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SHORT COMMUNICATION

VARIATIONS IN SECONDARY METABOLITES IN SOME ARID ZONE MEDICINAL PLANTS IN RELATION TO SEASON AND PLANT GROWTH

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In the present investigation, attempt has been made to find out variations in total alkaloids and phenols due to different seasons and plant growth stages in *Asparagus racemosus, Boerhavia diffusa* and *Sida cordifolia*. Results revealed that maximum accumulation of alkaloids and phenols occurred in summer season in all the three plant species. Peak concentrations of alkaloids and phenols were observed in flowering stage in all the plants except in *A. racemosus* that showed maximal accumulation of phenols in the vegetative stage. Interestingly, no alkaloids or phenols accumulated in the seedling stage.

Key words: **Alkaloids, *Asparagus racemosus*, *Boerhavia diffusa*, phenols, *Sida cordifolia***

Secondary plant products are the major sources of important chemicals such as drugs, insecticides, dyes, flavours and phytomedicines. There is an optimum time for harvest when plants contain maximum amount of secondary metabolites. The rates of biosynthesis and catabolism reflected in the pool size of alkaloids in plants tissues vary with respect to physiological states of plant development, diurnal variation and the functionally different parts of the plant (Waller and Nowacki 1978).

It is important to realize that while plant alkaloids comprise of about 15.6% of the known natural products, they constitute almost 50% of the plant-derived natural products of pharmaceutical and biological significance (Cordell *et al*. 2001). Phenolic constituents are involved as active principles in a number of medicinal plants (Harborne 1997). In the phenolic compounds, several flavonoids (such as scutellarin, icariin, isorhamnetin and puerarin) and coumarins (such as daphnetin and daphnoretin) are stated to show cardiovascular activity (Craker and Simon 2002). Although the levels of phenols and alkaloids biosynthesis are gene-governed, there are remarkable fluctuations in the concentrations and contents of alkaloids due to environmental influences.

Given that the quantitative estimation of secondary metabolites can determine the optimal harvesting time, the temporal (summer, rainy and winter seasons) and physiological (seedling, vegetative and flowering growth stages) variability in alkaloids and phenols was evaluated in three important medicinal plants of the Indian Thar desert, i.e. *Asparagus racemosus* (Shatawar; Fam.: Asparagaceae), *Boerhavia diffusa* (Punarnava; Fam.: Nyctaginaceae) and *Sida cordifolia* (Bala; Fam.: Malvaceae).

The plant materials were collected randomly from the natural habitats of J.N. Vyas University Campus, Jodhpur (Rajasthan) in different seasons (rainy: July-September; winter: December-February; and summer: April-June) and at physiological stages during 2004 & 2005. Total phenols (µg g^{-1} d.wt.) were estimated with the help of Folin-Ciocalteau reagent (Bray and Thorpe 1954), taking note of the colour produced by the reaction

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of phenols with phosphomolybdic acid in Folin-Ciocalteau reagent in the alkaline medium. The standard was prepared with caticol phenol. Total alkaloids (%) were quantified according to Higuchi and Bodin (1961) and their identities were confirmed by positive colour tests with Dragendroff's, Mayer and Wagner's reagent (Daniel 1991). The mean values of data obtained from six replicates during the two years (2004 & 2005) for each parameter were analyzed statistically as per the methods described by Gomez and Gomez (1984).

The data on accumulation of total phenols and alkaloids during different seasons and plant growth stages are presented in Tables 1 and 2, respectively. In *A. racemosus* and *B. diffusa*, the total phenolic contents vary with seasons, being highest during summer and lowest in winter season. However, in *S. cordifolia* the maxima occurred during summer whereas minima in rainy season. The phenolic contents also varied significantly in different physiological stages, being greater in vegetative stage than in flowering stage of *A. racemosus* (Table 1). In *B. diffusa* and *S. cordifolia*, the reverse was the case. No phenolic contents were recorded in the seedling stage in any of the species investigated. Analysis of variance showed that the temporal and physiological variations were significant (P<0.01).

Table 1. Variations in total phenols (µg g^{-1} dw) during different seasons and physiological stages of plant growth (values are the mean of six replicates).

	A. racemosus	*B. diffusa*	*S. cordifolia*
Seasons			
Summer	1464.86	1621.86	1804.32
Rainy	925.56	1168.83	687.25
Winter	367.03	241.66	972.72
CD	50.19**	58.21**	26.19**
Physiological stages			
Seedling	-	-	-
Vegetative	736.28	526.52	349.65
Flowering	480.60	1027.96	622.71
CD	25.92**	16.69**	19.17**

** = Significant at (P<0.01) level, - = No formation of phenols

Table 2. Variations in total alkaloides (% dw) during different seasons and physiological stages of plant growth (values are the mean of six replicates).

	A. racemosus	*B. diffusa*	*S. cordifolia*
Seasons			
Summer	1.453	0.246	1.201
Rainy	0.593	0.140	0.306
Winter	0.706	0.180	0.853
CD	0.151**	0.0536**	0.083**
Physiological stages			
Seedling	-	-	-
Vegetative	0.77	0.155	0.463
Flowering	1.60	0.218	0.946
CD	0.111**	0.0123**	0.0744**

** = Significant at (P<0.01) level, - = No formation of alkaloids

The quantitative estimation of the total alkaloids revealed that summer season was good for their production. The temporal variation in the alkaloid concentration followed the order: summer >winter >rainy seasons. With reference to the physiological stages of the plant, maximum alkaloids accumulated during flowering stage followed by vegetative stage, while no accumulation occurred in seedling stage in any of the three plant species. These variations were statistically significant (P<0.01).

Plant growth and development are complex biological phenomena that depend upon genetic and environmental variables (Waller and Nowacki 1978). Plants and their environment form a complex system with the multiplicity of factorial interactions. The magnitude of fast interacting external factors affect the plant life. A moderate water deficit is believed to stimulate the secondary metabolites synthesis (Horner 1990). Since the arid zone climate consists of not only drought but also extreme variations in temperatures and scanty rainfall that may wash out the secondary metabolites and the interaction of these factors affects the plant's adaptability to its environment.

The age and the stage of growth of the plant generally have an impact on phenolic contents (Wong

1973). Seasonal variation and disappearance at certain stages suggest degradation of phenolic compounds in the plant (Wong 1973). Senea *et al*. (2001) observed increased phenolic production in *Sorghum* due to water stress. In the present study, maximum phenolic contents were recorded in summer in all the three plant species. The minimum values were observed during winter in *A. racemosus* and *B. diffusa*, while during rainy season in *S. cordifolia.* No phenolic contents could develop in the seedling stage. Higher amounts in *A. racemosus* were observed in the vegetative stage than in the flowering stage. In *B. diffusa* and *S. cordifolia*, higher phenolic contents were reported in flowering stage than in the vegetative stage.

Many cases have been reported where the alkaloid contents of the plant show seasonal variations and in quite a few species it is reported to be maximum during or just prior to flowering (Waller and Nowacki 1978). The rates at which alkaloids accumulate vary with the environmental and nutritional conditions under which the plants are grown and often also with the stage of plant development. In younger plants the alkaloid content is low, gradually increasing with age upto a certain period followed by a decreasing tendency (Waller and Nowacki 1978). Bernath (2002) reported that in *Nicotiana tabacum*, the total alkaloids and proportion of nicotine were the highest under warm conditions. Sreevalli *et al*. (2001) reported maximum leaf alkaloid in periwinkle plants irrigated only once in three weeks, suggesting that water-deficit conditions increased alkaloid accumulation. Nandi (1996) reported maximum accumulation of alkaloids in the roots of *Rauvolfia serpentina* and *Catharanthus roseus* and in the leaves of *Datura innoxia*, *Atropa belladonna* and *Hyoscyamus niger* during reproductive (flowering) stage of plants, showing a positive correlation with the flower formation. In the present study, maximum alkaloids were found in the summer and minimum in rainy season in all the three plants investigated. This is because dry conditions favour a high accumulation of alkaloid contents. Alkaloid synthesis was also found to be correlated with flower formation during reproductive stage of the plants. Among the physiological stages, the maximum alkaloids were found in flowering stage as compared to vegetative stage, while no accumulation in the seedling stage in any of the three plant species. Thus, it could be stated that the rate of alkaloids synthesis remained associated with the reproductive stages. Finally, it is concluded that these medicinal plants behave differently under different seasonal conditions and growth stages in the Indian Thar desert.

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