Pak. J. Bot., 49(SI): 201-210, 2017.

SPATIO-TEMPORAL VARIATIONS IN NUTRITIONAL AND MEDICINAL PROPERTIES OF THORN APPLE (*SOLANUM INCANUM* L.), A RARE MEDICINAL PLANT SPECIES OF THE SALT RANGE, PAKISTAN

IFTIKHAR AHMAD[1], MUMTAZ HUSSAIN[2], M. SAJID AQEEL AHMAD[2], M. ASIM SULTAN[1]
M. YASIN ASHRAF[3], MISHAL IFTIKHAR[1], ATTIA NOREEN[1] AND AHMAD MUNEEB[1]

[1]*Department of Botany Sciences, University of Sargodha, Sargodha, Pakistan*
[2]*Department of Botany, University of Agriculture, Faisalabad*
[3]*Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan*
[*]*Corresponding author's email: iak8767@yahoo.com*

Abstract

In this study, Thorn Apple (*Solanum incanum* L.) plants collected during different seasons from various sites (Khabeki, Khoora, Dape Sharif, Anga, Jallar) in Soon Valley (Salt Range) were analyzed for some of the nutritional and medicinal components to investigate the spatio-temporal variations in the synthesis/accumulation of these compounds. The aim was to select the suitable harvesting season and site for deriving maximum quantity of these compounds from the plant. Partial RDA ordination biplot regarding the effects of seasons on biochemical attributes of *S. incanum* shoots showed that moisture and alkaloidal contents and most of the nutrients such as P, K, Ca and Fe strongly correlated with spring (growing) season. Fats and phenols were correlated with winter and autumn. Total minerals, Zn, Mg, fibers, proteins and N were associated with summer. The spatial effects of different sites on biochemical parameters indicated that dry matter, fibers, Na, Ca, Zn and NFES (nitrogen free extractable substances) were strongly correlated with nutrients and high field capacity at Khabeki site. Alkaloids, phenols, fats and K showed almost equal correlation with drier Dape Sharif and Jallar sites. However, in *S. incanum* fruits, Zn, Mg and Fe showed no significant correlation with any specific site, whereas alkaloids, flavonoids, dry matter, K and P were strongly associated with salt and drought stressed area of Jallar, whereas Ca, Na and fibers were weakly correlated. Protein, nitrogen, fats, phenols and minerals were strongly correlated with nitrogen containing clay loam soils of Khabeki and Jallar sites. NFES was also correlated with fruits collected from Khoora site whose soil was inversely correlated with N contents that is why NFES were higher at this site. In roots of *S. incanum,* phenols and alkaloids were correlated with high soil pH and more availability of minerals during spring. Therefore for *S. incanum,* summer season is the most favorable season for the synthesis of nutritional components and spring season for medicinal components, whereas areas with stressed and high pH soil (Jallar and Dape Sharif) are the most suitable sites for collecting *S. incanum* plants possessing maximum quantity of medicinal as well as nutritional components. However, for fruit collection of *S. incanum*, stressed (Jallar) site is the most suitable for medicinal components and high mineral containing (Khabeki) site for nutritional components.

Keywords: Alkaloids, Medicinal components, Phenols, Salt Range, Thorn apple

Introduction

Biological diversity is universally recognized as an important part of the world's natural heritage and an essential component for the sustainability of global ecosystems. Plant diversity has a creditable importance as a source of pharmaceutically active substances (Pearce & Puroshothaman, 1992; Principe, 1991; Dushenkov & Raskin 2008; Kurek-Górecka *et al*., 2014). According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional herbal medicine for their primary health-care needs (Inglis, 1994; Harvey, 2009; Singh *et al*., 2014). These traditional systems are culturally and psychologically more tolerable in most of the societies as compared to western allopathic medicines. In addition, being the natural plant products, they are considered to be the safest way of treating diseases with least side effects on human health as compared to allopathic or homeopathic medicines (Prescott-Allen & Prescott-Allen, 1982; Al-Arifi, 2013; Samojlik *et al*., 2013).

Salt Range has a very rich floral diversity and a treasure of valuable natural resources. People of the area have sufficient conventional knowledge of plant uses and their administration at local level. The area has a mountainous dry subtropical climate with semi-evergreen forests, typically with *Acacia modesta, Dodonaea viscosa, Olea ferruginea, Reptonia buxifolia and Salvadora oleoides*. The Soon Valley, present in the center of the Salt Range is regarded as the heart of the Salt Range, and 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 (Afzal *et al*., 1999). Large numbers of plant species are traditionally popular as curing agents and have been used by indigenous people since long. These plants pose therapeutic or exert beneficial pharmacological effects due to having a number of secondary metabolites including alkaloids, phenols, glycosides, tannins, volatile oils and minerals that show medicinal properties (Edeoga *et al*., 2005; Okwu, 2004).

Solanum incanum (Solanaceae) is a wild, herbaceous and perennial shrub found mostly in barren areas of Salt Range. Its leaves, fruits and roots are used as an expectorant, carminative analgesic and febrifuge. It may grow up to 1 m in height. It produces flowers and fruits mostly in autumn and winter, but also present in other seasons. *Solanum incanum* is distributed in Asia, Africa and in most of the Arab countries on low hills and waste-lands. This plant is used for various medicinal purposes due to the presence of alkaloids, phenols, saponins and glycosides (Ghazanfar 1996). Its leaves, stem, roots and fruits are used for bruised fingers, dyspepsia, earache, hemorrhoids and tooth ache (Hassan-Abdallah *et al*., 2013;

Mwonjoria *et al*., 2014). Mostly all parts of the plant are used for medicinal purposes (Ghazanfar, 1996).

The present work was planned to study the spatio-temporal variations in the synthesis/accumulation of nutritional and medicinal components and to select the suitable harvesting season and site for deriving maximum quantity of these components from *S. incanum* plants.

Material and Method

Meteorological data for the entire study period were recorded at the Horticultural Research Station, Soon Valley (already published Ahmad *et al*., 2012).

Selection of sites: On the basis of a preliminary survey, six ecologically diverse study sites, namely Khabeki (32.35N and 72.12 E, elevation 774 m asl, slope 30-35%, asepect western), Khoora (32.23N and 72.11 E, elevation 866 m asl, slope 40-45%, aspect northern), Dape Sharif (32.30N and 72.04 E, elevation 890 m asl, slope 35-40%, aspect western), Anga (32.35N and 72.05 E, elevation 821 m asl. slope 30-35%, aspect northern) and Jallar (32.27N and 72.09 E, elevation 996 m asl, slope 50-60%, aspect eastern) were selected.

Growth and biochemical analysis of selected medicinal plants: The plants were collected from their natural habitats at different sites in Soon Valley during all four seasons (autumn, winter, spring, and summer). For autumn, winter, spring and summer seasons, the ecological data were recorded during the last weeks of September, December, March, and June, respectively. Plants used for medicinal purpose (shoots, roots or fruits) were collected from each site in each season, and their fresh weights recorded. These were then analyzed for biochemical attributes and laboratory analysis.

Moisture contents, dry matter, crude fibers, mineral contents, fats, nitrogen free extractable substances (NFES) and net free energy were estimated according to the standard methods of Anon. (1984) and Anon. (2001).

Crude proteins were estimated using N content determined through the micro-Kjeldahl method (Hiller *et al*., 1948) and multiplying N with a factor of 6.25.

Macro- and micro-nutrients were determined through the acid digestion method following Wolf's sulphuric acid – hydrogen peroxide method (Wolf, 1982). The extract was filtered and used for the analysis of different nutrients.

Nutrient analysis was performed from the above digested material. Sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) contents were analyzed using a flamephotometer (Jenway PFP-7). Iron, copper, zinc and magnesium were analyzed using an atomic absorption spectrophotometer (Analyst 300, Perkin Elmer, Germany).

Phosphorus contents were estimated with spectrophotometer following Jackson (1962). Values of phosphorus were calculated using a standard curve. Barton reagent was prepared as described by Ashraf *et al*., (1992).

Phenolic contents were determined following Julkunen-Tiitto (1985).

Flavonoid contents were determined following Dewanto *et al*., (2002).

Alkaloidal contents were determined gravimetrically using the Harborne (1973) method.

Statistical analysis: The data for growth and biochemical attributes of individual species were analyzed using the Partial Redundancy Analysis (*p*RDA) keeping seasons as a variable and sites as a co-variable and *vice versa*. All parameters were centered and standardized by species and samples (season or site) and those having no data were removed from the analysis. The Multivariate Direct Gradient Model was fitted and all variables (nominal) were plotted on *p*RDA Axis 1 and 2.

Results

Solanum incanum was found at Khabeki and Jallar sites during all four seasons, however, it was not found during spring and winter at Dape Shaif and Anga, respectively. At Khoora, it was found only during spring and was absent at Knotti garden in all four seasons. It is apparent from 1-2 that most of the biochemical parameters varied significantly during different seasons and at different sites. Maximum dry matter of shoots was found during winter in *S. incanum* plants of Jallar, which was followed by summer at Khabeki and Anga sites. At all sites, minimum dry matter was observed during the spring season. Maximum moisture contents in plants were observed during spring at Khabeki and Jallar, which was followed by spring at Anga and Khoora sites. The moisture contents varied from 60 to 70 percent during different seasons and at different sites. Table 1 shows that total fiber and total mineral contents varied significantly during different seasons and at different sites. A maximum amount of total fibers was found in plants of Khoora during spring, which was followed by plants of Anga and Jallar collected during spring, respectively. In contrast, a minimum quantity of total fibers was observed in the plants from Dape Sharif during autumn.

A maximal amount of minerals was found in *S. incanum* plants during autumn collected from Anga and Jallar followed by those collected during autumn at Khabeki and Dape Sharif. Overall, at most of the sites, maximal amounts of minerals were observed during autumn. However, the lowest amounts of minerals were observed during spring at Khabeki and Anga. Table 1 shows that total fat and total protein contents in *S. incanum* plants varied significantly during different seasons and at different sites. A maximal amount of total fats was found during winter in plant samples collected from Dape Sharif, which was followed by the plants collected during spring and winter from Jallar. However, a mnimal quantity of total fats was observed in plants during summer at Khabeki. Overall, the fat contents were higher in winter and lower in summer.

Protein contents in plants were considerably higher during summer at Dape Sharif and during spring at Anga and Jallar followed by spring at Khabeki and summer at Anga. A minimal amount of protein contents was observed in samples collected during winter at Khabeki site. Overall, spring and summer showed higher protein contents, whereas winter showed lower protein contents at different sites.

Table 1. Spatiotemporal variation in some shoot physio-chemical characteristics of *Solanum incanum* L.

Characteristics	Seasons	Khabeki	Khoora	Dape Sharif	Anga	Jallar
Dry matter (%)	Autumn	39.3	NR	36.6	33.0	NR
	Winter	34.2	NR	36.6	NR	48
	Spring	26.5	31.9	NR	30.3	25.8
	Summer	46.8	NR	33.2	39.4	32.1
Moisture content (% fresh wt.)	Autumn	60.7	NR	63.4	67.0	NR
	Winter	61.2	NR	63.4	NR	48.3
	Spring	73.5	68.1	NR	69.7	74.2
	Summer	47.2	NR	66.8	60.6	67.9
Total fibers (% dry wt.)	Autumn	40.7	NR	35.0	33.5	NR
	Winter	33.3	NR	37.3	NR	37.2
	Spring	48.0	55.3	NR	51	48.2
	Summer	50.8	NR	43	43.7	39
Total minerals (% dry wt.)	Autumn	10.3	NR	9.3	11.4	NR
	Winter	5.8	NR	8.6	NR	7.8
	Spring	4.2	5.3	NR	4.4	5.4
	Summer	5.3	NR	6.3	5.4	11.7
Total fats (% dry wt.)	Autumn	5.3	NR	3.0	5.7	NR
	Winter	4.9	NR	7.9	NR	6.3
	Spring	6.1	3.3	NR	4.0	6.6
	Summer	1.0	NR	3.9	3.8	3.1
Total proteins (% dry wt.)	Autumn	14.4	NR	12.9	12.6	NR
	Winter	8.6	NR	12.8	NR	10.3
	Spring	17.7	13.4	NR	18.8	18.9
	Summer	11.8	NR	18.8	18.2	16.4
Nitrogen free extract (% dry wt.)	Autumn	29.2	NR	39.7	36.7	NR
	Winter	47.4	NR	33.3	NR	38.4
	Spring	23.9	22.7	NR	21.7	20.9
	Summer	31.0	NR	27.9	28.9	29.8
Total free energy (K cal g^{-1})	Autumn	135.8	NR	121.1	137.8	NR
	Winter	124.3	NR	152.7	NR	134.2
	Spring	151.4	108.7	NR	137.5	157.3
	Summer	91.9	NR	142.8	140.5	127.6
Na^+ content (mg g^{-1})	Autumn	5.4	NR	3.9	5.3	NR
	Winter	3.8	NR	4.0	NR	3.9
	Spring	2.2	2.4	NR	1.7	1.8
	Summer	1.5	NR	2.0	1.3	1.1
Ca^{2+} content (mg g^{-1})	Autumn	18.5	NR	14.0	15.8	NR
	Winter	6.5	NR	6.4	NR	6.5
	Spring	14.3	16.8	NR	16.1	14.3
	Summer	4.1	NR	7.5	7.1	6.6
N content (mg g^{-1})	Autumn	2.3	NR	2.1	2.0	NR
	Winter	1.3	NR	2.0	NR	1.6
	Spring	2.6	2.1	NR	3.0	3.0
	Summer	1.8	NR	3.0	2.9	2.6

Table 1 shows that nitrogen free extractable substances and net free energy varied significantly during different seasons and at different sites. Higher amounts of nitrogen free extractable substances were found in the samples collected during winter from Khabeki followed by autumn at Dape Shaprif and winter at Jallar site. In contrast, lower amounts of NFES were observed in samples collected during spring from Anga. Maximum values of NFES were observed mostly in the samples collected during winter and autumn, whereas minimum values observed during spring and summer at different sites. High amount of net free energy was found in samples collected during spring at Khabeki followed by spring at Jallar and winter at Dape Sharif. Overall *S. incanum* showed highest values of net free energy during spring and winter and lowest values during autumn and summer at different sites.

It is evident from Table 2 that Na and Ca contents in *S. incanum* shoots varied significantly. High amounts of Na were found in the samples collected during autumn at Khabeki and Anga sites, followed by the samples collected during spring from Dape Sharif. In contrast, a minimal amount of Na was found in samples collected during summer from Jallar. Ca contents showed a similar pattern with respect to different sites, as high amounts Ca were observed in samples collected during autumn from Khabeki followed by spring at Khoora. In contrast, low amount of Ca was observed during summer at Khabeki. Overall, higher values for Ca contents were observed during autumn and spring, and lower values during summer and winter.

It is apparent from Table 2 that concentrations of macronutrients in *S. incanum* also varied significantly during different seasons and at different sites. Highest amount of N was foundin the samples collected during summer from Dape Sharif and during spring from Anga and Jallar. In contrast, lowest amount of N was observed during winter at Khabeki. Overall, high amount of N was found during autumn and spring, and low during winter.

Highest amount of P was found in the samples collected during spring and winter from Jallar followed by during spring and winter at Anga and Dape Sharif. In contrast, low amount of P was observed during summer and spring at Jallar and Khoora. Generally, higher values of P were found during spring and winter and lower during autumn. Potassium contents were found maximum in the samples collected during spring from Jallar followed by spring at Anga and during autumn at Anga and Khabeki. In contrast, lowest values of K were observed in the samples collected during summer from Khabeki site. Zinc contents were found maximum in plant samples collected during spring from Khabeki followed by spring at Khoora However, lowest levels of Zn were found during winter at Khabeki. Magnesium (Mg) was found maximum in the plant samples collected during summer at Anga followed by in the same season at Dape and Khabeki. In contrast, lowest amount of Zn was found during winter at Jallar. A maximal amount of Fe was found in the plant samples collected during summer at Dape Sharif followed by during summer and spring at Khabeki. The Fe levels were the lowest during winter at Jallar. Copper (Cu) was maximum also during summer at Dape Sharif followed by during the same season at Anga and Khabeki, whereas lowest amount of Cu was found during winter at Jallar and Khabeki.

Concentrations of bioactive substances (Table 2) in shoots of *S. incanum* also varied significantly during different seasons and at different sites. Highest amount of phenolic compounds was found in the samples collected during autumn from Dape Sharif followed by in winter at Khabeki and Jallar sites. A minimal amount of phenolics was found in the samples collected during summer from Anga. Overall, higher amount of phenols in *S. incanum* shoots was found during autumn and spring and lower during summer. However, high amounts of phenolics were found in the roots of *S. incanum* during spring and low amount during summer at Dape Sharif. Overall, higher levels of phenolics were found during spring and of different sites at Jallar.

Total alkaloid contents in *S. incanum* shoots were found maximum during spring at Anga, followed by spring at Jallar. The minimal values of alkaloids were found during autumn at Anga. Overall, higher values of alkaloid contents in *S. incanum* were found during spring and lower during autumn and summer. Similar results were found in the roots of *S. incanum* wherein high amounts of alkaloids were found during spring at Khabeki site which were followed by spring at Khoora and Jallar. In contrast, low quantity of alkaloids was observed during autumn at Dape Sharif. Flavonoids were found maximum in samples collected during summer at Jallar which differed non-significantly from the samples collected during spring from the same site and during autumn from Dape Sharif. However, lowest amount of flavonoids was found during autumn at Anga site.

The fruits of *S. incanum* were found only during early winter at three sites (Khabeki. Khoora and Jallar). Fruit data was recoded in only one season, therefore, only spatial comparison was possible. Significant spatial variations were found for various biochemical aspects of *S. incanum* fruits (Table 3).

Discussion

Partial RDA ordination biplot regarding the effects of seasons and sites (both as variable and co-variable) on biochemical attributes of *Solanum incanum* shoots is presented in Fig. 1. It is evident that moisture and alkaloid contents in *S. incanum* shoots are strongly correlated with those of spring season, whereas dry matter is inversely correlated to spring season. Higher moisture contents and lower dry matter during spring might have been due to more water availability due to heavy rainfall during spring in the study period (Ahmad *et al*., 2011). Correlation of alkaloid contents to spring season might be attributed to high pH as most of the enzymes involved in alkaloid synthesis are active at high pH (Demeyer & Dejaegere, 1996). Another reason could be that high moisture availability also facilitates more mineral absorption and increase in mineral concentration also increases alkaloid synthesis (Augustus & Rutgers, 2006).

Table 2. Spatiotemporal variation in some shoot and root physio-chemical characteristics of *Solanum incanum* L.

Characteristics	Seasons	Khabeki	Khoora	Dape Sharif	Anga	Jallar
Shoot P content (mg g^{-1})	Autumn	5.1	NR	6.5	6.0	NR
	Winter	8.0	NR	10.3	NR	11.0
	Spring	9.5	4.7	NR	10.1	11.1
	Summer	5.1	NR	7.0	9.5	4.3
Shoot K^+ content (mg g^{-1})	Autumn	18.5	NR	14.0	15.8	NR
	Winter	6.5	NR	6.4	NR	6.5
	Spring	14.3	16.8	NR	16.1	14.3
	Summer	4.1	NR	7.5	7.1	6.6
Shoot Zn^{2+} content (mg g^{-1})	Autumn	23.6	NR	26.5	26.6	NR
	Winter	17.0	NR	21.6	NR	22.3
	Spring	18.8	29.6	NR	28.3	17.5
	Summer	32.6	NR	28.0	29.0	23.3
Shoot Mg^{2+} content (mg g^{-1})	Autumn	0.8	NR	1.6	1.6	NR
	Winter	0.8	NR	0.9	NR	0.7
	Spring	1.4	1.5	NR	1.4	1.8
	Summer	1.9	NR	1.9	2.1	1.8
Shoot Fe^{2+} content (mg g^{-1})	Autumn	120.0	NR	125.0	118.3	NR
	Winter	110.6	NR	115.6	NR	105.6
	Spring	128.0	121.3	NR	123.6	116.0
	Summer	128.0	NR	135.0	127.6	NR
Shoot Cu^{2+} content (mg g^{-1})	Autumn	15.6	NR	17.3	21.0	NR
	Winter	12.0	NR	14.6	NR	11.0
	Spring	17.0	18.6	NR	19.0	17.3
	Summer	21.0	NR	23.6	22.0	NR
Shoot total phenols (mg g^{-1})	Autumn	8.1	NR	10.5	6.8	NR
	Winter	9.0	NR	8.2	NR	9.1
	Spring	7.4	4.1	NR	6.4	8.2
	Summer	3.1	NR	5.5	3.0	3.8
Shoot total alkaloids (mg g^{-1})	Autumn	12.4	NR	7.1	7.3	NR
	Winter	12.5	NR	13.8	NR	16.3
	Spring	27.1	18.6	NR	30.7	29.5
	Summer	8.7	NR	9.6	8.9	12.0
Shoot total flavonoids (mg g^{-1})	Autumn	0.3	NR	0.5	0.2	NR
	Winter	0.3	NR	0.3	NR	0.3
	Spring	0.2	0.2	NR	0.2	0.5
	Summer	0.2	NR	0.2	0.2	0.5
Root total phenols (mg g^{-1})	Autumn	1.8	NR	1.7	2.0	NR
	Winter	1.4	NR	1.3	NR	2.1
	Spring	1.6	2.0	NR	2.1	2.6
	Summer	1.6	NR	1.4	1.7	2.2
Root total alkloids (mg g^{-1})	Autumn	10.7	NR	6.0	6.3	NR
	Winter	13.8	NR	12.6	NR	13.4
	Spring	27.7	18.9	NR	16.1	19.1
	Summer	9.1	NR	12.3	12.4	9.5

Table 3. Spatial variation in some fruit physio-chemical characteristics of *Solanum incanum* L.

Characteristics	Khabeki	Khoora	Jallar
Fruit dry matter (%)	57.7	56.8	63.0
Fruit moisture content (% fresh wt.)	41.9	42.6	37.0
Fruit total fibers (% dry wt.)	28.7	21.6	31.0
Fruit minerals (% dry wt.)	9.0	7.3	8.6
Fruit total fats (% dry wt.)	8.4	7.6	8.0
Fruit total proteins (% dry wt.)	16.2	14.3	13.8
Fruit nitrogen free extract (% dry wt.)	37.6	49.1	38.4
Fruit total free energy (K cal g^{-1})	176.2	172.9	163.4
Fruit Na^+ (mg g^{-1})	8.1	9.3	10.3
Fruit Ca^{2+} (mg g^{-1})	1.6	2.3	2.4
Fruit N (mg g^{-1})	2.6	2.3	2.2
Fruit P(mg g^{-1})	20.0	20.6	23.5
Fruit K^+ (mg g^{-1})	15.8	15.5	18.3
Fruit Zn^{2+} (mg g^{-1})	18.3	17.6	18.3
Mg^{2+} (mg g^{-1})	0.8	0.8	0.7
Fe^{2+} (mg g^{-1})	176.3	173.3	169.6
Cu^{2+} (mg g^{-1})	13.6	16.6	13.6
Total phenols (mg g^{-1})	4.7	4.5	4.7
Total alkaloids (mg g^{-1})	17.0	19.4	33.6
Tatal Flavonoids (mg g^{-1})	0.2	0.2	0.4

Most of the nutrients as P, K, Ca and Fe were also correlated with spring, which also may have been due to the availability of moisture which may have accelerated their absorption (McDowell, 2003). Another reason might be that during spring *S. incanum* plants were at the vegetative stage and high uptake of nutrients is believed to occur in many plants during this stage. After dry winter, moisture availability due to raining also plays a role in mineral absorption (McDowell, 2003) during spring. Sodium was correlated with winter and autumn that may be due to accumulation of Na salts in the upper layers of soil or in the root zone during these seasons. The plants were mature during winter because at maturity, plants contain maximum amount of soluble salts and most of the water soluble compounds contain higher Na contents (Tattini *et al*., 2006).

Fats and phenols were also correlated with winter and autumn seasons, because fats are the end-products produced during winter at maturity of the plant (Akingbade *et al*., 2001; Fiengul & Ertan, 2002). Phenols may be produced due to stress conditions, e.g. due to low temperature stress during winter (Matern & Grimmig, 1994; Harborne & Williams, 2000). Tannins or phenols are often higher at maturity and harvesting stage (Bussotti *et al*., 1998). During these seasons *S. incanum* was at maturity so the higher contents of phenols could be expected.

Total minerals had equal association with autumn and summer, whereas Zn and Mg were associated with summer which might have been due to the availability of optimum moisture and mineral contents in the soil during this season. High transpiration during these seasons is also important to regulate the levels of nutrients in plant tissues (McDowell, 2003; Ramirez *et al*., 2006).

Fibers, proteins and N were associated equally with summer and spring. These components generally produce when the plant growth is high. So, *S. incanum* plants showed maximum growth during summer and spring, which could be attributed to the presence of high levels of minerals during these seasons. Flavonoids and Cu contents. However, showed no significant relation to any specific season.

The spatial effects of different sites on biochemical parameters (Fig. 1) showed that dry matter, fibers, Na, Ca, Zn and NFES were strongly correlated with Khabeki and less so with Khoora, which may have been due to their soil texture and chemical composition as the soil of Khabeki was clayey loam enriched different types of mineral nutrients (Ahmad *et al*., 2010).

Alkaloids, phenols, fats and K showed a similar correlation with Dape Sharif and Jallar which may have been due to the abiotic stresses (Ali & Abbas, 2003; Miranda-Ham *et al*., 2007) prevalent at both sites, whereas Mg, moisture, protein, N, P and NFE were closely correlated with Dape Sharif that may have been due to its specific climatic conditions and variation in soil composition which favoured the uptake of N and P and synthesis of protein and NFE. Copper and Fe had a positive correlation with Anga and Dape Sharif which may have been due to the presence of micronutrients in reasonable amount in the soils of these sites, which may have been easily absorbed by the plants from the sandy clay soil.

Partial RDA ordination biplot showing the spatial effects of different sites on *S. incanum* fruits are presented in Fig. 3, which showed that Zn, Mg and Fe were centroid and equally correlated with all sites, whereas alkaloids, flavonoids, dry matter, K and P were strongly associated with Jallar, and Ca, Na and fibers were weakly correlated. It might have been due to salt and drought stress (Ali & Abbas, 2003; Miranda-Ham *et al*., 2007) prevalent at Jallar during the study period. Higher K and P might have been due to high field capacity of the soil or availability of these nutrients in balanced concentration.

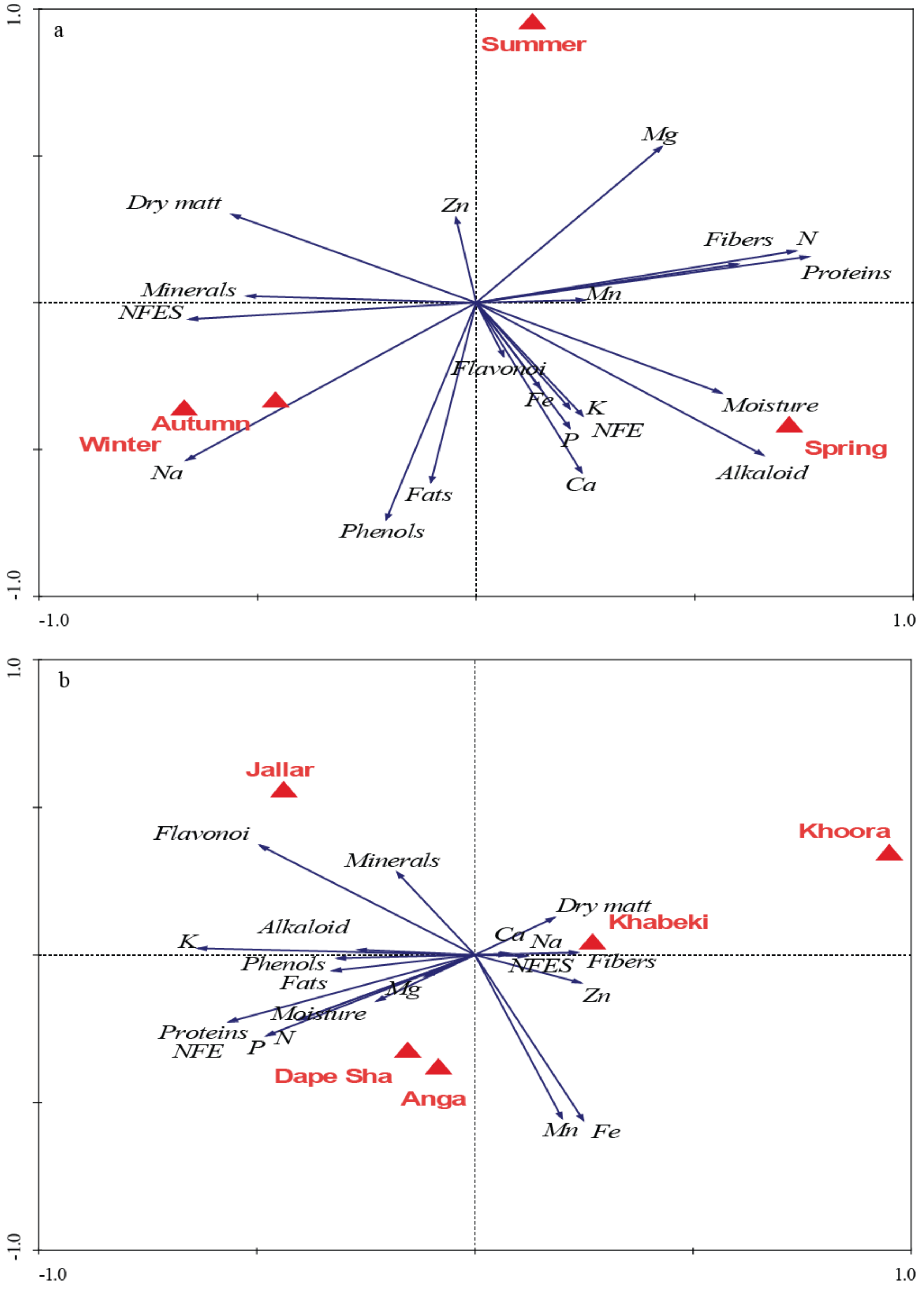

Fig. 1. Partial RDA ordination biplot showing the effect of seasons (a) and sites (b) on biochemical attributes of *Solanum incanum* L. shoots collected from Soon Valley of Salt Range.

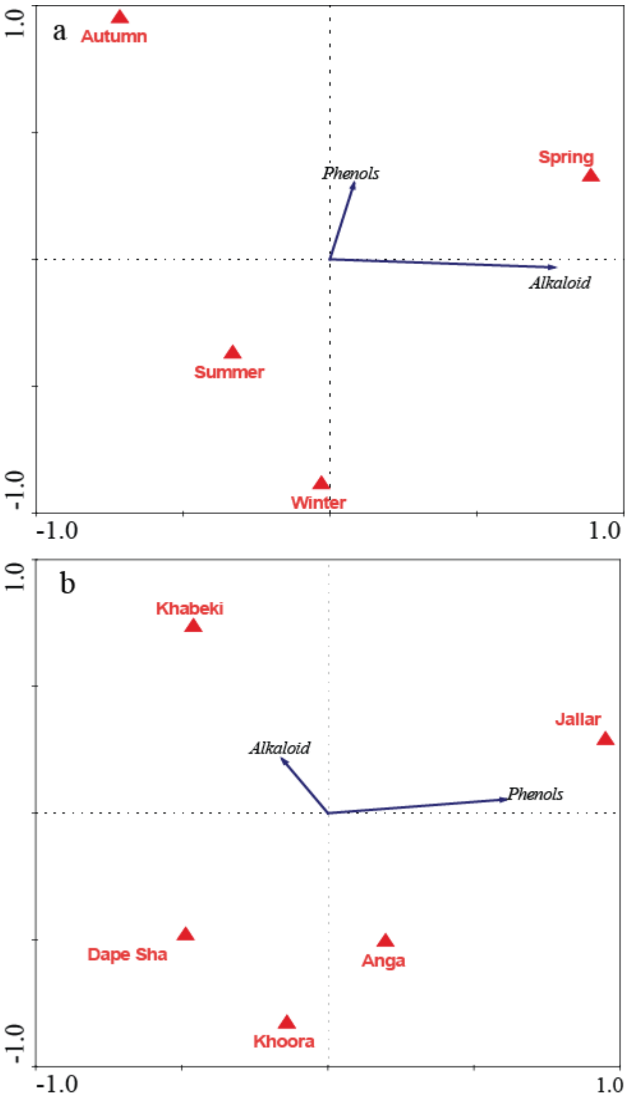

Fig. 2. Partial RDA ordination biplot showing the effect of seasons (a) and sites (b) on biochemical attributes of *Solanum incanum* L. roots collected from Soon Valley of Salt Range.

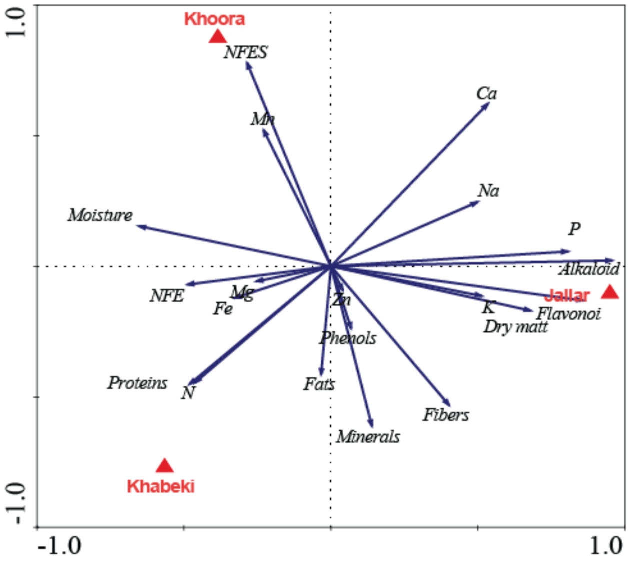

Fig. 3. RDA ordination biplot showing the effect of sites on biochemical attributes of *Solanum incanum* L. fruit collected from Soon Valley of Salt Range.

Protein and N were strongly correlated with Khabeki which was due to some association of N with the soils of Khabeki (Fig. 1). Fats, phenols and minerals were equally correlated with Khabeki and Jallar. Fats and minerals were associated with the availability of minerals, moisture content and soil texture. However, phenols may be produced due to some kind of injury (Barroso *et al*., 2001). Copper and NFES were strongly correlated with Khoora and Khabeki which might have been due to deficiency of N and excess of Cu in the soils of these sites.

Fruit chemical analysis clearly shows that the fruit collected from Jallar contained maximal amount of organic and inorganic constituents. Fruits from this site had higher Na, K, P, Ca, alkaloids fats and dry matter than those from other sites. The plants of these sites enjoyed the suitable environmental conditions, fertile soil and optimum moisture contents, which favored the uptake of P and K and other organic constituents. The pH and EC of the soil favored the synthesis of fats, alkaloids, phenols, accumulation of minerals, and high fiber contents which are typical properties of plants growing on marginally saline soils. The plants growing on such type of soil often have high dry matter due to accumulation of minerals, alkaloids, flavonoids, and phenols (Kováčik *et al*., 2011).

Moisture, N, Cu, proteins, and NFE were positively correlated with Khabeki for fruit chemical composition which may have been the result of availability of high moisture and high N uptake during fruit formation at the site. As the soil is clay loam with high field capacity, so it can effectively retain high moisture content for long time. Higher availability of water might have favored the uptake of N as a result of which most of the N containing compounds like proteins were synthesized in the fruit. As the soils of this site had higher FC, so the moisture remained available for a longer period during the fruit formation. Thus, the fruits growing on soils had high moisture content.

As Cu was correlated with Khoora soils which may have high amount of Cu contents which were easily available due to high moisture in the soil, so it was easily translocated to the fruits. NFES was also correlated with the fruits collected from Khoora, the soil of which is inversely correlated with N contents. This is the reason why NFES were higher at the site. It can be concluded that the site Jallar favored more accumulation of organic and inorganic constituents in the f fruit than at other sites.

Partial RDA ordination biplot showing the effects of seasons and sites (both as variable and covariable) on *S. incanum* roots is presented in Fig. 2. This shows that phenols and alkaloids were correlated with spring. During spring, high levels of alkaloids might have been due to high soil pH and availability of more minerals (Demeyer & Dejaegere, 1996) as during these conditions more alkaloids are produced. Phenols may accumulate due to some stress conditions (Harborne & Williams, 2000; Ali & Abbas, 2003).

Spatial variation (Fig. 2) showed that alkaloids were almost centroid having correlation with all the seasons, but more with Khabeki. This site had higher pH and more mineral contents which may have favored synthesis of alkaloids (Augustus & Rutgers, 2006). Phenols were more associated with Jallar than with other sites. This association may have been due to salt and drought stress at Jallar (Harborne & Williams, 2000; Ali & Abbas, 2003). Fruits were recorded only in a single season, therefore, only special variation was recorded which showed distinct correlations of Jhallar with most of the physio-chemical characteristics (Fig. 3).

It is evident from above discussion that in *S. incanum,* summer season is the most favorable one for the synthesis of nutritional components and spring for medicinal components, whereas Jallar and Dape Sharif are the most suitable sites for plant collection for achieving maximum quantity of medicinal as well as nutritional components. However, for fruit collection, Jallar site is the most suitable one for medicinal components and Khabeki for nutritional components.

References

Afzal, S., M. Younas and K. Hussain. 1999. Physical and chemical characterization of the agricultural lands of the Soan Sakesar Valley, Salt Range, Pakistan. *Aust. J. Soil Res.*, 37: 1035-1046.

Ahmad, I., M.S.A. Ahmad, M. Hameed, M. Hussain, M.Y. Ashraf, M. Farooq and H. Malik. 2012. Status of plant diversity in the Soone Valley, Salt Range, Pakistan. *Pak. J. Bot.*, 44(SI): 59-62.

Ahmad, I., M.S.A. Ahmad, M. Hussain, M. Ashraf, M.Y. Ashraf and M. Hameed. 2010. Spatiotemporal aspects of plant community structure in open scrub rangelands of sub-mountainous Himalayan plateaus. *Pak. J. Bot.*, 42(5): 3431-3440.

Ahmad, I., M.S.A. Ahmad, M. Hussain and M.Y. Ashraf. 2011. Spatiotemporal variations in soil characteristics and nutrient availability in open scrub type semi-arid rangelands of typical sub-mountainous Himalayan tract. *Pak. J. Bot.*, 43: 565-571.

Akingbade, A.A., I.V. Nsahlai and C.D. Morris. 2001. Seasonal variation in forage quality and mimosine contents of two varieties of *Leucaena leucocephala*. *Afr. J. Range & Forage Sci.*, 18: 131-135.

Al-Arifi, M.N. 2013. Availability and needs of herbal medicinal information resources at community pharmacy, Riyadh region, Saudi Arabia. *Saudi Pharma. J.*, 21: 351-360.

Ali, R.M. and H.M. Abbas. 2003. Response of salt stressed barley seedlings to phenylurea. *Plant, Soil & Environ.*, 49: 158-62.

Anonymous. 1984. Official Methods of Analyses, 14th ed., Arlington, Virginia 22209; Association of official Analytical Chemist, USA.

Anonymous. 2001. FAO in partnership with Support unit for International Fisheries and Aquatic Research, SIFAR. Torry Research Station, PO Box 31, 135 Abbey Road, Aberdeen AB9 8DG.

Ashraf, M.Y., A.H. Khan A.R. Azmi. 1992. Cell membrane stability and its relation with some physiological processes in wheat. *Acta Agronomica Hungarica*, 41: 183-191.

Augustus, E. and D. Rutgers. 2006. Influence of various concentrations of nutrients on growth and alkaloid synthesis in *Datura stramonium*. *J. Pharma. Sci.*, 50: 676-678.

Barroso, F.G., T.F. Martinez, T. Paz, A. Parra and F.J. Alarcon. 2001. Tannin content of grazing plants of southern Spanish arid lands. *J. Arid Environ.*, 49: 301-314.

Bussotti, F., E. Gravano, P. Grossoni and C. Tani. 1998. Occurrence of tannins in leaves of beech trees (*Fagus sylvatica*) along an ecological gradient, detected by histochemical and ultrastructural analysis. *New Phytologist*, 138: 469-479.

Demeyer, K. and R. Dejaegere. 1996. The Effect of total mineral dose and pH on alkaloid accumulation in *Datura stramonium* L. J. *Herbs Spices Med. Plants*, 3: 35-44.

Dewanto, V., X. Wu, K.K. Adom and R.H. Liu. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agri. & Food Chem.*, 50: 3010-3014.

Dushenkov, V. and I. Raskin. 2008. New strategy for the search of natural biologically active substances. *Russian J. Plant Physiol.*, 55: 564-567.

Edeoga, H.O., D.E. Okwu and B.O. Mbaebie. 2005. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.*, 4: 685-688.

Fiengul, B.L.G.N. and O. Ertan. 2002. Selected chemical constituents and their seasonal variations in *Flabellia petiolata* (Turra) Nizam. and *Halimeda tuna* (Ellis and Sol.) J.V. Lamour. in the Gulf of Antalya (North-eastern Mediterranean). *Turk. J. Bot.*, 26: 87-90.

Ghazanfar, S.A. 1996. *Handbook of Arabian medicinal plants*. Publishers, CRC press, USA. pp. 197-199.

Harborne, J.B. 1973. *Phytochemical methods*, London. Chapman and Hall, Ltd. pp. 49-188.

Harborne, J.B. and C.A. Williams. 2000. Advances in flavonoid research since 1992. *Phytochemistry*, 55: 481-504.

Harvey, A.L. 2009. The continuing value of natural products for drug discovery. *Revista de Salud Animal*, 31: 8-12.

Hassan-Abdallah, A., A. Merito, S. Hassan, D. Aboubaker, M. Djama, Z. Asfaw and E. Kelbessa. 2013. Medicinal plants and their uses by the people in the region of Randa, Djibouti. *J. Ethnopharm.*, 148: 701-713.

Hiller, A., J. Plazin and D.D. Van-Slyke. 1948. A study of conditions for Kjeldahl determination of nitrogen in proteins. *The J. Biol. Chem.*, 176: 1401-1420.

Inglis, J. 1994. Introduction. *Nature & Resour.*, 30: 3-4.

Jackson, M.L. 1962. *Soil chemical analyses*. Constable and company Ltd., England.

Julkunen-Tiitto. 1985 Phenolic constituents in the leaf of northern willows: method for the analyses of certain phenolics. *J. Agri. & Food Chem.*, 33: 213-217.

Kováčik, J., B. Klejdus, F. Štork J. Hedbavny. 2011. Nitrate deficiency reduces cadmium and nickel accumulation in chamomile plants. *J. Agri. & Food Chem.*, 59: 5139-5149.

Kurek-Górecka, A., A. Rzepecka-Stojko, M. Górecki, J. Stojko, M. Sosada and G. Świerczek-Zięba. 2014. Structure and antioxidant activity of polyphenols derived from Propolis. *Molecules*, 19: 78-101.

Matern, U. and B. Grimmig. 1994. Natural Phenols as stress metabolites. *Acta Horticulturae*, 381: 448-462.

McDowell, L.R. 2003. Minerals in Animal and Human Nutrition (2nd ed.), Elsevier, *The Netherlands*, pp: 340.

Miranda-Ham, M.D. Lourdes, Islas-Flores, Iqnacio, Vazquez-Flota and Felipe 2007. Accumulation of mono terpenoid Indole Alkaloids in Periwinkle seedlings (*Catharanthus roseus*) as amodel for the study of plant environment interaction. *Biochem. & Mol. Biol. Edu.*, 35: 206-210.

Mwonjoria, J.K., J.J. Ngeranwa, H.N. Kariuki, C.G. Githinji, M.N. Sagini and S.N. Wambugu. 2014. Ethno medicinal, phytochemical and pharmacological aspects of *Solanum incanum* (Lin.). *Int. J. Pharm. & Toxicology*, 2: 17-20.

Okwu, D.E. 2004. Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. *J. Sustainable Agri. & The Environ.*, 6: 30-34.

Pearce, D.W. and S. Puroshothaman. 1992. Protecting Biological Diversity: The Economic Value of Pharmaceutical Plants. CSERGE Global Environmental

Change Working Paper 92-27, Centre for Social and Economic Research on the Global Environment, University College, London and University of East Anglia.

Prescott-Allen, R. and C. Prescott-Allen. 1982. What's Wildlife Worth? Economic Contributions of Wild Animals and Plants to Developing Countries London: Earthscan.

Principe, P.P. 1991. Valuing the Biodiversity of Medicinal Plants. In *The Conservation of Medicinal Plants, Proceedings of an International Consultation*, (Eds.): O. Akerele, V. Heywood and H. Synge. 21-27 March 1988, Chiang Mai, Thailand. Cambridge: Cambridge University Press, pp. 79-124.

Ramirez, R.G., H. Gonzalez-Rodriguez, R. Ramirez-Orduna, M.A. Cerrillo-Soto and A.S. Juarez-Reyes. 2006. Seasonal trends of macro and micro minerals in 10 browse species that grow in northeastern Mexico. *Animal Feed Sci. & Technol.*, 128: 155-164.

Samojlik I, V. Mijatović, N. Gavarić, S. Krstin and B. Božin. 2013. Consumers' attitude towards the use and safety of herbal medicines and herbal dietary supplements in Serbia. *Int. J. Clinical Pharmacy*, 35: 835-840.

Singh, B., S.K. Borthakur and S.J. Phukan. 2014. A survey of ethnomedicinal plants utilized by the indigenous people of Garo Hills with special reference to the Nokrek Biosphere Reserve (Meghalaya), India. *J. Herbs, Spices & Med. Plants*, 20: 1-30.

Tattini, M., D. Remorini, P. Pinelli, G. Agati, E. Saracini, M.L. Traversi and R. Massai. 2006. Morpho-anatomical, physiological and biochemical adjustments in response to root zone salinity stress and high solar radiation in two Mediterranean evergreen shrubs, *Myrtus communis* and *Pistacia lentiscus*. *New Phytologist*, 170: 779.

Wolf, B. 1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Comm. in Soil Sci. & Plant Analysis*, 13: 1035-1059.

(Received for publication 15 January 2016)