INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2024, Vol. 3, NO. 1, 37-49.
doi: 10.56612/ijaaeb.v3i1.80
https://eminentscientists.com/

Spatio-temporal variation in synthesis of some nutraceutical components of thorowax foldwing (*Dicliptera bupleuroides* Nees)

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Abstract
The present research was carried out to determine the spatiotemporal variation in accumulation of different nutritional and medicinal components in *Dicliptera bupleuroides* from the Soone valley in the Salt Range of Punjab, Pakistan. Most of the biochemical attributes were higher in the spring season than those in the other seasons; this might have been due to high soil pH during spring. High amounts of minerals and high pH are necessary for the synthesis of alkaloids, phenols, flavonoids, and fibers. Proteins, N, dry matter, fats and nitrogen free extract (NFE) were higher in summer, which might have been due to considerable availability of macro-nutrients during this season due to high moisture content because of high rainfall during the season. Spatial variation showed that phenols and flavonoids were associated with the population from Khabeki site (despite enough minerals and moisture availability) which might have been due to physical injuries caused by grazing and cutting. Minerals, proteins and N were also associated with the Khabeki population probably due to large amounts of minerals and moisture retention in the soil of the site and prevalent optimum environmental conditions therein. Alkaloids, fats, moisture and some minerals were associated with Anga and Knotti Garden sites which might have been due to high pH and prolonged salt stressed conditions at both sites.

SECTION
Plant Biology (PB)

HANDLING EDITOR
Athar, H.R. (PB)

ARTICLE HISTORY
Received: 12 Jan, 2022
Accepted: 30 Aug, 2023
Published: 2 Jan, 2024

KEYWORDS
Alkaloids;
Flavonoids
Medicinal plant;
Nutrients;
Soone Valley

Introduction

Medicinal plants provide major constituents of most of the indigenous drugs as well as several allopathic medicines (Sagger et al., 2022). Large numbers of plants have been investigated for their biological activities and antioxidant compounds such as phenolics and flavonoids (Vitale et al., 2022). In particular, medicinal plants generally enriched with natural antioxidants in their different plant parts including fruit, have been broadly studied. For example, it has been advocated that the medicinal plants

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TO CITE THIS ARTICLE: Ahmad, I., Ahmad, M.S.S.A., Hameed, M., Muneeb, A., Imran, M., Hussain, M., Farooq, M., Ahmad, F., Asghar, A., Basharat, S., Fatima, S., Parvez, I., Shah, S.M.R. (2024). Spatio-temporal variations in synthesis of some nutraceutical components of thorowax foldwing (*Dicliptera bupleuroides* Nees). *International Journal of Applied and Experimental Biology* 3(1): 37-49.

are major sources of nutritional and medicinal molecules, some of which exhibit antioxidant and antimicrobial properties which can protect the human body against oxidative stresses and pathogens attack (Rathor, 2021) Therefore, it is very important to characterize different types of medicinal plants for their antioxidant potential. Medicinal and aromatic plant species have been used for thousands of years by many cultures to increase the flavor and aroma of foods (Sengu et al., 2009; Giannenas et al., 2020).

Of a number of medicinal plants known so far, herbs have a major role for providing many pure antioxidants (Xu et al., 2017). For example, *Dicliptera bupleuroides* is a small herb growing under trees and other shady places in the Salt Range (Riaz et al., 2019). The inhabitants of the Salt Range, particularly residing the Soone Valley, use this plant as a tonic, perhaps due to its high mineral contents. It starts growing during late spring, grows well in summer and dries in autumn. It is mostly present during rainy seasons of summer, autumn and rarely in spring (Safdar et al., 2021). It is widely distributed in Pakistan, Nepal, Afghanistan, Bangladesh, India to Indo-China, Bhutan and West China. Its flowering starts in June and ends in October (Nasir and Ali, 1988). It is traditionally used as a treatment of various eye diseases (Akbar et al., 2021). Its fresh leaves are crushed and applied on the affected portion of the body usually thrice a day for one week in eczema (Arya and Agarwal, 2008). However, nutritional and medicinal properties of *D. bupleuroides* have been less investigated, so it was the rationale of conducting the present studies. Thus, the present study was conducted to evaluate the essential components from *D. bupleuroides* for medicinal use and nutritional purpose, and to explore its spatiotemporal heterogeneity in the Soone Valley.

Material and Methods

The studies were conducted to determine the distributional pattern and growth of vegetation particularly medicinal plant diversity at different seasons and sites within the Salt Range region. Meteorological data for the entire study period were recorded at the Horticultural Research Station, Soone Valley. The data for rainfall, and maximum and minimum temperatures are presented in **Table 1**.

Site selection

Based on a preliminary survey, six ecologically different study sites, Khabeki, Khoora, Dape Sharif, Anga, Knotti Garden and Jallar were mainly focused. These sites were selected based mainly on the differences in their environmental attributes, especially variations in elevation, slope, aspect, altitude, topography, soil composition, habitat, vegetation type, and plant community (**Table 1-3**).

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

Sites	Coordinates	Elevation (m)	Slope (%)	Aspect
Khabeki	32.35N and 72.12 E	774	30-35	Western
Khoora	32.23N and 72.11 E	866	40-45	Northern
Dape Sharif	32.30N and 72.04 E	890	35-40	Western
Anga	32.35N and 72.05 E	821	30-35	Northern
Knotti 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
Khabeki	Moderate slopes	Dominant large shrubs with grasses	*Justicia adhatoda*
Khoora	Hills with steep slopes	Shrubs, herbs and grasses	*Dodonaea viscosa*
Dape Sharif	Almost plain area	Shrubs and grasses	*Dodonaea viscosa*
Anga	Hills with steep slopes	Dominant grasses with shrubs	*Acacia modesta*
Knotti Garden	Hills with steep slopes	Mixture of herbs, grasses and shrubs	*Justicia adhatoda and Acacia farnesiana*

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

Season	20014-15			2015-16		
	Average Temperature °C		Rainfall (mm)	Average Temperature °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

Soil analysis

Soil texture was determined using the hygrometer method (Dewis and Freitas, 1970). Electrical conductivity, pH and elemental ions of soil saturation extracts were determined according to Rhoades (1982) and Jackson (1962). The physico-chemical characteristics are presented in **Tables 4** and **5**.

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

Sites	Field Capacity (%)				Soil ECe (dS m^{-1})			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
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
Dape 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
Knotti Garden	39	38	35	35	2.9	4.4	1.2	1.6

Sites	Soil pH				Soil Texture
	Autumn	Winter	Spring	Summer	
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
Dape Sharif	7.82	8.29	8.1	7.9	Sandy clay
Anga	7.78	7.89	7.9	7.8	Sandy clay with stones
Knotti Garden	7.73	7.87	8.1	8.1	Sandy clay

Table 5: Soil ion concentrations of the sites selected in the Soone Valley of the Salt Range

Sites	Na^+ (mg/g dry soil)				K^+ (mg/g dry soil)			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
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
Dape 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
Knotti Garden	0.051	0.042	0.027	0.041	0.03	0.07	0.01	0.02

Sites	P (mg/g dry soil)				N (mg/g dry soil)			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
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
Dape 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
Knotti Garden	8.5	16.5	16.5	19.5	0.09	0.10	0.11	0.09

Growth and biochemical analysis of *D. bupleuroides*

Populations of *D. bupleuroides* were collected from different sites within the Salt Range, and from each plant, shoots, roots and fruit were separated, and their fresh weights recorded. All samples were properly dried in an oven at 65 ^{o}C until their constant weights. All these were then analyzed in the laboratory for the determination of biochemical attributes.

Determination of physicochemical parameters

Shoot moisture contents**,** dry matter, crude fibers, mineral contents, fat contents, and nitrogen free extractable substances (NFES) were determined using the formulae given by AOAC (Anon, 1984; 1990). Macro- and micro-nutrients were determined by the acid digestion method following Wolf's sulphuric acid – hydrogen peroxide method (Wolf, 1982). The extracts so resulted after proper digestion were filtered and used for the analysis of different nutrients. Sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) contents were analyzed using a flame photometer (Jenway PFP-7). Iron, copper, zinc and magnesium were analyzed using an atomic absorption spectrophotometer (Perkin Elmer A-100). Nitrogen was estimated by the micro-Kjeldahl method (Bremner, 1965). Phosphorus contents were estimated spectrophotometrically (Jackson, 1962) using the Barton reagent as described by Ashraf et al. (1992). Phenolic contents were determined spectrophotometrically as described elsewhere (Julkunen- Tiitto, 1985). Flavonoid contents were determined using the standard method of Dewanto et al. (2002). Alkaloidal contents were determined gravimetrically as described elsewhere (Harborne, 1973).

Statistical analysis

The data for different ecological and biochemical attributes were analyzed using the Canoco Computer Package for Windows [Version 4.5]. The LSD values (5%) were used to test the significance of means values. The data for growth and biochemical attributes 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 season or site. The Multivariate Direct Gradient Model was fitted and all variables (nominal) were plotted on *p*RDA Axis 1 and 2.

Results

Dicliptera bupleuroides is a small herbaceous plant that grows under trees and other shady places in the Salt Range. Its growth period starts from late spring, grows luxuriously in summer and dries in autumn. It mostly spreads during rainy seasons of summer, autumn and rarely in spring. The plant samples were collected during different seasons such as autumn, spring and winter at Khabeki and Knotti Garden sites, during summer at Khoora and Anga, and during autumn alone at Dape Sharif site. However, it was not found from Jallar site during any season. It is clear from **Figures 1-9** that different biochemical parameters varied significantly during different seasons and at different sites. Maximum dry matter was recorded during summer at Khoora site which was followed by autumn at Dape Sharif and autumn and spring at Khabeki site. Minimum dry matter was recorded during spring in the ecotype from Knotti Garden. Maximum moisture contents were observed during spring in the Knotti Garden population which was followed by autumn in the population collected from Khabeki site. The minimum moisture contents were observed during summer in the Khoora population. Higher values of moisture contents were noted during autumn and spring and low values during summer.

It is evident (**Figure 2**) that total fiber and total mineral contents in *D. bupleuroides* shoots varied significantly at different sites and during different seasons. Maximum total fibers were observed during summer in the Khoora population which was followed by that from Anga in summer and that in spring in the Knotti Garden population. Minimum fibers were found during autumn in the ecotype from Dape Sharif. Maximum mineral contents were recorded during spring in the ecotype growing at Khabeki site which was followed by in the same population during autumn and summer. Minimum mineral contents were observed during summer and autumn in the Anga and Knotti Garden ecotypes, though these values did not differ significantly. Overall, higher mineral contents were found in the Khabeki population and lower in the Knotti Garden ecotype.

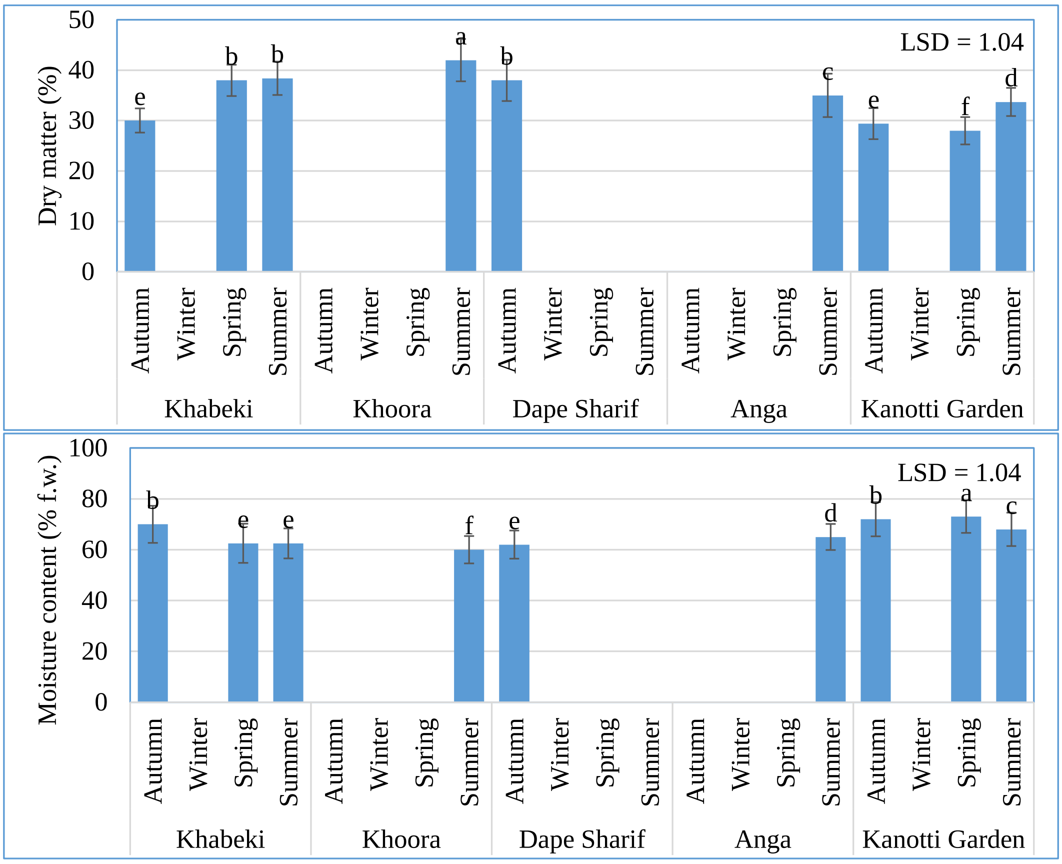

Figure 1. Spatiotemporal variation in dry matter and moisture contents in the shoot of *Dicliptera bupleuroides* collected from different sites within the Salt Range

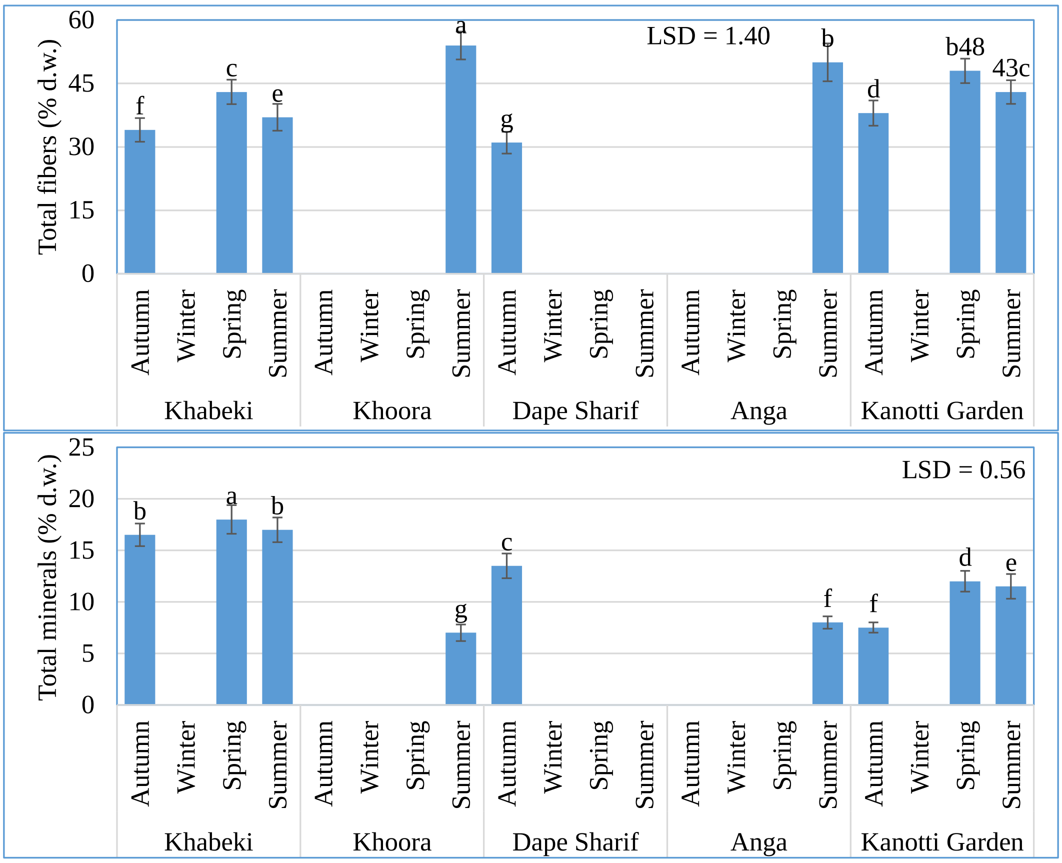

Figure 2. Spatiotemporal variation in total fibers and total minerals in the shoot of *Dicliptera bupleuroides* collected from different sites within the Salt Range

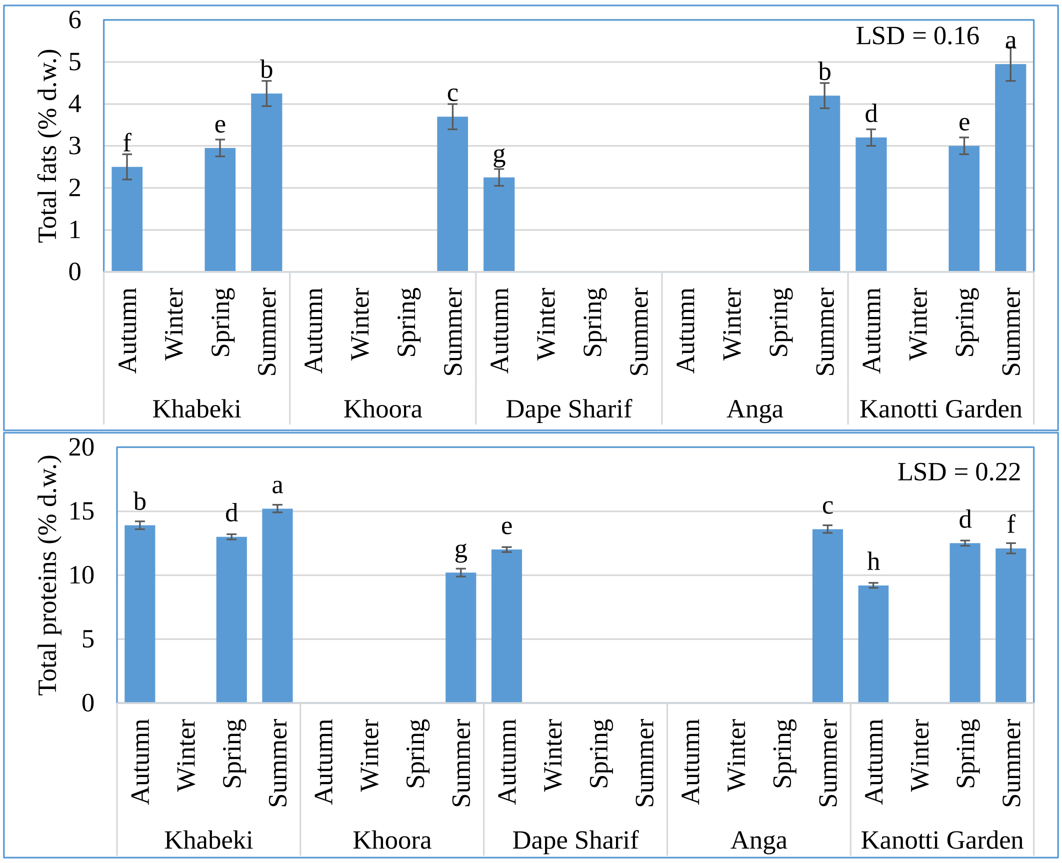

Figure 3. Spatiotemporal variation in total fats and total proteins in the shoot of *Dicliptera bupleuroides* collected from different sites within the Salt Range

Figure 3 shows that fats and protein contents in the *D. bupleuroides* shoots varied significantly at different sites and during different seasons. Maximum fat contents were found during summer in the Knotti Garden ecotype which was followed by that in the populations from Anga and Khabeki sites during summer, which though did not differ significantly. Minimum fat contents were found during autumn in the Dape Sharif ecotype. Maximum protein contents were determined during summer in the Khabeki ecotype, which was followed by that in the same population during autumn. Minimum protein contents were found during summer in the Khoora population. The Khabeki population showed higher values than those from the other populations. It is clear from **Figure 4** that N-free extractable substances varied significantly during different seasons and at different sites. Maximum NFES were found during autumn in the populations from Dape Sharif and Knotti Garden, which though did not differ significantly and these were followed by those in the Khabeki population during autumn. The minimum value was observed during spring in the plants growing at Khabeki.

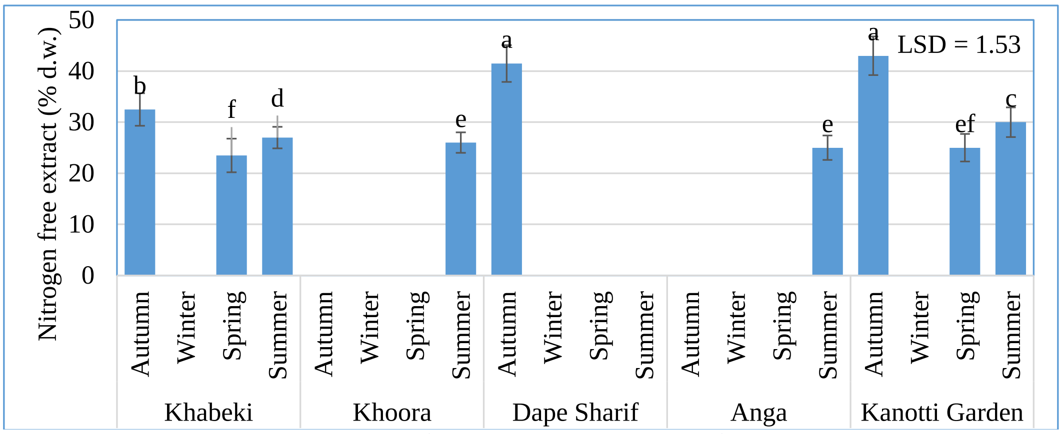

Figure 4. Spatiotemporal variation in nitrogen free extract in the shoot of *Dicliptera bupleuroides* collected from different sites within the Salt Range

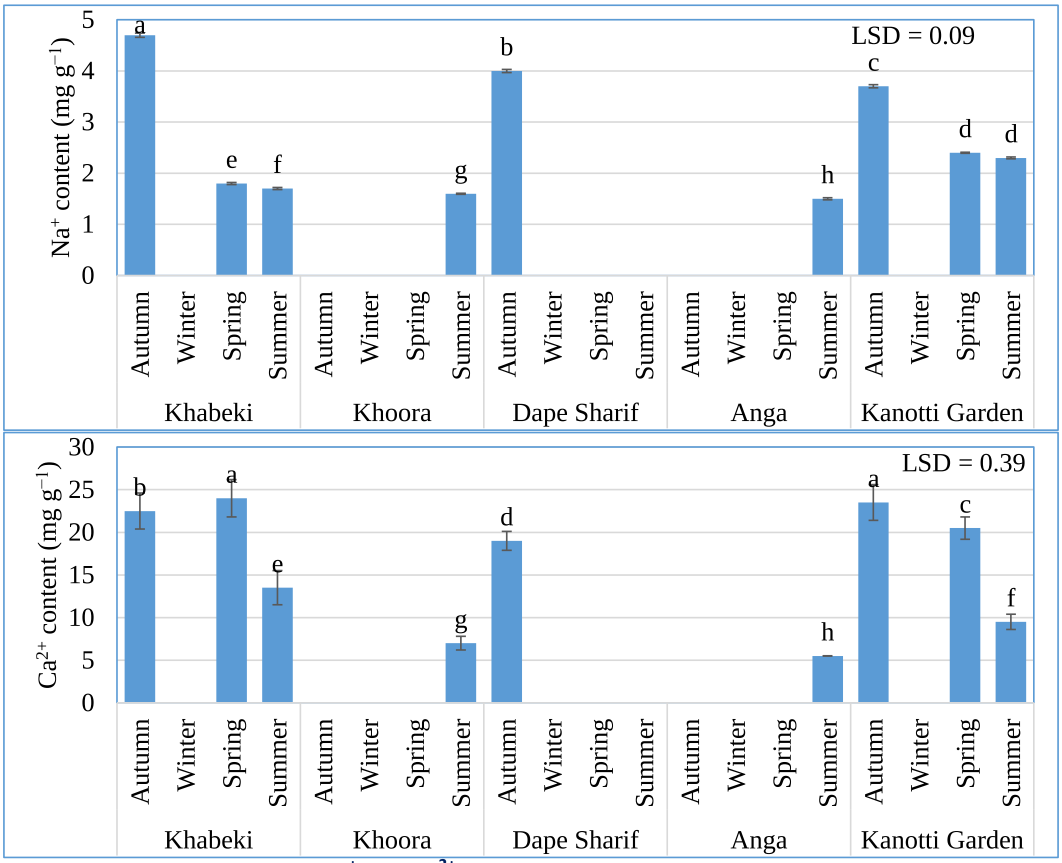

Figure 5. Spatiotemporal variation in Na^+ and Ca^{2+} contents in the shoot of *Dicliptera bupleuroides* collected from different sites within the Salt Range

Figure 5 indicates that Na^{+} and Ca^{2+} contents in the shoots of *D. bupleuroides* varied significantly during different seasons and at different sites. Maximum Na^{+} contents were found during autumn in the plants growing at Khabeki, which was followed by those in the Dape Sharif population during the same season. Minimum Na^{+} contents were found during summer in the plants inhabiting Anga. Similar results were observed for Ca^{2+}, however, maximum Ca^{2+} contents were found during autumn and spring in the Knotti Garden and Khabeki ecotypes, which though differed non-significantly. The minimum concentration of calcium was observed during summer in the Anga ecotype.

It is evident from **Figure 6** that macronutrients in the shoots of *D. bupleuroides* varied significantly during different seasons and at different sites. Maximum N contents were noted during summer in the plants growing at Khabeki and it was followed by that during spring in the same population. Minimum N value was observed during autumn in the plants inhabiting the Knotti Garden site. Maximum P contents were observed during spring in the Knotti Garden population and it was followed by that in the plants growing at Dape Sharif during autumn. Minimum P value was obtained during summer in the plants inhabiting Khoora. Maximum K^{+} contents were observed during spring in the Knotti Garden population and they were followed by those during autumn in the populations from Knotti Garden and Khabeki sites.

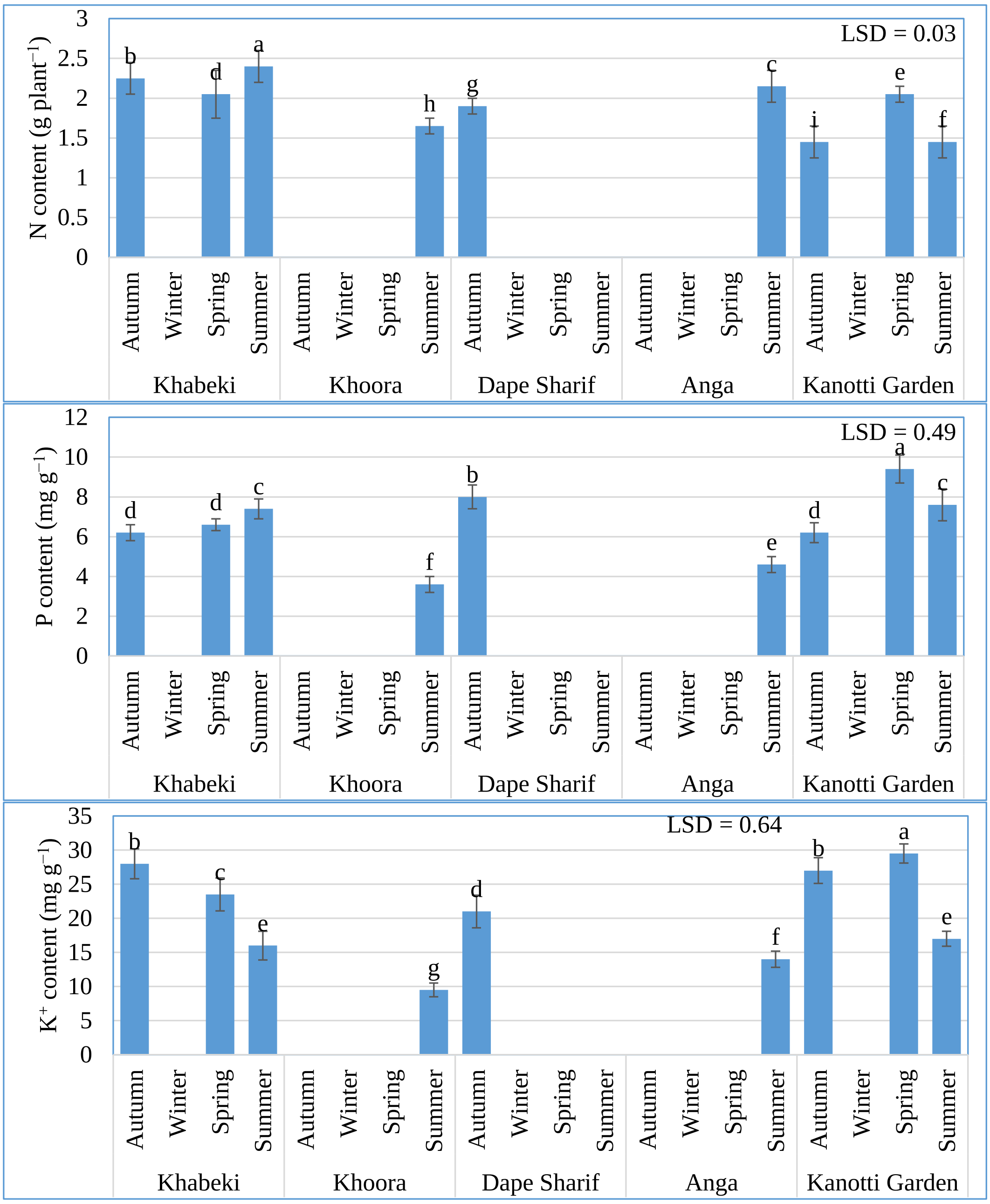

Figure 6. Spatiotemporal variation in N, P and K contents in the shoot of *Dicliptera bupleuroides* collected from different sites within the Salt Range

Leaf Zn^{2+} concentration during summer was the highest in all populations collected from different sites (**Figure 7**). Leaf Mg^{2+} was also highest in all the plant populations except that from Khabeki. Leaf Fe^{2+} was also highest during summer in all populations, whereas Cu^{2+} was only highest in the population from Anga (**Figure 8**).

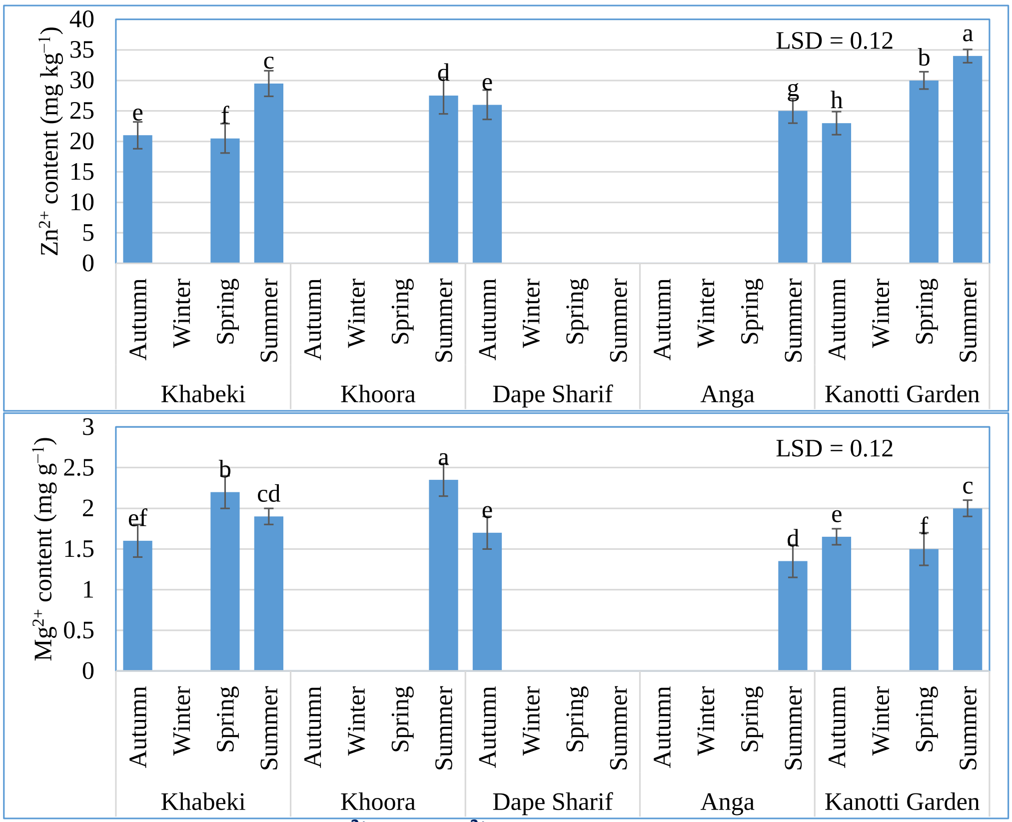

Figure 7. Spatiotemporal variation in Zn^{2+} and Mg^{2+} in the shoot of *Dicliptera bupleuroides* collected from different sites within the Salt Range

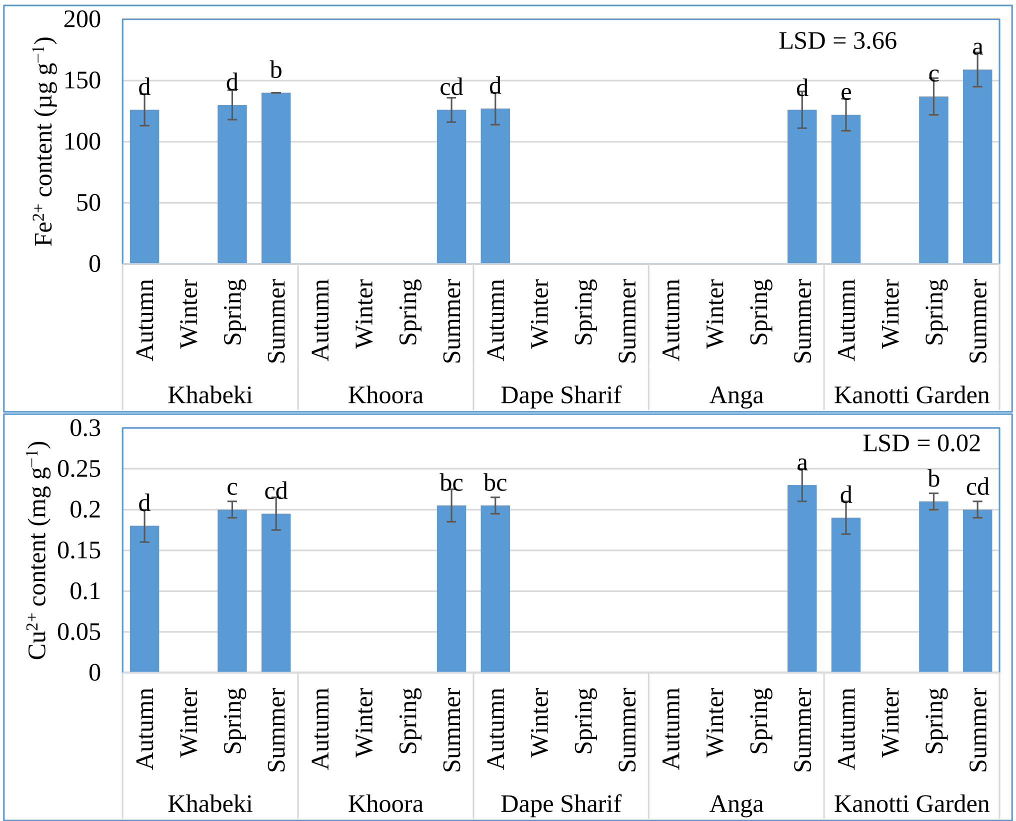

Figure 8. Spatiotemporal variation in Fe^{2+} and Cu^{2+} in the shoot of *Dicliptera bupleuroides* collected from different sites within the Salt Range

Figure 9 shows that organic metabolites also varied significantly during different seasons and at different sites. Maximum phenolic compounds in the shoots of *D. bupleuroides* were found during summer in the plants inhabiting Khabeki, which were followed by those during autumn in the Dape Sharif ecotype, whereas the minimum phenolic compounds were found during summer in the Knotti Garden population. Maximum phenolic compounds were recorded in the roots of *D. bupleuroides* collected from Knotti garden during summer, which was followed by those during spring in the same population. Minimum phenolic compounds were found during summer in the Khabeki ecotype. Total alkaloids in the shoots of *D. bupleuroides* were recorded maximum during summer in the ecotype from Anga, followed by those during spring in the Khabeki ecotype. Minimum alkaloidal contents were found during summer and autumn in the populations from Khoora and Dape Sharif, which though did not differ significantly.

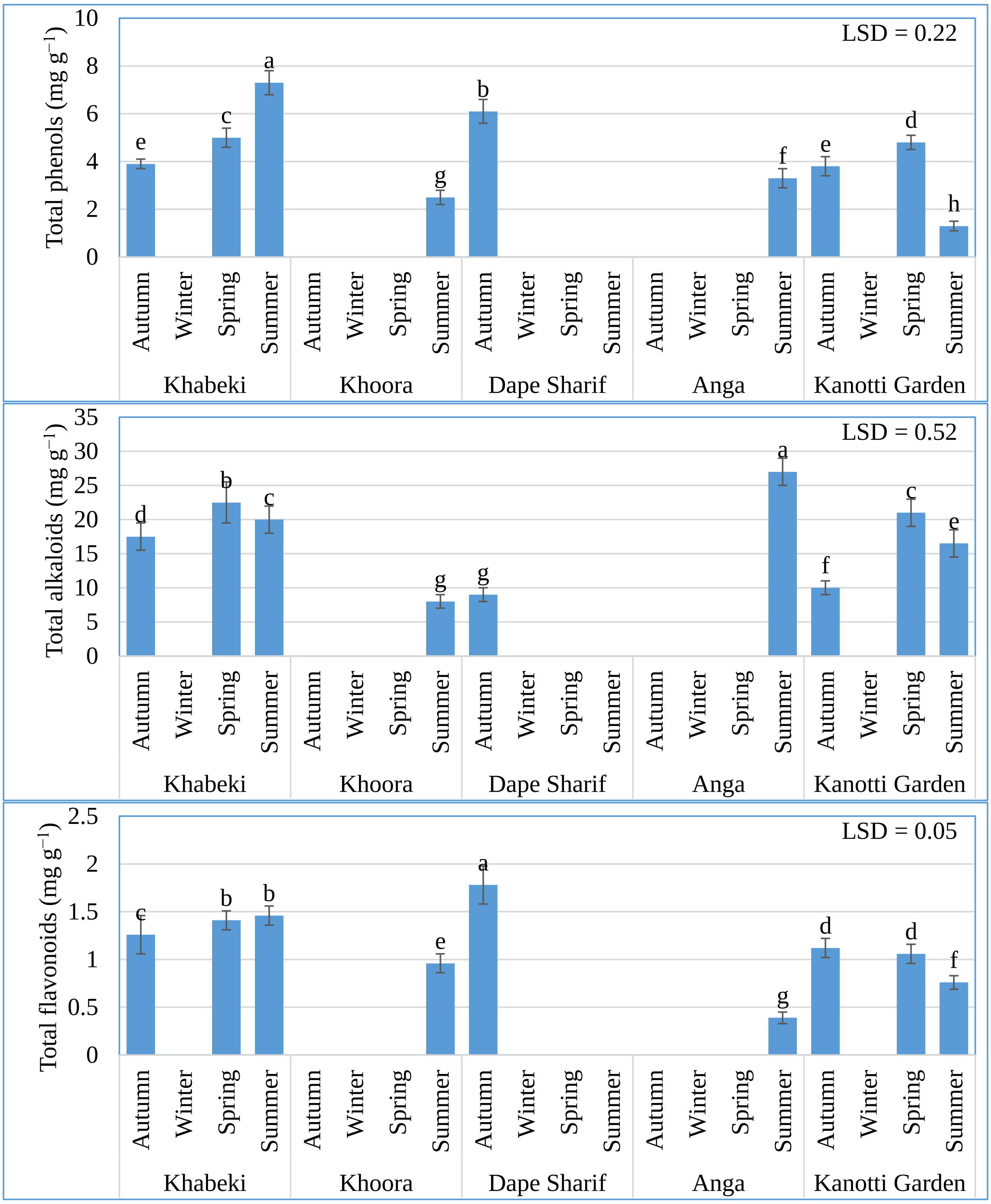

Figure 9. Spatiotemporal variation in total phenols, total alkaloids and total flavonoids in the shoot of *Dicliptera bupleuroides* Nees collected from different sites within the Salt Range

Discussion

A partial RDA ordination biplot showing the effect of seasons and sites on biochemical attributes of *D. bupleuroides* shoots is presented in **Figure 10**. The seasonal variation showed that most of the biochemical attributes were associated with spring and summer seasons. The association of alkaloids and minerals might have been due to high soil pH during spring which favors the accumulation of minerals; also, high pH is necessary for the synthesis of alkaloids (Ahmad et al., 2017). Moreover, the uptake of minerals might have been high during spring, which might have occurred due to the availability of high amounts of moisture contents due to high intensity of spring rainfalls (Ramírezet at al., 2016). Phenols, flavonoids and fibers were higher during spring probably due to stress conditions caused by higher pH and EC or some other environmental stresses (Sarker et al., 2018). The soil analysis showed that pH and EC were reasonably high during spring (**Figure 1**).

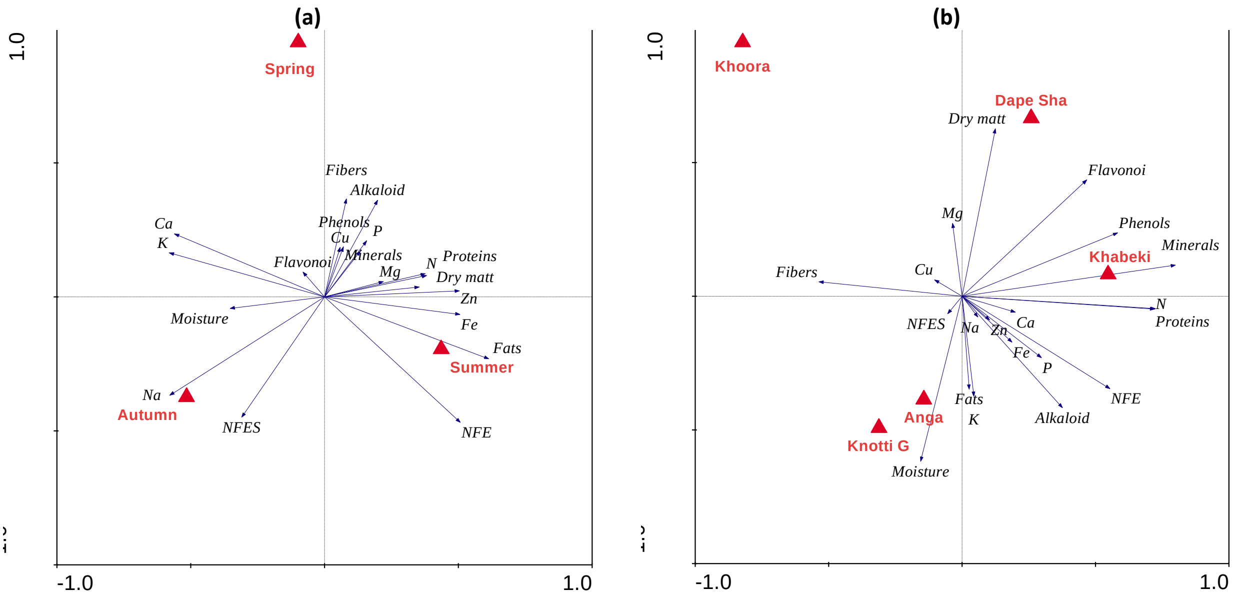

Figure 10. Partial RDA ordination biplot showing the effect of seasons (a) and sites (b) on biochemical attributes of *Dicliptera bupleuroides* shoot collected from the Soone Valley of the Salt Range

Proteins, N, dry matter, fats and NFE were associated with summer, which might have been due to more availability of macro-nutrients during summer (**Figure 1**); because a lot of rain usually occurs during summer due to monsoon (**Table 4**), which might have been one of the factors for increased synthesis of proteins (Tripathi et al., 2014) and other organic compounds such as fats. NFE also increased due to high protein and fat contents during this season. Maturity of plants also plays a significant role to increase the synthesis/accumulation of these compounds during summer and most of plant species contain these compounds reasonably high at maturity (Niedbala et al., 2022).

Moisture content, Na^+, and K^+ were associated with autumn perhaps due to higher moisture availability during this season, because of high rainfall. Nouri et al. (2021) reported that moisture content is strongly associated with growth of the plant (**Table 4**). Another reason could be that plants at the maturity stage generally accumulate high amounts of different minerals which result in lowering the intrinsic osmotic potential, hence a factor that triggers plants to absorb more water. Association of NFES with autumn could have been due to N deficiency usually occurring during autumn (**Table 5**) as N is remobilized from old or senescing leaves to other plant parts so as the plant can reuse it during the active growth period (Fataftah et al., 2022).

Spatial variation showed that phenols and flavonoids were associated with the population from the Khabeki site despite the fact that this population had significantly high amounts of minerals and moisture content compared with those in the other populations. This might have been due to stress conditions probably caused by grazing and cutting (Yeshi et al., 2022) which are common factors at this site. Some other environmental conditions of this site might have also favored the enhanced synthesis of phenols and flavonoids in this population. Minerals, proteins and N were also associated with the Khabeki population perhaps due to the presence of reasonable amounts of minerals and moisture content in the soil of the site (**Figure 1**) as well as the optimum environmental conditions therein. Alkaloids, fats, NFE, moisture and some of the minerals were associated with the populations from Anga and Knotti Garden which might have been due to higher pH of the site soil (Ahmad et al., 2008) as well as salt stress conditions prevailing at these sites. Some soil and environmental interactions may also affect these parameters (Reynolds et al., 2015).

Some of the minerals such as Na, Zn, Ca, and Fe etc. are considered important due to the reason that herbs growing in saline environment absorb most of the minerals from all soils for their active growth (Quasso, 2018). The same role of minerals was noted in *D. bupleuroides.* Fibers and dry matter were more in the Khoora and Dape Sharif populations than those in the other populations. As with other such sites, the stress conditions prevalent at these two sites might have triggered the plants to accumulate more fibers, and hence dry weight (Essafi et al., 2006). Phenols were also correlated with spring that may have been due to high soil EC and pH during this season and other environmental stresses prevalent therein (Bautista et al., 2016). Spatial variation showed a uniform trend as already observed for shoot growth; alkaloids were correlated with the ecotype from the Anga site, and phenols with the Dape Sharif ecotype (Ahmad et al., 2017). Overall, the spring season and Khabeki site are the most favorable season and site, respectively, for plant collection particularly for attaining maximum nutritional and medicinal components.

Acknowledgement
This paper has been extracted from PhD Thesis of the first author.

Supplementary material
No supplementary material is included with this manuscript.

Conflict of interest
The authors declare no conflict of interest.

Source of funding
Declared none

Contribution of authors
Research superior(s): MH, Conduction of experiment: IA, Formal statistical analysis: MSAA, Conceptualization and designing the study: MH, FA, Resource availability: FA, Moderation of laboratory activities: AM, IP, SMRS, Instrumentation and analysis: SB, SF, Preparation of initial draft: MI, AA.

Ethical approval
This study did not involve human/animal subjects, and thus no ethical approval was needed.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All authors contributed in designing and writing the entire review article. All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

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