymes in isolated chloroplasts. I. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*. **24** 1-15.

Bates LS, Waldren RP and Teare ID 1973 Rapid determination of free proline for water stress studies. *Plant Physiol* **41** 205-207.

Bawa AK 1992 Ecological studies of some arid zone grasses. Ph D. Thesis, J N Vyas University, Jodhpur.

Bhamburdekar SB and Chavan PD 2011 Effect of some stresses on free proline content during Pigeon pea (*Cajanas cajan*) seed germination. *Jour. Stress Physiol.* and *Biochem*. **7** (3) 236-241.

Bhandari MM 1990 *Flora of Indian Desert*. MPS REPROS, Jodhpur, 433.

Biswal B, Joshi PN, Raval MK and Biswal UC 2011 Photosynthesis, a global sensor of environmental stress in green plants: stress signaling and adaptation. *Curr. Sci*. **101**(1) 47-56.

Gomez KA and Gomez AA 1984 *Statistical Procedures for Agricultural Research* (2nd ed.). John Wiley and Sons, New York.

Janardhan KV, Murthy ASP, Giriraj K and Panchaksharairah S 1975 A rapid method for determination of osmotic potential of plant cell sap. *Curr. Sci.* **44** (11) 390-391.

Kedia S, Kasera PK and Mohammed S 2008 Phytochemical analysis during different seasons and growth stages in *Phyllanthus fraternus*, an important medicinal plant of the Indian arid zone. *J. Plant Biol.*, **35** (1) 47-51.

Kedia S, Kasera PK and Mohammed S 2009 Ecophysiology of *Peganum harmala* L. during different seasons and growth stages. *Proc. Nat. Acad. Sci. India*, Sect. B, **79** Pt. III 267-270.

Khare CP 2001 *Indian Medicinal Plants - An Illustrated Dictionary*. Springer-Verlag, Berlin, New York, USA, 812.

Khatun S, Absar N and Ashraduzzaman M 2003 Changes in physiochemical compositions and activities of some hydrolytic and oxidative enzymes in two types of Sajna (*Moringa oleifera* lamk.) leaves at different maturity levels. *Indian J. Plant Physiol*. **8** 6-11.

Liu X and Huang B 2001 Seasonal changes and cultivars differences in turf quality, photosynthesis and respiration of creeping bentgrass. *Hort. Sci*., **36** 1131-1135.

Mathur M and Sundaramoorthy S 2006 Ecological and aphrodisiac properties of *Blepharis sindica. Nig. J. Nat. Prod. and Med*. **10**17-25.

Mishra S and Bhatt RK 2004 Varietal differences in photosynthetic pigments and biochemical constituents in *Leucaena leucocephala*. *Indian J. Plant Physiol.* **9** 86-89.

Mohammed S, Kasera PK, Chawan DD and Sen DN 1998 Ecophysiology of *Cassia italica* (Mill.) Lamk. Ex. Anders. in Indian desert. *Sci. Cult.*, **64** 233-234.

Mohammed S, Lal P and Kasera PK 2012 Proline accumulation in *Blepharis sindica* T. Anders: A vulnerable medicinal plant growing in the Indian Thar desert. *Asian Journal of Plant Science and Research*, **2** (5) 588-592.

Mohammed S and Sen,DN 1994. Seasonal variations in sugar and protein contents of halophytes in Indian desert. *Annals Arid Zone*, **33** 249-251.

Naidu CV and Swami PM 1994 Seasonal variation in N, P and K contents in different plants in tropical deciduous tree species. *Indian J Plant Physiol* **37** 224-228.

Nath AK, Kumari S and Sharma DR 2005 *In-vitro* selection and characterization of water stress tolerant cultivars of bell pepper. *Indian J Plant Physiol* **10** 14-19.

Patel HE, Mahatama MK, Patel NJ, Bhatnagar R and Jageda GC 2005 Differential response of pearl millet hybrids to water stress in relation to antioxidant enzymes and proline. *Indian J plant Physiol* **10** 344-348.

Peach K and Tracey MV 1955 *Modern Methods of Plant Analysis*. Springer-Verlag, Berlin.

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

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 and Research* **2** (4) 409-413.

Rontein D, Gilles B and Andrew DH 2002 Metabolic engineering of osmoprotectant accumulation in plants. *Metabolic. Eng.* **4** 49-56.

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

Singh V, Wadhwanii AM and Johari BM 1996 *Dictionary* of Economic Plants in India. ICAR, New Delhi.

UNDP, 2010 *Rajasthan Red Listed Medicinal Plants.* http://www. frlht.org, Banglore.

Vahid B, Behrouz S, Mahmood K and Abolfazal R 2011 Protein electrophoretic profiles and physiochemical indicator of salinity tolerance in Sorghum (*Sorghum bicolor* L.). *African Jour. of Biotech*. **10** (14) 2683-2697.