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SPATIO-TEMPORAL VARIATIONS IN PHYSIOCHEMICAL ATTRIBUTES OF *ADIANTUM CAPILLUS VENERIS* FROM SOONE VALLEY OF SALT RANGE (PAKISTAN)

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Abstract

In this study, spatio-temporal variations in biochemical and physicochemical parameters of *Adiantum* shoots during different seasons and sites of Soone Valley in Salt Range were investigated. *Adiantum* was located only at two sites *i.e.* at Knotty Garden during all the four seasons and at Dape Sharif site only during spring. All the parameters studied varied significantly during different seasons and at both sites. It was observed that dry matter, fiber and total mineral contents were the maximum during winter whereas, moisture, fat and protein contents were the highest during autumn at Knotti Garden. In contrast, NFES and NFE were found higher during spring at Dape Sharif. Among the ionic contents studied, Ca, P and Fe were higher during spring at Dape Sharif where as N and K contents were more during autumn at Knotti Garden. Mg and Zn were the maximum during summer and spring at Knotti Garden respectively. Partial RDA of the data revealed that phenols and flavonoids were associated with spring at Knotti Garden where as, alkaloids were associated with spring at Dape Sharif. It was concluded that association of phenolics and falvonoids with Knotti Garden was due to low temperature stress, where that of alkaloids with Dape Sharif with high pH and EC of the site.

Introduction

Pakistan represents a unique ecological zone of the world as it contains a wide variety of ecologically diverse habitats. It contains almost all types of well-known ecosystems e.g., deserts, snow-caped mountains and large water-bodies, temprate decidious forests, evergrenn forests and a wide variety of rangelands. Among the rangelands of Pakistan, the Soone Valley of Salt range (co-ordinates 72°00 and 72°30 E and 32°25 and 32°45 N) covers an area of 300 km^2, 20 km in length and 5 km in breadth and with average elevation of 762 m. Relatively low annual rainfall (20 inches or less), average minimum temperature as 1 ^{O}C (during January) and average maximum temperature as 36 ^{O}C (during June) are the unique charistics of this area that supports a rich biodiversity of this area (Afzal *et al.*, 1999).

A large number species loaced in this area are traditionally popular as healing agents and have been used by indigenous people. These plants that possess therapeutic or exert beneficial pharmacological effect have a number of secondary metabolites like alkaloids, phenols, flavonoides, tannins, volatile oils (Edeoga *et al*., 2005; Okwu, 2004).

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Adiantum capillus veneris is one on the most common species located in this area that is of a potential importance for midicnal purpose. Its shoots are commonly used for different health problems by the indigenous peoples of Salt Range especially in Soone valley. *Adiantum* species are used for chest complaints, cough, expectorant, increase lactation, colds, aid kidney function, antiparasitic, dandruff, general cure-all. The fresh or dried leafy fronds are antidandruff, antitussive, astringent, demulcent, depurative, emetic, weakly emmenagogue, emollient, weakly expectorant, febrifuge, galactogogue, laxative, pectoral, refrigerant, stimulant, sudorific and tonic(Grieve,1985). In Nepal, a paste made from the fronds is applied to the forehead to relieve headaches and to the chest to relieve chest pains. The plant is best used fresh, though it can also be harvested in the summer and dried for later use **(**Chiej, 1984; Launert, 1981).

The present study was planned to determine the effect of different sites and seasons on physiological attributes of *Adiantum* in Soone valley. Moreover, we aimed to draw relationships between different physiochemical attributes and the environmental variables (seasons and sites)

Material and Method

Sites: This study was conducted to access the spetio-temoral variations in the active ingrideints of the vegetation located in the Soone Valley of salt range. In this study, the six sites (Khabeki, Khoora, Dap Sharif, Anga, Knotty Garden and Jaller) were selected. However, *Adiantum* was located on two sites with the following attributes:

Meteorological data: Meteorological data were recorded at Horticultural Research Station, Soon Valley for the entire study period. The data about rain fall, maximum and minimum temperature have been presented in table 3.1.

Soil analysis: Soil texture was determined by using hygrometer method (Dewis & Freitas, 1970). The physicochemical characteristics are presented in tables 3.4 and 3.5. Electrical conductivity pH and ions of saturation extracts were determined according to Rhoades (1982) and Jackson (1962).

Physicochemical parameters: Moisture contents**,** dry matter, crude fibers, mineral contents, fat contents, Nitrogen Free Extractable Substances (N.F.E.S.) and net free energy were calculated using following the method of AOAC (AOAC, 1984).

Determination of macro- and micro-nutrients: From the digested material Sodium (Na^+) Potassium (K^+) and Calcium (Ca^{2+}) contents were analyzed using flame photometers (Jenway PFP-7). Iron, manganese, zinc and magnesium were analyzed using atomic absorption spectrophotometer (Perkin Elmer).

Nitrogen estimation: Nitrogen was determined using micro Kjeldahl method (Bremner, 1965). The phosphorus contents were estimated by spectrophotometer (Jackson, 1962).

Flavonoids: Flavonoid contents of samples were determined by using the standard method of Dewanto *et al*., (2002). One milliliter of aqoues extract containing 0.1 g/mL of

Table 1: Geographical aspects of the sites selected in the Soone Valley of the Salt Range

Sites	Coordinates	Elevation (m)	Slope (%)	Aspect
Dap Sharif	32.30N and 72.04 E	890	35-40	Western
Knotty Garden	32.40N and 72.14 E	783	30-35	Northern

Table 2: Plant community Structure of the sites selected in the Soone Valley of the Salt Range

Sites	Habitat Description	Vegetation Type	Plant Community
Dap Sharif	Within the valley	Shrubs and grasses	*Dodonea viscose*
Knotty Garden	Hills with steep slops	Mixture of herbs grasses and shrubs	*Justicia adhatoda and Acacia fernesiana*

Table 3: Meteorological data of different seasons in the Soone Valley of the Salt Range

Season	2004 -05			2005- 06		
	Average Temperature ^{o}C		Rainfall (mm)	Average Temperature ^{o}C		Rainfall (mm)
	Maximum	Minimum		Maximum	Minimum	
Autumn	32.00	20.00	31.50	32.4	20.2	96.6
Winter	19.06	3.16	23.6	12.8	4.16	0.00
Spring	20.9	10.45	111.5	22.6	11.4	82.0
Summer	37.6	22.56	38.1	33.8	23.8	61.0

Table 4: Soil characteristics of the sites selected in the Soone Valley of the Salt Range

Sites	Field Capacity (%)				Soil ECe (dS m^{-1})			
	Aut.	Win.	Spr.	Sum.	Aut.	Win.	Spr.	Sum.
Khabeki	49	54	51	51	1.7	2.7	2.1	1.3
Khoora	25	23	30	32	0.9	3.8	0.9	1.2
Dap Sharif	40	39	38	41	7.6	8.7	1.7	2.2
Anga	39	39	34	37	1.4	3.9	1.2	1.4
Knotty Garden	39	38	35	35	2.9	4.4	1.2	1.6
Jaller	46	50	48	45	1.1	3.4	5.6	4.2

Sites	Soil pH				Soil Texture
	Aut.	Win.	Spr.	Sum.	
Khabeki	7.69	7.69	8.3	7.7	Clay loam with Stones
Khoora	7.76	7.96	8.1	7.8	Sandy Clay with Stones
Dap Sharif	7.82	8.29	8.1	7.9	Sandy Clay
Anga	7.78	7.89	7.9	7.8	Sandy Clay with Stones
Knotty Garden	7.73	7.87	8.1	8.1	Sandy Clay
Jaller	7.82	7.92	8.1	8.2	Clay Loam

Table 5: Ionic concentrations in soil of the sites selected in the Soone Valley of the Salt Range

Sites	Na^+ (mg/g dry soil)				K^+ (mg/g dry soil)			
	Aut.	**Win.**	**Spr.**	**Sum.**	**Aut.**	**Win.**	**Spr.**	**Sum.**
Khabeki	0.120	0.096	0.100	0.040	0.03	0.17	0.10	0.03
Khoora	0.013	0.037	0.047	0.040	0.02	0.06	0.03	0.04
Dap Sharif	0.260	0.240	0.121	0.201	0.04	0.14	0.02	0.06
Anga	0.140	0.210	0.180	0.350	0.02	0.03	0.07	0.02
Knotty Garden	0.051	0.042	0.027	0.041	0.03	0.07	0.01	0.02
Jaller	0.326	0.360	0.356	0.390	0.02	0.02	0.01	0.02
Sites	**P (mg/g dry soil)**				**N (mg/g dry soil)**			
	Aut.	**Win.**	**Spr.**	**Sum.**	**Aut.**	**Win.**	**Spr.**	**Sum.**
Khabeki	5.1	13.0	6.5	22.5	0.09	0.09	0.09	0.17
Khoora	6.9	7.5	8.0	11.1	0.06	0.05	0.11	0.10
Dap Sharif	9.0	8.5	7.2	15.5	0.13	0.05	0.12	0.11
Anga	12.5	8.5	4.5	17.5	0.12	0.14	0.12	0.20
Knotty Garden	8.5	16.5	16.5	19.5	0.09	0.10	0.11	0.09
Jaller	7.2	8.75	7.5	9.5	0.08	0.08	0.11	0.18

dry matter was taken in a 10 mL volumetric flask, and 5 mL of distilled water and 0.3 mL of 5% $NaNO_2$ (Merck) were added and mixed. After 5 min, 0.6 mL of 10% $AlCl_3.6H_2O$ (Merck) was added. After 5 minutes 2 mL of 1 mol/L NaOH (Merck) was added and the volume was made up to 10 mL with distilled water. The solution was mixed well and the absorbance was measured immediately at 510 nm on spectrophotometer. Flavonoid contents were calculated using a standard calibration curve developed by using the known concentrations of epicatechin (200-800 ppm). The results were expressed as mg per g equivalent to epicatechin.

Alkaloidal contents: The alkaloidal contents of samples were determined gravimetrically by using Harborne (1973) method. Dried finely ground 5 g of the sample was weighed into a 250 ml beaker and 200 mL of 10% acetic acid in ethanol was added. Beakers were covered and allowed to stand for 4 hours. Then extract was taken by filtering the entire material and was concentrated on a water bath to one-quarter of the original volume. Then concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete and fumes stopped to evolve. The whole solution was allowed to settle and the precipitate was collected on already oven dried and weighed filter papers after washing with dilute ammonium hydroxide. Then the precipitate was dried in the oven at 40-45 ^oC till constant weight was obtained on the filter papers. The residue is the alkaloid, which was calculated as mg per g of the dry plant material. The method was optimized by comparing with spectrophotometric method (Sreevidya & Mehrotra, 2003).

Statistical analyses: Data recorded were analyzed statistically by using Partial Redundancy Analysis (*p*RDA) with the help of Canoco Package for windows version 4.5 (2002). The Multivariate Direct Gradient Model was fitted and all variables were plotted on CCA Axis 1 and 2. LSD (5%) was used to compare mean values.

Results

Adiantum capillus veneris was found to be one of the rarest species as it was found at relatively fewer sites as well as seasons. It was observed that *Adiantum* was found only at Knotti Garden during all the four seasons and at Dape Sharif only during spring season. It is clear that different physiological parameters varied significantly during different seasons and at different sites. Maximum DM was found at Knotti Garden during winter season followed by summer season. During spring season almost equal DM production was observed at Knotti Garden and Dape Sharif whereas lowest DM was observed at Knotti Garden during autumn season.

Moisture contents also varied significantly during the seasons and Sites. Maximum MC were observed at Knotti Garden during autumn followed by spring at Dape Sharif and Knotti Garden. Minimum MC were observed during winter at Knotti Garden.

It is evident that total fiber contents varied significantly at both sites and during all the seasons. Maximum fiber contents were found during winter at Knotti Garden which differed non-significantly with summer at same site, followed by spring at Knotti Garden and Dape Sharif. Minimum fiber contents were observed during autumn at Knotti Garden.

Total mineral percentage varied significantly during all the seasons and at both sites. Maximum mineral contents were observed during winter at Knotti Garden site followed by summer and spring at the same site. Minimum mineral contents were observed during

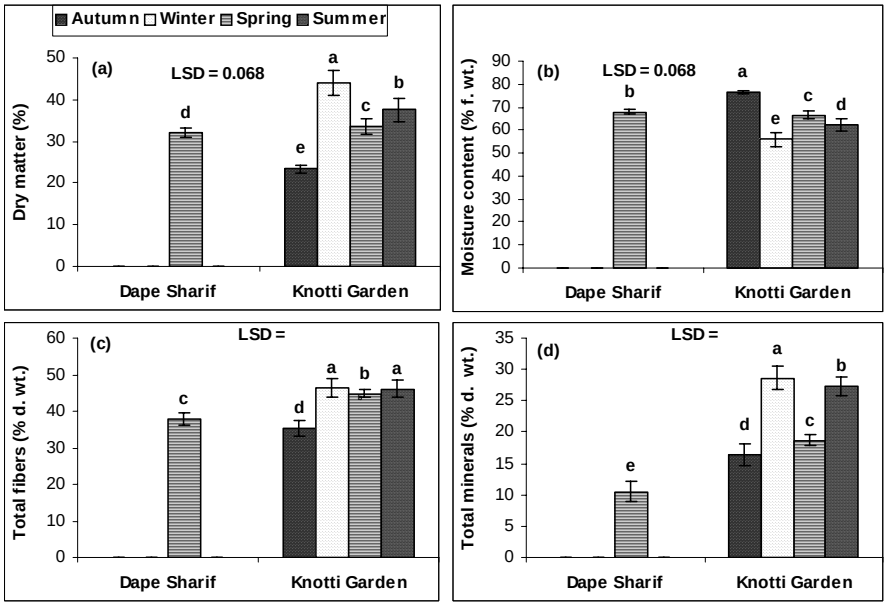

Fig. 1: Spatiotemporal variation in (a) dry matter (b) moisture contents (c) total fibers (d) total minerals in shoot of *Adiantum capillus-veneris* L.

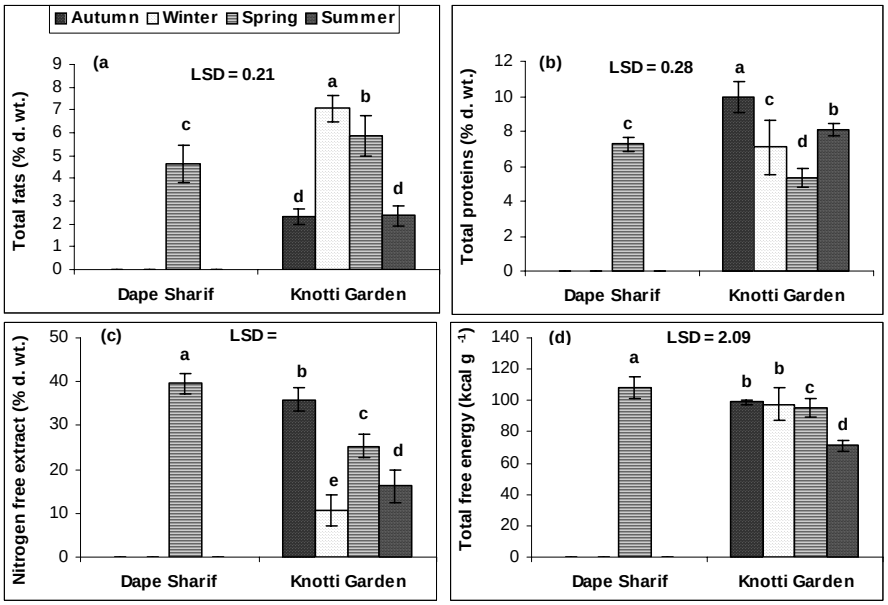

Fig. 2: Spatiotemporal variation in (a) total fats and (b) total proteins, (c) nitrogen free extract and (d) net free energy in shoot of *Adiantum capillus-veneris* L.

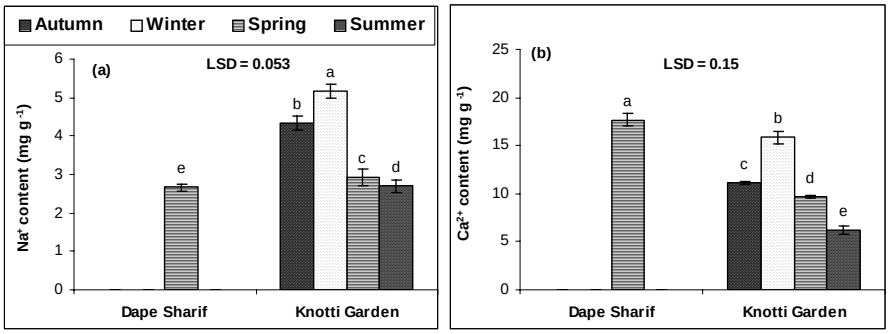

Fig. 3: Spatiotemporal variation in (a) Na^+ and (b) Ca^{2+} contents in shoot of *Adiantum capillus-veneris* L.

spring at Dape Sharif. Similarly, total fat percentage differed significantly during different seasons at both sites. Maximum fat contents were observed during autumn at Knotti Garden followed by spring at Knotti Garden and Dape Sharif. Minimum and almost equal fat contents were observed during autumn and summer at Knotti Garden.

Maximum protein contents were observed during autumn at Knotti Garden followed by summer at the same site. Non-significant and almost equal protein contents were observed during winter at Knotti Garden and during spring at Dape Sharif. Minimum protein contents were found during spring at Knotti Garden. It is evident that Nitrogen free extractable substances varied significantly during different seasons and at both sites.

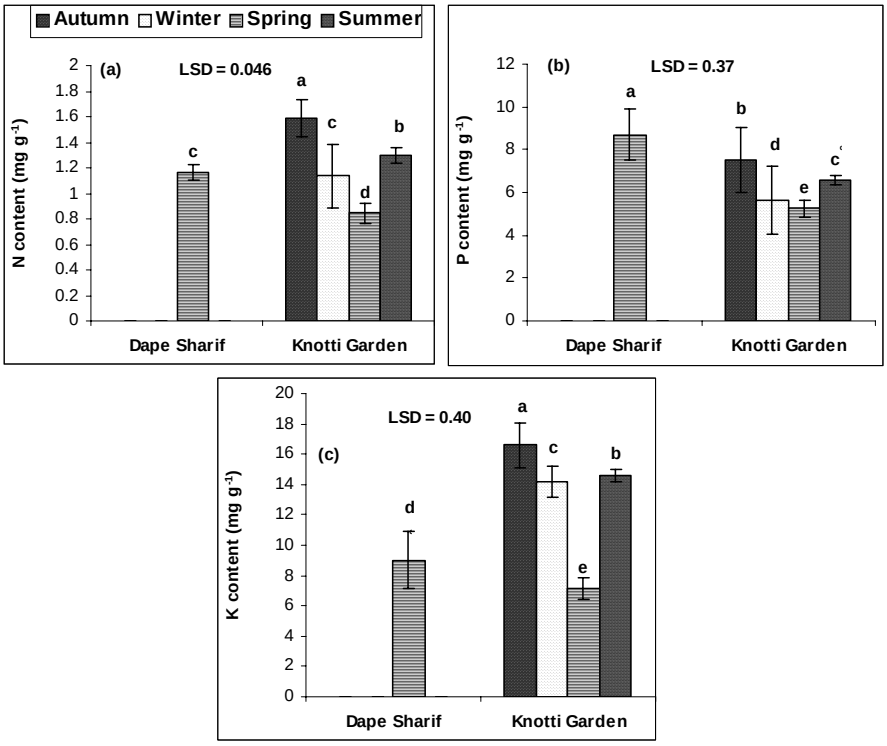

Fig. 4: Spatiotemporal variation in (a) N, (b) P and (c) K contents in shoot of *Adiantum capillus-veneris* L.

Maximum NFES were found during spring at Dape Sharif followed by autumn, spring and summer at KG. Minimum NFES were found during winter at Knotti Garden. Net free energy also differed significantly during different seasons and at the two sites. Maximum net free energy was observed during spring at Dape Sharif followed by autumn, winter and spring at Knotti Garde. However, net free energy during autumn and winter at Knotti Garden varied non-significantly. Minimum net free energy was observed during summer at Knotti Garden.

Sodium and calcium contents during different seasons and at different sites varied significantly. Maximum sodium contents were observed during winter at Knotti Garden followed by autumn, spring and summer at Knotti Garden. Minimum sodium contents were found during spring at Dape Sharif. Maximum Ca contents were observed during spring at Dape Sharif followed by winter and autumn at Knotti Garden. Minimum Ca contents were found during summer at Knotti Garden.

It is evident that Nitrogen, phosphorus and Potassium contents in *Adiantum* shoots varied significantly at different sites and seasons. Maximum Nitrogen contents were found during autumn at Knotti Garden followed by summer at the same site. Plants collected during spring and winter from Dape Sharif and Knotti Garden respectively showed non-significant and almost equal nitrogen contents. Minimum nitrogen contents were observed during spring at Knotti Garden. Maximum Phosphorus contents were

found during spring at Dape Sharif followed by autumn, summer and winter seasons at Knotti Garden. Minimum Phosphorus contents were observed during spring at Knotti Garden. Maximum potassium contents were found during autumn at Knotti Garden followed by summer and winter which differed non-significantly. Minimum potassium contents were observed during spring at Knotti Garden. It is evident that Mg and Zn contents of *Adiantum* shoots varied significantly during different seasons and at different sites.

Mg contents were the maximum during summer at Knotti Garden followed by spring at Knotti Garden and Dape Sharif. Minimum Mg contents were found during winter at Knotti Garden. Maximum Zn contents were observed during spring at Knotti Garden followed by during spring at Dape Sharif. During summer significantly more Zn content than autumn were observed at Knotti Garden. Minimum Zn contents were found during winter at Knotti Garden. Fe and Cu showed significant variations at different sites and during different seasons. Maximum Fe contents were observed during spring at Dape Sharif followed by summer and spring at Knotti Garden. Minimum Fe contents were observed during winter at Knotti Garden which differed non-significantly with autumn season at Knotti Garden.

Accumulation of bioactive substances as phenols, alkaloids and flavonoids in *Adiantum* varied significantly during different seasons and at different sites. Maximum phenolic contents were found during spring at Knotti Garden followed by during spring

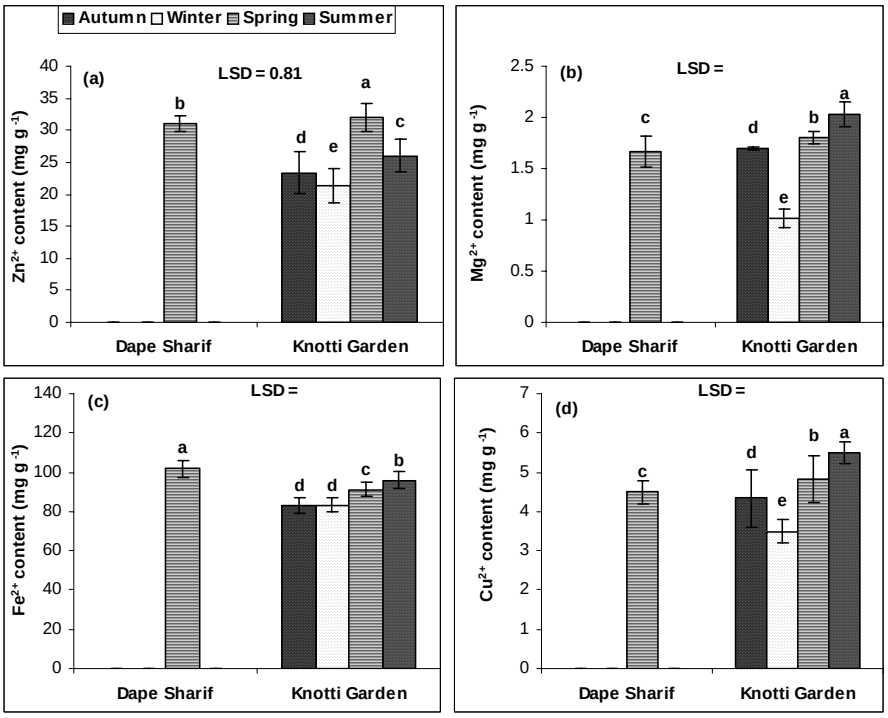

Fig. 5: Spatiotemporal variation in (a) Zn^{2+}, (b) Mg^{2+}, (c) Fe^{2+} and (d) Cu^{2+} in shoot of *Adiantum capillus-veneris* L.

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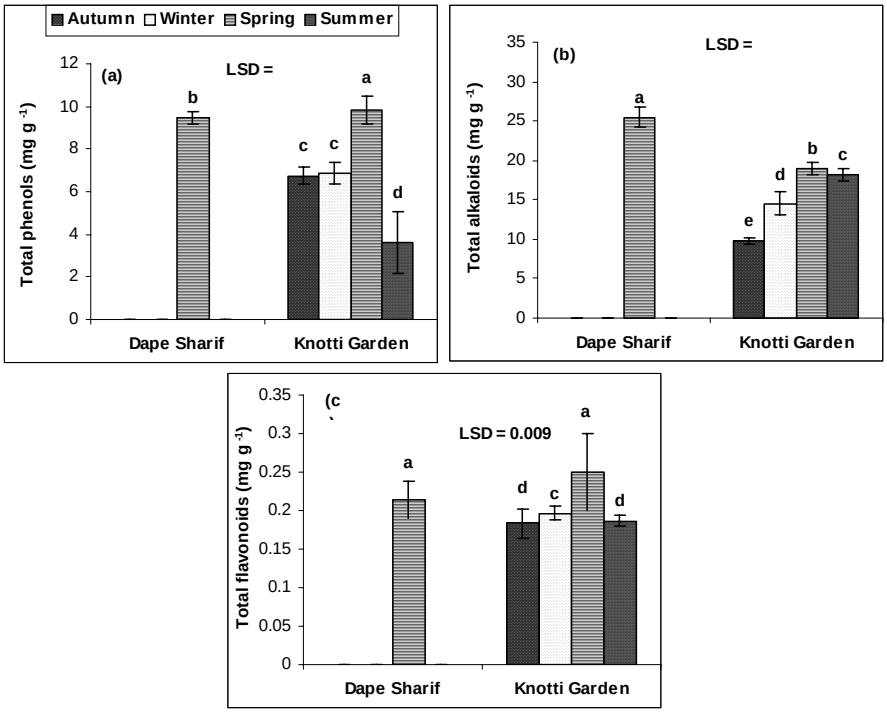

Fig. 6: Spatiotemporal variation in (a) total phenols, (b) total alkaloids and (c) total flavonoids in shoot of *Adiantum capillus-veneris* L.

Dape Sharif. Autumn and winter at Knotti Garden showed almost equal phenolic contents. Minimum phenols were found during summer at Knotti Garden. Maximum alkaloids were found during spring at Dape Sharif followed by spring, summer and winter at Knotti Garden. Minimum quantity of alkaloids was observed during autumn at Knotti Garden. Maximum and almost equal flavoniods were found during spring at Knotti Garden and Dape Sharif followed by winter at Knotti Garden. Minimum and non-significantly different quantity of flavonoids was observed at autumn and summer at Knotti Garden.

Discussion

Partial RDA ordination biplot showing the effect of seasons and sites on biochemical attributes of *Adiantum* shoots is presented in Fig. 7 which indicated that most of the minerals and moisture contents were associated with summer and autumn (Ramirez *et al*., 2006) which might be due to higher transpiration rate at high temperature during summer and autumn. *Adiantum* is found at moist places. So enough moisture and minerals are available during all the seasons at Knotti Garden and Dape Sharif. The absorption rate is thus controlled with temperature or with increase in transpiration rate.

Similarly higher protein contents during autumn may be due to the more absorption of N during this season. Phenol, flavonoid and fiber contents were more associated with

Table 6: Summary of the partial RDA showing the effect of seasons and sites on biochemical attributes of *Adiantum capillus-veneris* L. shoot collected from Soone Valley of Salt Range

Parameters and data	Axes		Total variance	F-ratio	*P* value
	1	2			
Seasons | Sites					
Eigenvalues	0.234	0.189	1.000	6.759	0.0020 ***
Sum of all canonical Eigenvalues	0.552				
Sites | Seasons					
Eigenvalues	0.090	0.061	1.000	3.304	0.0020 ***
Sum of all canonical Eigenvalues	0.090				

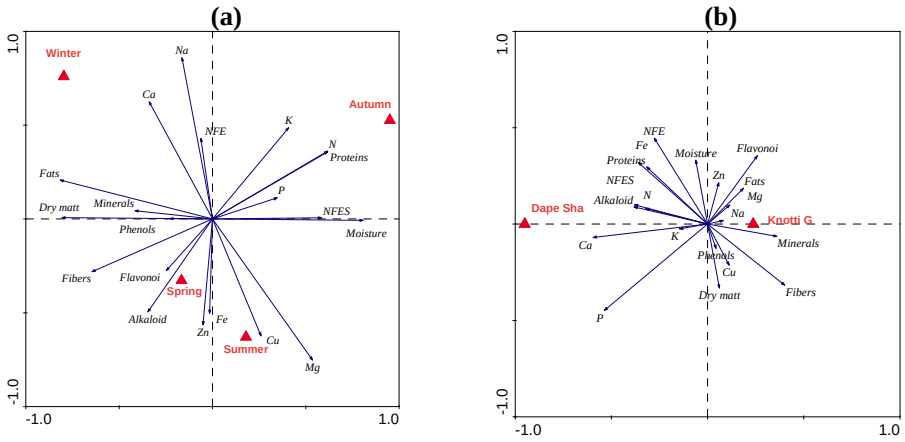

Figure 7: Partial RDA ordination biplot showing the effect of seasons (a) and sites (b) on biochemical attributes of *Adiantum capillus-veneris* L. shoot collected from Soone Valley of Salt Range.

spring. According to Palo *et al*., 1985, the proportion of phenols are enough during spring but decreases dramatically after spring when leaves start emerging and growth is initiated and most of the breakdown products are used up in growth. It may also be due to stressed conditions as salt stress or low temperature stress of late winter.

Alkaloids were strongly correlated with spring due to high pH and mineral contents during this season as with increase in pH and mineral supply alkaloidal synthesis increases (Demeyer & Dejaegere, 1996). Fats, minerals, dry matter and NFE were associated with winter as most of the plants in Soone Valley completes their life cycle before severe winter and these compounds were mostly found maximum at maturity in different plants.

Spatial variation showed that most of the micro nutrients and moisture contents were associated with Dape Sharif which might be due to the high transpiration at an open area of the site whereas other site is a narrow small valley surrounded by high mountains with less availability of sunlight. Moreover availability of nutrients and high pH and EC also interact with the absorption of nutrients.

Phenols, flavonoids, dry matter and NFE were almost equally associated with both sites due to presence of similar soil and environmental conditions. Moreover, higher moisture contents buffers most of the stresses and established a balanced environment, therefore, stress related compounds were produced in equal quantities. High alkaloidal contents at Dape Sharif might be due to high pH associated with this site (Demeyer & Dejaegere, 1996). Proteins were higher at this site due to more supply of macro nutrients especially of N.

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