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SPATIO-TEMPORAL VARIATIONS IN SOME MEDICINALLY IMPORTANT BIOCHEMICAL CONSTITUENTS OF *PEGANUM HARMALA* (HERMAL)

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Abstract

Peganum harmala is an annual herb commonly found in the Salt Range during spring and summer seasons. Spatiotemporal variations in accumulation of some medicinally important biochemical constituents in different parts of *P. harmala* during different seasons and at different sites of Soone Valley in the Salt Range were investigated. Dry matter and phosphorus contents were strongly correlated with autumn, whereas minerals, fats, flavonoids and K contents were the maximum in winter and fibers in summer. All other parameters as phenols, alkaloids, proteins, moisture and most of the minerals as Ca, N, K, Cu, Mg, Zn, Na, NFES (nitrogen free extractable substances), and NFE (net free energy) were associated with spring. Spatial variation showed that moisture, dry matter, NFES, NFE, N and proteins were centroid as their accumulation was not affected due to variation in the study sites, however, alkaloids, K, and Fe were associated with Dape Sharif site having water springs and water channels passing through the valley and soil with higher electrical conductivity, Fibers, Mg and fats were associated with Jallar and Khoora sites (cultivated areas with high amount of salts). Phenols, Zn and flavonoids were associated with high pH soil of Anga site. In seeds, maximum organic and inorganic constituents were found at nutrient rich Khabeki site. Fats and NFES were found maximum in seeds of the plants growing at Anga site, and phenols, flavonoids, NFE and K were higher in seeds of Khoora site. Spring was found to be the most suitable harvesting season for maximum quantity of nutritional as well as medicinal components in *P. harmala*. Among sites, high pH soils of Anga and higher nutrient containing soils of Khabeki are good for maximum quantity of phenols, flavonoids, alkaloids, mineral and nutritional components.

Introduction

The Soone Valley, present in the center of the Salt Range is regarded as the heart of Salt Range. This Valley lies between longitudes 72°00 and 72°30 E, and latitudes 32°25 and 32°45 N, surrounded by two parallel east west longitudinal ridge systems, covering an area of 300 km^2. The average elevation of the area is 762 m and the highest adjacent point is the Sakesar Top being 1522 m above sea level. It is 20 km in length and 5 km in breadth (Afzal *et al.*, 1999). The climate of the Valley is characterized by a relatively low annual precipitation (500 mm) and average minimum temperature of 1 ºC during January, while average maximum temperature is 36 ºC during June. Hot dry winds and prolonged periods of drought are frequent, whereas winters are accompanied by frost (Hussain, 2002; Ahmad *et al.*, 2008a, b).

The dominant vegetation of this Valley comprises *Achyranthus aspara, Acacia modesta, A.acia nilotica, Albizzia lebbeck, Melilotus alba, Capparis decidua, Chenopodium album, Calotropis procera, Datura metel, Fumeria indica, Justicia adhatoda, Mentha longifolia, Olea ferruginea, Peganum harmala*, and *Prosopis glandulosa* (Ahmad *et al*., 2002; Hussain, 2002). These and many other such species are traditionally popular as healing agents and have been under use by the indigenous people since very long. These plants possess therapeutic or exert beneficial pharmacological effects due to having a number of secondary metabolites like alkaloids, phenols, flavonoids, tannins, volatile oils and minerals (Okwu & Okwu, 2004; Edeoga *et al*., 2005).

Peganum harmala (Zygophyllaceae) is a much branched deciduous or short-lived perennial herb or under-shrub growing to 0.6 m in height. It is found on dry steppes, especially where grazing is heavy, and dry waste places. It is often found in basic (alkaline) or saline soils (Chevallier, 1996). *Peganum harmala* is present in salt-affected wastelands and along the road sides in Soon Valley. Its root has been used as a parasiticide to kill body lice and leaves are used for treating rheumatism and nervous conditions where as seeds are used externally in the treatment of haemorrhoids and baldness (Asgarpanah & Ramezanloo, 2012). The fruit and seeds are also used as digestive, diuretic, hallucinogenic, narcotic and uterine stimulant (Bown, 1995). It is widely used as an effective analgesic, anti-inflammatory and antibiotic due to the presence of high quantity of alkaloids.

Manu plants including medical one growing under natural conditions undergo significant spatio-temporal changes in their biochemical and medicinal ingredients that affect their medicinal values. Such changes are most commonly a result of variation in their soil environment and other meteorological conditions during different seasons (Ahmad *et al.*, 2010, 2011a, b). Thus, it was pertinent to determine the variation in medicinally important biochemical ingredients in shoot, root and seeds of *Peganum harmala* growing at various sites of Soone Valley of the Salt Range during different seasons. The outcome of this study will help formulate recommendations to the stake holders who utilize this plant for harvesting of *Peganum harmala* for maximum recovery of its medicinal component.

Material and Methods

Study sites: This study was conducted to assess the spatiotemporal variations in accumulation of some medicinally important biochemical constituents in *Peganum harmala* in Soone Valley of the Salt Range. In this study, six ecologically diverse study sites namely Khabeki (higher macronutrients and field capacity), Khoora (around cultivated area, fertile soil), Dape Sharif (water spring and water channels passing through the valley, soil with high EC), Anga (high pH), Knotti Garden (macronutrients, water springs and water channel), and Jallar (saline area with steep slopes) were studied.

Meteorological data: Meteorological data were recorded at the Horticultural Research Station, Soone Valley for the entire study period. The data for rainfall and maximum and minimum temperatures have already been reported in one of our previous publications (Ahmad *et al*., 2008a, b).

Soil analysis: Soil texture was determined using the hygrometer method (Dewis & Freitas, 1970). The physicochemical characteristics of soil are presented elsewhere (Ahmad *et al*., 2009). Electrical conductivity, pH and ions of saturation extracts were determined according to Jackson (1962) and Rhoades (1982).

Physicochemical parameters: Moisture contents**,** dry matter, crude fibers, mineral contents, fat contents, nitrogen free extractable substances (NFES) and net free energy (NFE) were calculated using standard formulae (Anon., 1984).

Determination of macro- and micro-nutrients: From the digested material, sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) contents were analyzed using a flame photometer (Jenway PFP-7, Japan). Iron, manganese, zinc and magnesium were analyzed using an atomic absorption spectrophotometer (A-Analyst-300, Perkin Elmer, Germany).

Nitrogen estimation: Nitrogen was determined using the micro-Kjeldahl method (Bremner, 1965). Phosphorus contents were estimated by a spectrophotometer (Jackson, 1962).

Phenolic contents: Were determined using standard spectrophotometeric method (Julkunen-Titto, 1985).

Flavonoids: Flavonoid contents were determined y using the standard spectrophotometric method of Dewanto *et al*., (2002).

Alkaloidal contents: The alkaloidal contents of the samples were determined gravimetrically using the Harborne (1973) method which was optimized by comparing with the spectrophotometric method (Sreevidya & Mehrotra, 2003).

Statistical analyses: Data recorded were analyzed statistically using redundancy analysis (RDA) for each species using Canoco Package for Windows version 4.5 (2002). The Multivariate Direct Gradient Model was fitted and all variables were plotted on RDA axis 1 and 2. The results of multivariate analysis are presented in Table 2.

Results and Discussion

Partial RDA showing the effects of seasons and sites (variable and co-variable) on biochemical attributes of *P. harmala* shoots is presented in Fig. 1. Seasonal variation showed (Fig. 1a) that dry matter and phosphorus were strongly correlated with autumn, whereas minerals, fats, flavonoids and K were associated with winter and fibers with summer. All other parameters as phenols, alkaloids, proteins, moisture and most of the minerals as Ca, N, K, Cu, Mg, Zn, Na, NFES and NFE were associated with spring (Table 1). Spatial variation (Fig. 1b) showed that moisture, dry matter NFES, NFE, N and proteins were centroid as they were least affected by different sites, however, alkaloids, K, minerals and Fe were associated with higher moisture and mineral containing soil of Dape Sharif. Fibers, Mg and fats were associated with stressed area of Jallar and Khoora. Phenols, Zn and flavonoids were associated with high pH soil of Anga site.

Seasonal and site variations were recorded for growth, physiological and biochemical attributes in all the species under study. If the variation for different attributes regarding physiological and biochemical parameters in *P. harmala* were compared, the maximum dry biomass was recorded during autumn that may be due to its high photosynthetic activity during summer (Zavodnik *et al.,* 1998) because during summer plants have longer period for trapping sunlight so the accumulation of biomass increased progressively and reached up to the maximum level during autumn. As the day length or photoperiod decreased its growth reduced. There are many reports which indicate that *P. harmala* maintains its high growth during summer and autumn (Darabpour *et al*., 2011). Similarly, when different biochemical parameters were compared, the maximum concentration of different compounds was recorded during spring, which was due to their sprouting and maximum biochemical activities expected during this period. Because of this, phenol, NFE, alkaloids, proteins, moisture were higher during spring (Stanley & Bouzaher, 1995). However, higher alkaloids during spring might be due to high soil pH (Demeyer & Dejaegere, 1996) and higher phenols due to environmental stresses (Harborne & Williams, 2000; Ali & Abbas, 2003) and high pH and EC.

Total minerals were higher during winter which is common in the perennial plants which disappear in winter, because maximum accumulation for minerals is within the plant body (Akingbade *et al.,* 2001). At the end of winter, *P. harmala* starts sprouting from seeds or root-stocks, so the maximum activities can be recorded during spring. During summer this species becomes photosynthetically active and produces maximum biomass at the end of the growth period i.e. autumn. In the present study, maximum macro- and micro-nutrients were recorded during spring and summer with few exceptions. These findings support the above-statement regarding the biochemically and photosynthetically active period (Ahmad *et al.*, 2010). It is obvious that maximum nutrient uptake can be recorded after sprouting and at high metabolic activities which were recorded during spring and summer. Another reason may be the availability of more moisture during these seasons (McDowell, 2003) due to rainfall which not only increases moisture contents but also facilitates absorption of minerals and other solutes. The literature supports the present findings that nutrient uptake would be maximum during early growth period and at high metabolically active period (Zavodnik *et al.,* 1998; Ahmad *et al.,* 2011a; b).

Table 1. Spatio-temporal variation in physio-chemical characteristics in shoot and root of *Peganum harmala* L.

Attribute	Khabeki	Khoora	Dape Sharif		Anga				Jallar			
	Summer	Spring	Autumn	Spring	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Shoot												
Dry matter (%)	32.50	26.07	36.64	23.50	28.92	54.50	35.00	37.43	36.45	47.67	22.20	47.77
Moisture content (% f. wt.)	67.50	73.93	63.36	76.50	71.08	45.50	65.00	62.57	63.55	52.33	77.70	52.23
Crude Fibers (% d. wt.)	49.67	27.67	24.33	29.00	29.67	25.50	26.33	56.00	34.50	30.50	33.33	46.00
Total Minerals (% d. wt.)	5.50	14.23	14.60	14.03	15.50	26.50	6.57	4.97	17.20	16.33	10.57	5.97
Total Fats (% d. wt.)	1.60	6.25	4.33	2.50	2.33	4.98	3.90	2.20	4.17	7.90	1.30	0.53
Total Proteins (% d. wt.)	12.15	19.16	17.50	24.33	21.27	17.58	21.55	15.27	11.91	20.01	28.05	14.79
NFES (% d. wt.)	31.09	32.69	39.23	30.13	31.23	28.77	41.65	21.56	32.22	25.26	26.75	32.71
NFE (kcal g^{-1})	98.23	167.65	151.78	159.13	145.15	146.76	169.06	107.45	118.32	176.91	163.10	103.43
Na^{+} (mg g^{-1})	10.00	14.33	6.58	3.67	14.67	7.42	7.00	9.17	3.58	5.17	16.00	6.17
Ca^{2+} (mg g^{-1})	3.17	15.17	18.92	15.17	16.08	13.50	17.83	4.83	18.42	20.83	12.83	8.17
N (mg g^{-1})	1.94	3.07	2.80	3.90	3.24	2.80	3.50	2.44	1.96	3.20	4.49	2.38
P (mg g^{-1})	2.17	8.00	10.50	7.17	8.58	6.67	5.17	5.50	9.33	8.00	7.17	6.33
K^{+} (mg g^{-1})	5.00	26.50	42.92	40.50	35.83	13.67	26.00	5.50	24.17	44.33	6.83	6.17
Zn^{2+} (mg g^{-1})	26.33	18.50	19.67	23.00	27.33	22.00	26.33	29.23	24.33	17.67	22.17	21.33
Mg^{2+} (mg g^{-1})	1.83	1.67	1.67	1.53	1.74	0.77	1.47	2.03	1.76	0.91	2.27	1.43
Fe^{2+} (mg g^{-1})	132.33	132.00	132.67	131.33	142.33	124.67	138.33	123.33	0.00	108.33	112.33	118.33
Cu^{2+} (mg g^{-1})	16.67	15.33	15.33	17.17	14.33	12.50	18.33	16.33	11.50	10.50	16.33	14.67
Phenols (mg g^{-1})	3.35	8.92	7.75	8.00	9.67	10.50	8.83	4.33	8.00	9.25	8.13	3.42
Alkaloids (mg g^{-1})	15.23	32.77	19.97	39.30	8.77	19.07	35.07	7.10	15.80	28.23	26.90	9.20
Flavonoids (mg g^{-1})	0.19	0.18	0.20	0.20	0.21	0.33	0.28	0.25	0.20	0.18	0.18	0.17
Root												
Phenols (mg g^{-1})	2.43	2.38	2.17	2.33	1.17	2.5	2.27	2.07	2.65	2.8	3.67	2.43
Alkaloids (mg g^{-1})	9.17	15.67	11.27	16.5	24.1	11.63	21.5	8.63	26	16	18.33	10.83

Table 2. Summary of the partial RDA showing the effect of seasons and sites on biochemical attributes of *Peganum harmala* L. shoot collected form Soone Valley of Salt Range.

Parameters and data	Axes		Total variance	F-ratio	*P* value
	1	2			
SHOOT					
Seasons | Sites					
Eigenvalues	0.178	0.134	1.000	9.159	0.0020 ***
Sum of all canonical Eigenvalues	0.370				
Sites | Seasons					
Eigenvalues	0.056	0.032	1.000	2.455	0.0020 ***
Sum of all canonical Eigenvalues	0.132				
ROOT					
Seasons | Sites					
Eigenvalues	0.311	0.106	1.000	11.794	0.0020 ***
Sum of all canonical Eigenvalues	0.416				
Sites | Seasons					
Eigenvalues	0.224	0.095	1.000	6.775	0.0020 ***
Sum of all canonical Eigenvalues	0.319				
FRUIT					
Sites					
Eigenvalues	0.315	0.192	1.000	3.497	0.0020 ***
Sum of all canonical Eigenvalues	0.567				

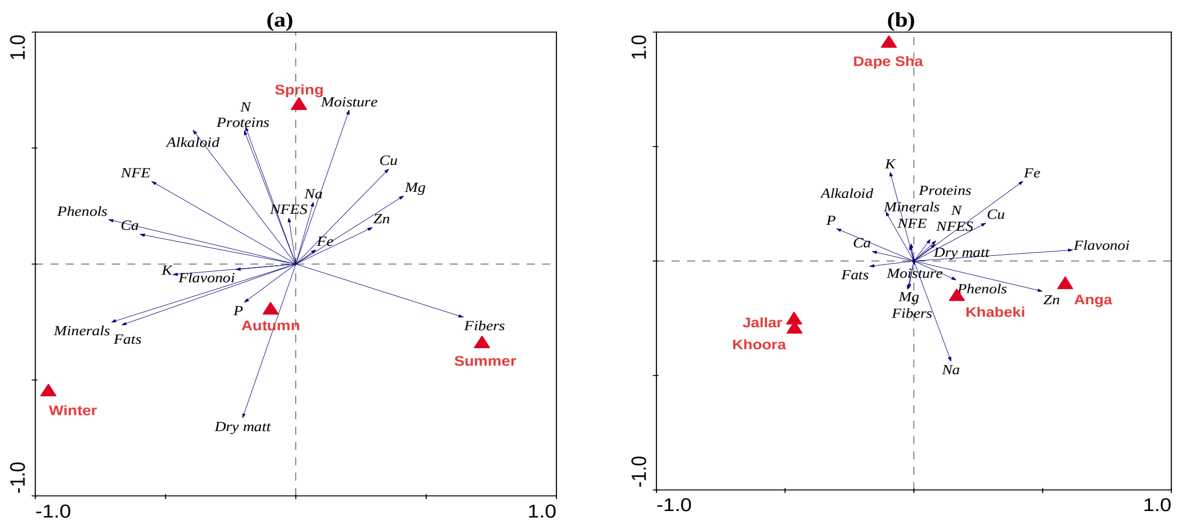

Fig. 1. Partial RDA ordination biplot showing the effect of seasons (a) and sites (b) on biochemical attributes of *Peganum harmala* L. shoot collected from Soone Valley of the Salt Range.

The maximum fibers were recorded during summer which also favors the results of different studies indicating that fiber synthesis increases in plants growing towards maturity (Anon., 2005). Fats and minerals were higher in plants growing during winter which was the end-product of most of the plant species at maturity (Akingbade *et al.*, 2001; Fiengul & Ertan, 2002), however, mineral contents were increased when plants grow under suitable environmental conditions of late summer when enough rainfall and minerals were available (McDowell, 2003, Ahmad *et al.*, 2011b).

Spatial variation indicated that most of the biochemical parameters were equally affected by all the sites, however, alkaloids, P, K, proteins, Fe, N and Cu were higher in plants growing at Dape Sharif while flavonoids, phenols and Zn were higher in plants collected from Anga site. Similarly, Na and fibers were correlated with Khabeki site. Spatial variations regarding physiochemical and biochemical parameters were noted by many workers (Spears, 1994; Ramirez *et al.*, 2006) because variation in uptake and synthesis of different biochemical compounds by plants depend upon genetic variation (Ashraf *et al.*, 1987) as well as soil (texture, soil chemical composition) and environmental conditions at different sites. As in the present study, different sites have different types of soils regarding their texture, composition and different environmental conditions (temperature, humidity, light) and available water (rain, spring). Therefore, spatial variations in different biochemical parameters are natural and can be supported by different reports available in the literature for different plant species (Skarpe, 1990; Ashraf, 2003; Demeyer & Dejaegere, 1996; Foroutan-pour *et al.*, 1997).

Eco-physiological analysis of seeds indicated large site to site variation (Table 3; Fig. 2). However, the maximum organic and inorganic constituents were found in the seeds of Khabeki site that may be due to the soil texture and environmental interactions, because its soil is clay loam with high K and P being which are necessary for most of the metabolic activities. Potassium is involved in activation of more than 40 metabolic enzymes (Taiz & Zeiger, 1991). Similarly, P stimulates the enzymes involved in protein synthesis so it is obvious that the soil containing high K and P is beneficial for optimum plant metabolic activities. Many reports confirm the present findings (Hu & Wang, 2004; Zhang *et al.*, 2004). In contrast, the moisture contents were high in the seeds of Jallar plants which may be due to the environmental conditions (high field capacity) or harvesting of the seeds at the time when there was maximum moisture contents. It is evident that premature seeds contain more water than mature ones and the seeds from humid environment contain more moisture. Fats and NFES were higher in seeds of the plants growing at Anga site. This may be due to limited availability of N at Anga site.

Table 3. Spatio-temporal variation in physio-chemical characteristics in fruit of *Peganum harmala* L.

Attribute	Khabeki	Khoora	Anga	Jallar	LSD
Dry matter (%)	81.03	80.86	78.13	74.67	3.63
Moisture content (% f. wt.)	18.96	18.63	21.7	28.67	2.3
Total fibers (% d. wt.)	39.86	31.67	33.8	35.13	2.04
Total minerals (% d. wt.)	8.67	8.33	9.00	13.00	1.07
Total fats (% d. wt.)	6.1	8.2	7.2	7.47	0.59
Total proteins (% d. wt.)	22.01	19.33	15.13	13.36	1.19
Nitrogen free extracts (% d. wt.)	23.36	32.97	34.87	31.04	1.8
Total free energy (kcal g^{-1})	169.97	183.92	5.13	13.35	6.49
Na^{+} content (mg g^{-1})	14.33	13.83	13.45	11.67	0.79
Ca^{2+} content (mg g^{-1})	2.73	2.27	2.4	2.4	0.28
N content (mg g^{-1})	2.63	2.97	2.3	2.17	0.19
P content (mg g^{-1})	23.33	16.83	19.5	22.67	1.35
K content (mg g^{-1})	16.5	18.17	16	11	0.91
Zn^{2+} content (mg g^{-1})	20	18.33	18.33	21.67	3.63
Mg^{2+} content (mg g^{-1})	0.76	0.6	0.8	0.73	0.13
Fe^{2+} content (mg g^{-1})	162.33	159.33	166.67	148.67	6.01
Cu^{2+} content (mg g^{-1})	21.33	18.33	20.67	19.33	3.05
Total phenols (mg g^{-1})	4.9	6	5.67	3.87	0.29
Total alkaloids (mg g^{-1})	15.03	11.67	14.63	15.63	1.19
Total flavonoids (mg g^{-1})	0.21	0.22	0.37	0.18	0.03

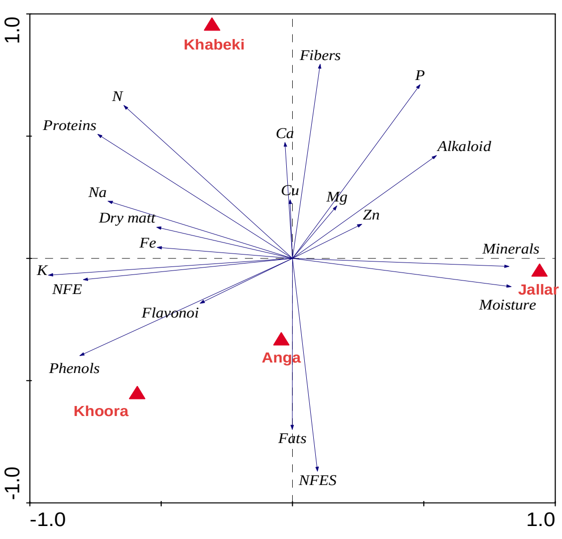

Fig. 2. RDA ordination biplot showing the effect of sites on biochemical attributes of *Peganum harmala* L., seeds collected from Soone Valley of the Salt Range.

Phenols, flavonoids, NFE and K were higher in seeds of Khoora that may be due to its specific stressful environmental conditions favouring the synthesis of phenols and flavonoids in *P. harmala* plants (Harborne & Williams, 2000; Ali & Abbas, 2003) and accelerating the uptake of K. Many reports indicate site to site variation in seed chemical composition due to its climate and soil composition (Adler & Kittelson, 2004; Sauer *et al.*, 2006). In roots, presence of maximum phenols in winter indicated that they faced low temperature and drought stress as in most of the plant species phenols are produced during stressed conditions (Smirnoff, 1993; Ali & Abbas, 2003). However maximum alkaloids were associated with spring and autumn due to maximum growth during these seasons (Fig. 3). Another reason is the more supply of minerals and suitable pH of the soil during these seasons which enhance alkaloid synthesis (Demeyer & Dejaegere, 1996).

Spatial variation showed that phenols were associated with plants of Jallar site which might be due to stressed conditions prevailing at this site (Harborne & Williams, 2000; Ali & Abbas, 2003). However, alkaloids were associated with Anga site which might be due to high soil pH and salt levels of the site (Demeyer & Dejaegere, 1996, Ahmad *et al.*, 2011b).

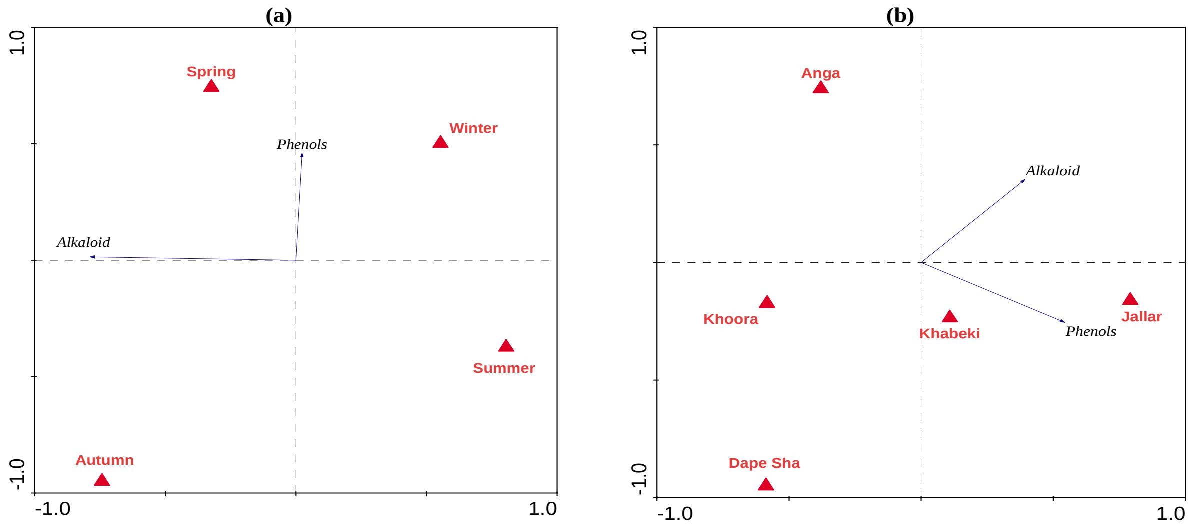

Fig. 3. Partial RDA ordination biplot showing the effect of seasons (a) and sites (b) on biochemical attributes of *Peganum harmala* L. roots collected from Soone Valley of the Salt Range.

Conclusion

It can be concluded that in *P. harmala,* spring is the most suitable harvesting season for maximum quantity of nutritional as well as medicinal components. Among sites, high pH soils of Anga and high nutrient containing soils of Khabeki are good for attending maximum quantity of phenols and flavonoids and for maximum quantity of alkaloids, and mineral and nutritional components. Mineral and water containing Dape Sharif is the most favourable site. However, seeds with maximum nutritional and alkaloidal components can be harvested from Khabeki site with high minerals and fibre content.

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