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Dependency of primary metabolites production and their variability against climatic factors in *Blepharis sindica* T. Anders: a vulnerable medicinal plant from the indian arid zone

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ABSTRACT:

The present paper deals with the quantitative production and pattern of variation in various primary metabolites of *Blepharis sindica* T. Anders (Acanthaceae) during different months of evaluation in response to prevailing environmental conditions. The metabolites, *viz.* leaf pigments (chl. *a*, chl. *b* and carotenoids), proline, sugars (soluble, insoluble and total), crude protein and phosphorus contents varied significantly according to different growth phases of the plants. The amount of water in and out of the plant body strongly influenced the biosynthesis rate of these metabolites primarily, whereas the growth stage and temperature affects were found secondarily. Higher values for accumulated proline, carotenoids and phosphorus contents were observed during the end of growing season, *i.e.* December; while chlorophylls (chl. *a*, *b* and total) during middle of season, *i.e.* August to October. Total sugars and crude protein values were highest during July with a clear negative correlation having proline accumulation.

Keywords:

Blepharis sindica, medicinal plant, primary metabolites, vulnerable, climatic factors.

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INTRODUCTION

Arid zones are well known for their poor vegetation cover in comparison to other habitats. The Indian arid zone has a unique composition of floristic diversity in a fine adjustment with climatic peculiarities of the area where water demand by vegetation and crops are high but availability is restricted by scanty rainfall and long dry periods throughout the year (Raghav and Kasera, 2012). Besides harsh conditions and much constrains on growth potentials, the plant species of arid zone synthesize and accumulate a variety of bioactive compounds which play a vital role in the treatment of different health problems worldwide. Habitat destruction, unscientific collection, ecological limitations, etc. are some potent threats which are pushing valuable medicinal plant diversity under the verge of extinction. *B. sindica* (Acanthaceae) is categorized as a vulnerable medicinal herb from Indian Thar desert (UNDP, 2010).

Blepharis sindica T. Anders (Family: Acanthaceae) is an annual, dichotomously branched, lignified herb (Fig. 1; a - c). Local communities know this plant as Billi Khojio/Bhangara/Unt-Katalo (Bhandari 1990). It grows on loose soils, along crop fencings and much especially on dune slopes having sandy soil with heavy percolation. During extreme winter and successive summer periods; the seed loaded capsules attached on the lignified dried stem within spiny spikes are the only interacting parts of its life cycle. The plant starts to grow by means of seeds after first rainfall which enables fruit wall to split explosively from the distal tapered end and release the seeds to absorb water. These plants fulfill their life-cycle in between the period of July to December. Seeds have compactly clothed hygroscopic hairs which are potent component of different herbal and aphrodisiac stimulants (Bhandari 1990; Mathur 2012). Roots of this plant are used for treating urinary discharging problems and dysmenorrhoea. The plant powder is applied on the infections of genitals and burns to heal the ailments (Khare, 2007). Flavonoides (apigenin, blepharin, prunine-6″-O-coumarate, and terniflorin), steroid (β-sitosterol) and triterpinoide-oleanolic acid are contained in the seed (Ahmad *et al*. 1984).

In the present investigation an attempt has been made to assess the physiochemical responses in this species under prevailing conditions of the desert climate of Churu region. The fluctuations in leaf pigments (chl. *a*, chl. *b* and carotenoides), proline, sugars (soluble, insoluble and total), crude protein and phosphorus contents during successive months of growing season constitute main aspects of the present study.

MATERIALS AND METHODS

Plant samples were collected during July to December, 2012 and 2013 from the open areas of Buntia village (10 km towards north-east direction from the College Campus) of Churu- a part of Indian Thar desert. The leaf samples were collected from the similar nodes of different plants to maintain sample equality. Healthy and mature fresh leaves were collected in the morning hours and were mixed randomly to estimate pigments and proline on the same day. Oven dried leaf samples (80^0C; 48 h), after grinding were used for estimation of sugars, crude protein and phosphorus. Leaf pigments were estimated using Arnon's method (1949), proline as suggested by Bates *et al*. (1973), sugars using Anthrone reagent method (Plummer 1971), protein by microkjeldahl method (Peach and Tracey 1955) and phosphorus by Allen *et al*. (1976). Soil samples at surface (0-5 cm) and depth (20 - 25 cm) levels were collected underneath the plant canopies and the available moisture at both levels were calculated in percentage using oven-dry weight loss basis. All the analyses were conducted in triplicate and repeated twice for confirmation. The pooled data of entire seasons were subjected to analysis of variance (ANOVA) as per suggested by Gomez and Gomez (1984) and mean values for both seasons are presented in tabular form. The

Table 1. Analysis of various primary metabolites parameters in *B. sindica* leaves during different months (values are mean of six replicates of two successive growth seasons)

Parameters	Months						CD
	Jul	Aug	Sep	Oct	Nov	Dec	
Chl. *a* (mg g^{-1} f. wt.)	0.965	1.444	1.802	2.711	2.686	1.920	0.4215^{ns}
Chl. *b* (mg g^{-1} f. wt.)	1.113	1.821	1.395	1.556	1.363	0.7005	0.4465^{ns}
Total chl. (mg g^{-1} f. wt.)	2.378	3.779	3.795	5.160	4.990	3.726	0.7735^{ns}
Carrot. (mg g^{-1} f. wt.)	0.300	0.514	0.598	0.893	0.941	0.983	0.1340^{ns}
Proline (µg g^{-1} f. wt.)	0.012	0.870	0.146	1.889	6.770	9.423	1.5447^{*}
Sol. sugar (mg g^{-1} d. wt.)	29.191	25.254	24.603	27.917	25.811	24.406	2.8651^{*}
Insol. sugar (mg g^{-1} d. wt.)	7.377	8.462	11.075	5.000	4.742	2.885	4.4150^{ns}
Total sugars (mg g^{-1} d. wt.)	36.568	33.716	35.678	32.917	30.553	27.291	6.3253^{*}
Crude protein (mg g^{-1} d. wt.)	7.357	4.753	5.625	5.091	4.766	3.047	0.6083^{ns}
Phosphorus (mg 100 g^{-1} d. wt.)	23.992	24.992	25.792	23.850	27.883	29.250	0.0555^{*}

*= Significant at $p< 0.05$; and ns = non-significant.

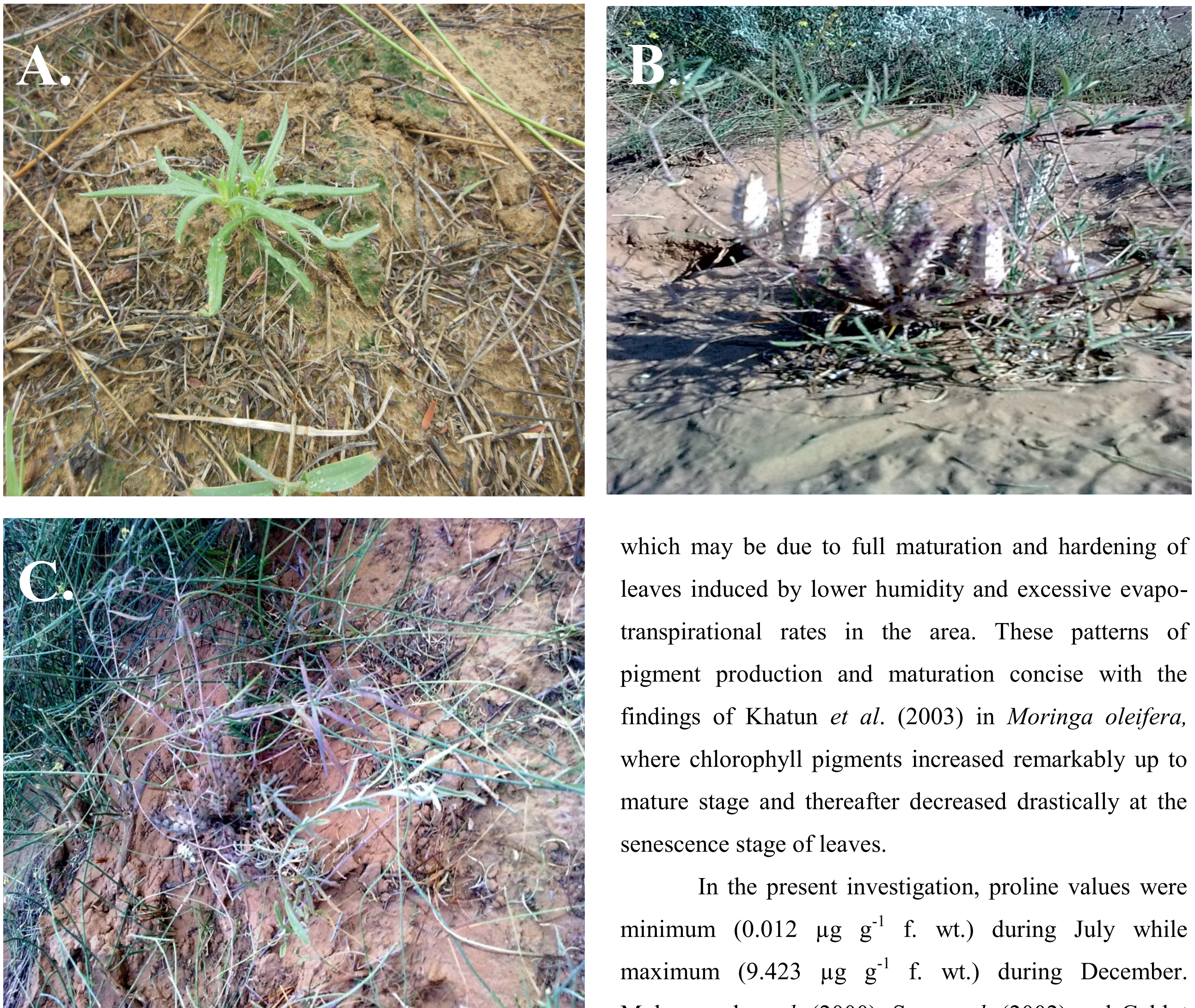
Table 2. Mean values of meteorological data during different months (source: Meteorological Centre, Jaipur, India) and soil moisture (%) at surface and depth levels at particular site from Churu

Parameters	Months					
	Jul	Aug	Sep	Oct	Nov	Dec
Minimum temperature (^{0}C)	27.70	26.40	24.10	18.55	13.25	5.30
Maximum temperature (^{0}C)	37.80	34.75	34.00	36.10	30.80	24.75
Minimum relative humidity (%)	55.50	66.00	61.00	31.00	44.50	43.00
Maximum relative humidity (%)	72.50	85.50	81.50	64.50	75.50	82.00
Total rainfall (mm)	90.65	237.10	105.70	0.00	7.65	15.90
Soil moisture at surface (0-5 cm)	4.93	2.91	1.19	0.62	0.69	0.58
Soil moisture at depth (20-25 cm)	4.62	6.24	5.34	2.79	2.89	2.56

RESULTS

The observed values for various primary metabolites in *B. sindica* during different months are presented in Table 1, while the mean values of meteorological data are shown in Table 2. It is evident from Table 1 that the values of these primary metabolites varied significantly during the growing season, *i.e.* July to December which indicates their interdependency to cope the environmental constrain along and over the life span of the species. Highest amounts of chlorophyll *a* (2.711 mg g^{-1} f. wt.) and total chlorophylls (5.160 mg g^{-1} f. wt.) were recorded during October, while chlorophyll *b* (1.821 mg g^{-1} f. wt.) in August. Carotenoids were maximum (0.983 mg g^{-1} f. wt.) at the ending of growth season, *i.e.* December. The amounts of total sugars were observed higher at the onset of growth season in July and lower at the commencement of plant death in December. Highest values for soluble (29.191 mg g^{-1} d. wt.) and total sugars (36.568 mg g^{-1} d. wt.) were found during July, while insoluble (11.075 mg g^{-1} d. wt.) in September. Crude protein values were highest (7.357 mg g^{-1} d. wt.) in July, while lowest (3.047 mg g^{-1} d. wt.) in December. Phosphorus contents were maximum (29.250 mg g^{-1} d. wt.) in December. The ANOVA showed that temporal variations were significant ($p<0.05$) for accumulated proline, soluble sugars, total sugars and phosphorus contents whereas pigment, insoluble sugars and crude protein values were found to be non-significant (Table 1). Periods of July- September, October-November and December-February were

Figure 1. ***Blepharis sindica*****: A juvenile plant on dune slope in July (a), fully developed plant with matured spikes on branching nodes and ready to detached leaves on branch endings during October-November (b), and plant in December with purplish colour leaves indicating the cold-stress intensity over the species.**



considered as rainy, post-monsoon and winter seasons, respectively for the area (Table 2.).

DISCUSSION

In the present study, the values for various pigments were found to be higher during the rainy months. This pattern is similar to that reported by Jain and Ahrodia (2007) in *Guazuma tomentosa*, Kedia *et al*. (2008 and 2009) in *Phyllanthus fraternus* and *Peganum harmala* and by Gehlot and Kasera (2013) in *Phyllanthus amarus*. As a slight deviation, the total chlorophylls were highest during October (post-monsoon/rainy season), which may be due to full maturation and hardening of leaves induced by lower humidity and excessive evapo-transpirational rates in the area. These patterns of pigment production and maturation concise with the findings of Khatun *et al*. (2003) in *Moringa oleifera,* where chlorophyll pigments increased remarkably up to mature stage and thereafter decreased drastically at the senescence stage of leaves.

In the present investigation, proline values were minimum (0.012 µg g^{-1} f. wt.) during July while maximum (9.423 µg g^{-1} f. wt.) during December. Mohammed *et al*. (2000), Sen *et al*. (2002) and Gehlot and Kasera (2013) reported maximum proline accumulation during winter season in *Trianthema triquetra, Zygophyllum simplex* and *Phyllanthus amarus*, respectively. In the present study, proline accumulation was also maximum during winter season, *i.e*. the month of December, which was in agreement with the above-mentioned investigations. The maximum values during December may be due to lower values of temperatures and soil moisture contents as compared to other months (Table 2). 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). Higher proline as well as lower protein contents in *B. sindica* clearly indicated proteiolytic adjustment in the late phases of plant growth. This pattern confirmed the biochemical breakdown of proteins to maintain cellular integrity by means of proline synthesis during plant senescence and hampered anabolism in severe cold.

Young seedlings of *B. sindica* exhibited the highest amount of sugars, that is in a clear relation with the pattern in *Leucaena leucocephala* (Mishra and Bhatt 2004) and *Phyllanthus fraternus* (Kedia *et al*., 2008). The decline in carbohydrate contents during late season might be a result from an imbalance between carbon assimilation by photosynthesis and consumption for 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). These observations clearly indicated about the slowdown of photosynthetic capabilities and maximal energy utilization for seed setting in old plants. Decreased values for total sugars during August (rainiest month) favoured its negative correlation with higher moisture levels (Table 2), which in-turn links the present findings with strong requisite for low moisture in the species (Lal *et al*., 2014).

Generally the crude protein contents decline during drought and saline conditions, as a result, increased proteolysis and decreased protein synthesis happens (Mohammed and Sen, 1994). Root and stem parts of *B. sindica* exhibited maximum protein contents in winter season as observed by Mathur and Sundaramoorthy (2006). In the present study, highest values of the crude protein were observed in leaves during the initial growth stages of the plant. This trend illustrated the correlation of photosynthetic efficiency with that of protein contents in leaves besides a separate protein pool of stem and root parts in this species. Gehlot and Kasera (2013) recorded higher crude protein values during rainy season than winter in *Phyllanthus amarus*.

Phosphorus values are considered to be linked with foliar sprouting and seed formation in plants. Bawa (1992) found maximum average phosphorus in the Indian desert grasses during rainy season and minimum in winter season. The concentration of phosphorus is usually considered with new foliage formation followed by a gradual decrease with the advancement of growing season up to leaf fall (Naidu and Swami 1994). Nutrient uptake and utilization efficiency studies revealed that this species seems to be a better nutrient efficient for phosphorus uptake (Mathur 2013). Present observation about phosphorus concentration in late season proves the fact observed by Mathur (2013), whereas low phosphorus amounts during October indicates the excessive mineral integration in DNA for seed developmental necessities.

CONCLUSION

The present study reveals that as a result of differences between inherent efficacy to utilize availed resources and hindrances caused by environmental limitations and plant ageing, the species has to adopt differential metabolic adjustments along the growing season with a clear aim to replenish seed pool of the area. Chlorophyll amounts seem to be a resultant of leaf maturation with is favoured by low humidity in and around the plants. Higher chlorophyll *b* amounts in contrast to chlorophyll *a* during August, and carotenoids during the end of the season reflected the role of accessory pigments to compensate photosynthesis loss caused by assimilation incompatibility by rains and cold temperature, respectively (Figure 1; c). Excessive accumulation of proline during late season indicates the effect of cold stress and hampered metabolism which is confirmative with protein breakdown. High level of phosphorus contents at the end of season further confirms the tendency of this lignified species for bio-magnification of minerals.

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