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Original Article

Chemical divergence of the *Juglans Regia* L. across districts Swat and Dir, Khyber Pakhtunkhwa, Pakistan

Divergência química do *Juglans Regia* L. através dos Distritos Swat e Dir, Khyber Pakhtunkhwa, Paquistão

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Abstract

Juglans regia L. are nutritious fruit bearing plants mostly found in Northern areas of Pakistan. The population of walnuts was explored from district Dir and Swat Khyber Pakhtunkhwa, Pakistan for their geographical, climatic and chemical divergence. The geographical differences such as altitude, latitude and longitude whereas climatic differences viz. soil EC, soil pH, precipitations, intensity of light, temperature and soil temperature. In both districts TPC ranged from 211.2±0.6 to 227.8±0.4 mg/100g, RSA ranged from 43.32±1.5% to 52.18±0.4%, conductivity ranged from 296.43±0.6 to 312.22±0.3 S/m and elemental composition such as iron, copper, calcium, zinc and magnesium in Dir differs from 0.312±0.032, 0.209±0.13, 20.0±0.313, 0.406±0.10 and 10.2±0.030 mg/L to 0.543±0.65, 0.698±0.82, 28.7±0.234, 0.685±0.15 and 17.6±0.015 mg/L respectively. Altitude and temperature showed a correlation with total phenolics contents and radical scavenging activity while soil pH, precipitations, soil temperature, soil Ec and light intensity indicated a weak correlation with chemical traits of walnuts. Further studies of walnuts are needed to explore their therapeutically important phytochemicals to succeed naturally pharmaceutical nutrients of the maximum significance for the health of human beings.

Keywords: Geographical, Northern areas, elemental composition, calcium.

Resumo

Juglans regia L. são plantas frutíferas nutritivas encontradas nas áreas do norte do Paquistão. A população de nozes foi explorada do distrito de Dir e Swat, Khyber Pakhtunkhwa, Paquistão por sua divergência geográfica, climática e química. As diferenças geográficas, como altitude, latitude e longitude, enquanto as diferenças climáticas viz. Ec do solo, pH do solo, precipitações, intensidade de luz, temperatura e temperatura do solo. Em ambos os distritos, o TPC variou de 211,2±0,6 a 227,8±0,4 mg/100g, o RSA variou de 43,32±1,5% a 52,18±0,4%, a condutividade variou de 296,43±0,6 a 312,22±0,3 S/m e composição elementar como ferro, cobre, cálcio, zinco e magnésio em Dir difere de 0,312±0,032, 0,209±0,13, 20,0±0,313, 0,406±0,10 e 10,2±0,030 mg/L a 0,543±0,65, 0,698±0,82, 28,7±0,234, 0,685±0,15 e 17,6±0,015 mg/L respectivamente. A altitude e a temperatura mostraram correlação com os teores de fenólicos totais e atividade de eliminação de radicais, enquanto o pH do solo, precipitações, temperatura do solo, Ec do solo e intensidade de luz indicaram uma correlação fraca com características químicas das nozes. Mais estudos de nozes são necessários para explorar seus fitoquímicos terapeuticamente importantes para obter naturalmente nutrientes farmacêuticos de máxima importância para a saúde dos seres humanos.

Palavras-chave: geográfica, áreas do Norte, composição elementar, cálcio.

1. Introduction

From earlier ages walnuts were used worldwide for humans as a source of nutrition. Their kernels contain more proteins and oil contents that make their fruit best for human health. In the FAO list walnuts for human nutrition is classified as a strategic species and it counted as a central concern plant (Gandev, 2007). Walnuts are rich in nutrients food stuff because it has vitamins, minerals fats, and proteins. It is also a good source of phenolic acids, flavonoids, sterols, pectic substances and interrelated polyphenols. Walnuts nutritive contents vary from cultivar

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to cultivar which may be affected by their genotype, different ecosystem, soil conditions, and cultivator (Muradoglu et al., 2010). The oil of walnut has several constituents that are α-linolenic acids, triacylglycerols (980 g/kg), linoleic acids, oleic acid, monounsaturated fatty acids, and polyunsaturated fatty acids. The word "nutraceutical" is usually used to determine food nutritional supplement that states a medical or health benefit to provide proper nourishment (Wong et al., 2015). They contain useful nutrients which are famous as a good remedy for chronic diseases (Luciano, 2014); their leaves, bark and nuts are ethnobotanically used for the cleansing of teeth (Irfan et al., 2018a, b), leaves are used for intestinal infection and eczema (Irfan et al., 2018c, d), bark is used in cleaning of teeth, toothache and in diabetes (Irfan et al., 2018e); fruits are rich in proteins used for cleaning of teeth and throat infections (Ahmad et al., 2016), bark is used in dry cough, decoction of leaves given for eczema and intestinal worms (Irfan et al., 2017a, b). The relationship between cognitive health and walnuts are inconclusive (Cahoon et al., 2021). Walnuts are an efficient nutrient that contains an important constituent in Mediterranean food and are considered as finest nutrition (Ley et al., 2014). Their green shells, bark, and kernel are mostly important for cosmetic and pharmaceutical industry (Adeel et al., 2017). They are single seeded, round stone fruits commonly used for their meat. Walnuts are ripening between September and November (Grant et al., 2011).

Generally, the nuts of *Juglans regia* L. are used for their nutritive features because it has vital antioxidants potential and is also considered as reserve nutrition elements (Martinez and Maestri, 2016). The seeds of walnuts are considered good for coronary and heart ailments throughout the world (Schwingshackl et al., 2017). It also has numerous nutritive aspects that indicate additional segments of the polyphenolics; therefore, it is assimilated into the nutritional food. Sanchez-Gonzalez et al. (2017) examined a number of phytochemicals in the walnuts such as fatty acids, melatonin, serotonin, and phenolics. Several phytochemicals in walnuts like hydrolyzable and condensed tannin have been studied (Figueroa et al., 2016; Irfan et al., 2019a). High oil contents as well as the protein of walnut kernels helps the fruits necessities for our nutrition. Food and agriculture organization has categorized walnuts as a deliberate candidate and phytomedicine for human food (Gandev, 2007). Generally, from fats energy can be produced from nuts that have nearby 66% and create more calories energy and considered the best nutrition (Tapsell et al., 2009). Monounsaturated fatty acids and triacylglycerols are mostly oleic acid (α-linolenic acids) and polyunsaturated fatty acids (PUFAs) (Martinez and Maestri, 2008). Linoleic acids in the oil of walnut are present in high amounts in all their genotypes. The omega-6 fatty acid is the central constituent of the walnuts which are usually known as linoleic acid. Walnut contains comparatively more quantities of the omega-3 fatty acids about 8-14% of the entire fat contented that is known as alpha-linolenic acids. Deckelbaum and Torrejon (2012) investigated those walnuts are a chief source of alpha-linolenic acids that reduces inflammation and augments blood composition. Muradoglu et al. (2010) examined the normal protein assessment that is up to 18.2% including 70% of the entire seed protein along with fewer amounts of the albumins (7%) globulins (18%) and prolamin about 5%. The proteins of walnuts contain all essential amino acids that are required for our healthy life. New taxa were added to the flora of Pakistan that had highly medicinal importance (Ali et al., 2017). Most of the medicinal plants showed highly antibacterial and antifungal potentials (Ullah et al., 2018; Iftikhar et al., 2019; Irfan et al., 2021, 2024; Ullah et al., 2022).

Amino acids exist in precise proportion in the walnuts especially the lysine and arginine ratio. Low lysine and arginine proportion declines atherosclerosis enlargement (Martinez et al., 2010). Similarly, walnuts contain a proper number of vitamins and minerals like magnesium (Mg), iron (Fe), Phosphorus (P), and Potassium (K) (Cosmulescu et al., 2009). Copper (Cu), Calcium (Ca), Sodium (Na), and Zinc (Zn) are present in accurate ratio (Siahnouri et al., 2013). Macronutrients are significant for the accurate development and growth of organisms (Jeszka-Skowron et al., 2017; Ilyas et al., 2020, 2022). Various macronutrients are involved in enzymatic response for the overall absorption of the organisms. The micronutrients help in the maintenance of heart diseases, bone function, immunity system, and nervous body function (Abdel-Aziz et al., 2016; Irfan et al., 2019b). The aim of the present study was to investigate the total phenolic contents, radical scavenging activity and some minerals contents of the newly selected walnuts genotypes from the Northern areas of Pakistan.

2. Materials and Method

2.1. Collection of walnuts

The nuts of the walnuts were collected for the biochemical research from 44 locations of the districts Swat and Dir. In Dir nuts were collected from 22 localities viz. Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar, Kasky Toormang, Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai while, from district Swat 22 locations were Malam Jaba, Baghderai, Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar.

2.2. Selection procedure

Forty-four mature walnuts plants were labelled in different localities of district Swat and Dir in January 2018. Walnuts plants were occurring naturally in the hilly region of district Swat and Dir. The location's genotype's name was based on their spot and designated by the alphabetical abbreviation. Selected plants were mature in cropping condition and healthy.

2.3. Nuts collection

From walnuts, nuts were collected from different locations of the district Swat and Dir in October 2018.

From each walnut tree in specific site (1 kg) of nuts were collected. Nuts were then dehydrated and kept in plastic bags at room temperature for further chemical analysis.

2.4. Nuts samples preparation

Nuts were collected from the selected walnuts and then they were treated for research investigations. Nuts were divided into two equal amounts and shells were removed and grinded by the blinder machine and transformed to the form of powder. The powdered were then stored in paper bags for further chemical analysis.

2.5. Biochemical study

2.5.1. Elemental composition

The elemental composition of nuts was determined by the wet digestion technique. By a flame photometer (Jenway PFP7) the minerals including calcium (Ca) and magnesium (Mg) were analysed (Khan et al., 2007). There investigated alkaline heavy metals such as calcium, zinc, magnesium and iron were through atomic absorption spectrometer with air/acetylene flame at the 2200–2400K (Photomultiplier tube detectors) (Hanlon et al., 1992).

2.5.2. Walnut total phenolics contents (TPC)

2.5.2.1. Principle of the assay

Chromogens were made when there Folin and Ciocalteu's phenol chemical react with non-phenolics and phenolics reducing resources. Chromogens were sensed spectrophotometrically at 515 nm. The color progress happens of the passage in electron due to their basic pH, to decrease phosphotungstic acids/ phosphomolybdic acids for making chromogens by metals that contain fewer valence.

2.5.2.2. Preparation of Folin's reagent

Two and half grams of sodium molybdate and 10 grams of sodium tungstate were dissolved in 70 mL of distilled water and 5 mL of 85% phosphoric acid and about 10 mL concentrated HCl were combined as a solution. Then the mixture was refluxed for ten hours and 5 mL of distilled water, 15 grams of lithium sulphate, and one droplet of the bromine was added to this mixture, and reflux yet again for 15 minutes. The mixture was then cool to room temperature and distilled water was added to reach their volume up to 100 mL.

2.5.2.3. Procedure

Nuts TPC was observed by using Folin–Ciocalteu reagents by the method of Slinkard and Singleton (1977). The reagents were prepared through dilution stock solution and then added distilled water (1/10, v/v). About 5 mL of Folin–Ciocalteu's phenol chemical, 1 mL of methanolic extractions of nut, and 4.0 mL of Na_2CO_3 (7.5%) were then mixed in the test tube. The absorbance was measured by spectrophotometer after the incubation for 1 hour at 765 nm (Shimadzu, Japan). Total phenolics contents was measured by calibration curve and showed in the mg of gallic acid equivalents/100 g of the walnuts.

2.5.3 Walnut conductivity

Nuts conductivity of *Juglans regia* L. was measured through conductivity meter. Similarly, every 15 minutes after the conductivity of nuts was measured and recorded until 60 minutes.

2.5.4. Walnut radical scavenging activity

2.5.4.1. DPPH solution preparation

The DPPH solution of ethyl acetate was prepared at 0.1 mM amounts dissolved in 7.88 mg DPPH in ethyl acetate of 200 mL.

2.5.4.2. Procedure

Five millilitres, 0.1 mM DPPH solution was mixed in the test tube with the 56.0 µL sample in 30 mL methanol. Thirty minutes after the combination of the solution in dark and then there use UV/Visible spectrophotometer (Japan). Sample mixture absorbance was observed at 515 nm. Radical scavenging activity towards the DPPH radicals was measured by variances from the absorbance of the DPPH solution of the sample. Percentage of the RSA was calculated according to Equation 1 (Lee et al., 2007). Percent RSA was alike as percent inhibition.

$$\%\text{RSA} = \frac{(\text{Ac-As}) \times 100}{\text{Ac}} \quad (1)$$

Here 'As' indicates test sample absorbance and 'Ac' shows control of the absorbance.

2.5.4.3. Statistical analysis

Data recorded were tabulated. The experimental work was organized in a randomized design and took three replications. For comparison data were analyzed on the mean values by sigma plot version. Minitab statistical software, version 14.20 was used for data analysis. Statistical set of the social science version 23 (SPSS Inc., U.S) according to (Norusis, 2015). There Pearson correlation coefficients were also used. The variance analysis (ANOVA) was used to investigate various environmental factors mainly precipitation and temperature effect on the walnut morphological characters. After the mean values of walnut characters correlation of various climatic factors was calculated according to Equation 2 (Fulekar, 2009).

$$r = \frac{\sum xy}{\sqrt{\sum x^2 \times \sqrt{\sum y^2}}} \quad (2)$$

Here 'y' = variable and 'r' = Correlation of coefficient

3. Results

The walnut biochemical parameters like radical scavenging activity, total phenolics contents, elemental

composition, and conductivity of walnuts were examined at various sites of districts Swat and Dir at diverse altitudes.

3.1. Walnuts total phenolics contents

Walnuts' total phenolics contents in the investigated regions districts Swat and Dir were noted at diverse altitudinal regions.

In district Dir total phenolic contents of walnut were noted such as 217.7±0.3, 221.1±0.4, 212.3±0.4, 221.6±0.5, 224.7±0.3, 226.2±0.5, 215.3±0.5, 221.2±0.4, 225.1±0.5, 216.7±0.4, 224.3±0.5, 223.8±0.2, 221.8±0.2, 218.7±0.6, 217.4±0.2, 215.6±0.6, 220.2± 0.7, 211.2±0.6, 213.2±0.1, 218.3±0.1, 219.2±0.2 and 219.2±0.6 mg/100g, in the locations Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar, Kasky Toormang, Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai respectively whereas in the district Swat it was noted such as 227.8±0.4, 214.5±0.2, 212.7±0.5, 218.7±0.3, 216.2±0.1, 219.7±0.3, 225.2±0.4, 212.3±0.4, 225.1±0.1, 224.4±0.7, 223.8±0.5, 219.2±0.2, 217.7±0.6, 223.8±0.5, 211.3±0.3, 223.8±0.7, 213.3±0.7, 220.2±0.7, 223.4±0.3, 216.4±0.4, 222.1±0.2 and 222.7±0.6 mg/100g, in Malam Jaba, Baghderai, Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar places respectively (Table 1).

It was decided that nuts at high altitudes which contain lower temperatures have higher total phenolic contents (Kalam and Laram) in both Districts investigated regions whereas in the low altitude which contain higher temperatures have less total phenolic contents (Rangila and Balo Rabat). There is a significant difference present in all research places (Table 1).

3.2. Conductivity of walnut

Conductivity of walnuts was investigated by the conductivity meter. Walnut's conductivity in both investigated regions districts Swat and Dir were noted at diverse altitudinal sites.

In districts Dir conductivity of walnuts was noted by conductivity meter after 15 minutes as 226.8±0.5, 221.1±0.3, 220.3±0.2, 226.0±0.6, 226.3±0.1, 230.2±0.7, 225.5±0.7, 224.1±0.6, 227.1±0.8, 225.2±0.2, 223.2±0.7, 222.4±0.4, 220.5±0.5, 226.5±0.7, 225.1±0.1, 225.3±0.3, 221.2±0.2, 215.3±0.1, 221.4±0.9, 222.4±0.4, 228.8±0.3 and 222.0±0.2 S/m, in their locations Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar, Kasky Toormang,

Table 1. Variations in total phenolic contents of nuts of district Swat and Dir.

S.No	Dir Sites	TPC (mg/100g)	Swat Sites	TPC (mg/100g)
1	Abi Shah	217.7±0.3 ^{B}	Malam Jaba	227.8±0.4 ^{D}
2	Asilo Rabat	221.1±0.4 ^{D}	Baghderai	214.5±0.2 ^{A}
3	Khanpur	212.3±0.4 ^{A}	Yakhtangy	212.7±0.5 ^{A}
4	Siagaonai	221.6±0.5 ^{D}	Nalcot	218.7±0.3 ^{B}
5	Babyawar	224.7±0.3 ^{D}	Tegako	216.2±0.1 ^{A}
6	Laram	226.2±0.5 ^{D}	Chuprial	219.7±0.3 ^{C}
7	Barimky	215.3±0.5 ^{A}	Milaga	225.2±0.4 ^{D}
8	Usherai Dara	221.2±0.4 ^{D}	Bihar	212.3±0.4 ^{A}
9	New Kaly	225.1±0.5 ^{D}	Bar Thana	225.1±0.1 ^{D}
10	Jabar	216.7±0.4 ^{A}	Kalam	224.4±0.7 ^{D}
11	Barawal	224.3±0.5 ^{D}	Ghaki Banda	223.8±0.5^{D}
12	Sia Wargar	223.8±0.2 ^{D}	Shakar Dara	219.2±0.2^{C}
13	Kasky Toormang	221.8±0.2 ^{D}	Qalagy	217.7±0.6^{B}
14	Sheringal	218.7±0.6 ^{B}	Bazkhela	223.8±0.5 ^{D}
15	Zeyam	217.4±0.2 ^{B}	Rangila	211.3±0.3 ^{A}
16	Parakot	215.6±0.6 ^{A}	Shawar	223.8±0.7 ^{D}
17	Rabat Dara	220.2±0.7 ^{C}	Sar Bala	213.3±0.7 ^{A}
18	Balo Rabat	211.2±0.6 ^{A}	Peochar	220.2±0.7 ^{C}
19	Dog Dara	213.2±0.1 ^{A}	Totanu Banda	223.4±0.3 ^{D}
20	Chokyatan	218.3±0.1 ^{B}	Baidara	216.4±0.4 ^{A}
21	Buth Qillah	219.2±0.2 ^{C}	Lalko	222.1±0.2 ^{D}
22	Bombolai	219.2±0.6 ^{C}	Murghozar	222.7±0.6 ^{D}

In column similar letters values no significant difference $\alpha < 0.05$.

Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai locations respectively whereas in District Swat it was noted as 228.5±0.8, 224.5±0.2, 227.3±0.6, 227.6±0.6, 223.4±0.7, 228.4±0.3, 226.5±0.7, 222.2±0.3, 226.2±0.7, 221.9±0.1, 225.3±0.3, 220.1±0.2, 227.5±0.4, 225.3±0.7, 219.5±0.5, 225.5±0.1, 222.7±0.2, 223.5±0.8, 227.1±0.9, 222.9±0.4, 226.2±0.4 and 222.2±0.7 S/m, in the Malam Jaba, Baghderai, Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar locations respectively (Table 2 & 3). In district Dir conductivity of walnuts was noted by conductivity meter after 30 minutes as 242.5±0.2, 243.7±0.4, 243.2±0.1, 242.0±0.5, 241.3±0.4, 251.7±0.9, 243.9±0.4, 240.1±0.9, 243.2±0.3, 245.5±0.7, 247.0±0.5, 241.4±0.2, 249.0±0.3, 245.7±0.8, 245.7±0.3, 243.4±0.2, 240.8±0.5, 238.4±0.1, 249.7±0.1, 242.4±0.3, 244.2±0.4 and 246.5±0.4 S/m, in their locations Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar, Kasky Toormang, Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai locations respectively whereas in district Swat it was noted as 250.3±0.3, 241.6±0.3, 243.6±0.7, 241.6±0.7, 240.7±0.3, 246.7±0.1, 240.7±0.3, 240.5±0.3, 247.9±0.3, 274.4±0.7, 244.2±0.3, 242.8±0.6, 241.4±0.7, 242.8±0.8, 238.5±0.2, 246.3±0.4, 248.1±0.2, 249.0±0.5, 242.2±0.1, 244.7±0.9, 242.5±0.4 and 241.1±0.4 S/m, in the Malam Jaba, Baghderai, Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar locations respectively (Table 2 & 3).

In districts Dir conductivity of walnuts was noted by conductivity meter after 45 minutes as 272.3±1.3, 277.5±1.6, 279.1±1.2, 273.1±0.4, 274.1±0.7, 280.7±0.9, 278.1±0.2, 279.2±0.1, 273.9±1.6, 273.6±1.3, 273.7±1.5, 273.9±0.4, 275.4±0.1, 271.2±1.6, 278.2±1.3, 272.6±1.2, 276.6±1.3, 269.5±0.4, 276.5±1.3, 270.0±1.4, 274.3±0.4 and 276.8±1.8 S/m, in their locations Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar, Kasky Toormang, Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai locations respectively whereas in District Swat it was noted as 280.9±1.2, 272.7±1.4, 279.3±1.2, 277.1±1.0, 271.6±1.3, 276.5±1.2, 271.2±1.7, 278.2±1.8, 273.6±1.4, 279.2±1.1, 276.1±1.9, 272.2±1.5, 275.2±1.5, 271.5±1.0, 268.6±0.3, 273.3±1.2, 272.8±1.8, 274.4±1.3, 274.6±0.2, 270.4±1.3, 273.6±1.3 and 272.5±1.6 S/m, in the Malam Jaba, Baghderai,

Table 2. Variations in conductivity of walnuts at 25°C temperature in Dir sites.

S.No	Dir sites	Conductivity (S/m)			
		15 minutes	30 minutes	45 minutes	60 minutes
1	Abi Shah	226.8±0.5 ^{DD}	242.5±0.2 ^{BA}	272.3±1.3 ^{CA}	302.7±0.5 ^{DA}
2	Asilo Rabat	221.1±0.3 ^{AA}	243.7±0.4 ^{BB}	277.5±1.6 ^{CC}	308.1±0.4 ^{DC}
3	Khanpur	220.3±0.2 ^{AA}	243.2±0.1 ^{BB}	279.1±1.2 ^{CD}	309.4±0.2 ^{DD}
4	Siagaonai	226.0±0.6 ^{DD}	242.0±0.5 ^{BA}	273.1±0.4 ^{CB}	302.2±0.4 ^{DA}
5	Babyawar	226.3±0.1 ^{DD}	241.3±0.4 ^{BA}	274.1±0.7 ^{CB}	302.1±0.5 ^{DA}
6	Laram	230.2±0.7 ^{DD}	251.7±0.9 ^{BD}	280.7±0.9 ^{CD}	312.2±0.3 ^{DD}
7	Barimky	225.5±0.7 ^{CC}	243.9±0.4 ^{BB}	278.1±0.2 ^{CC}	304.2±0.1 ^{DB}
8	Usherai Dara	224.1±0.6 ^{CC}	240.1±0.9 ^{BA}	279.2±0.1 ^{CD}	206.5±0.3 ^{DC}
9	New Kaly	227.1±0.8 ^{DD}	243.2±0.3 ^{BB}	273.9±1.6 ^{CB}	300.7±0.9 ^{DA}
10	Jabar	225.2±0.2 ^{CC}	245.5±0.7 ^{BB}	273.6±1.3 ^{CB}	304.4±0.2 ^{DB}
11	Barawal	223.2±0.7 ^{BB}	247.0±0.5 ^{BC}	273.7±1.5 ^{CB}	309.6±0.8 ^{DD}
12	Sia Wargar	222.4±0.4 ^{BB}	241.4±0.2 ^{BA}	273.9±0.4 ^{CB}	306.5±0.4 ^{DC}
13	Kasky Toormang	220.5±0.5 ^{AA}	249.0±0.3 ^{BD}	275.4±0.1 ^{CB}	307.0±0.4 ^{DC}
14	Sheringal	226.5±0.7 ^{DD}	245.7±0.8 ^{BB}	271.2±1.6 ^{CA}	308.2±0.4 ^{DC}
15	Zeyam	225.1±0.1 ^{CC}	245.7±0.3 ^{BB}	278.2±1.3 ^{CC}	305.1±0.7 ^{DB}
16	Parakot	225.3±0.3 ^{CC}	243.4±0.2 ^{BB}	272.6±1.2 ^{CA}	301.3±0.1 ^{DA}
17	Rabat Dara	221.2±0.2 ^{AA}	240.8±0.5 ^{BA}	276.6±1.3 ^{CC}	310.5±0.7 ^{DD}
18	Balo Rabat	215.3±0.1 ^{AA}	238.4±0.1 ^{BA}	269.5±0.4 ^{CA}	296.4±0.6 ^{DA}
19	Dog Dara	221.4±0.9 ^{AA}	249.7±0.1 ^{BD}	276.5±1.3 ^{CC}	298.8±0.2 ^{DA}
20	Chokyatan	222.4±0.4 ^{BB}	242.4±0.3 ^{BA}	270.0±1.4 ^{CA}	303.5±0.7 ^{DB}
21	Buth Qillah	228.8±0.3 ^{DD}	244.2±0.4 ^{BB}	274.3±0.4 ^{CB}	304.1±0.7 ^{DB}
22	Bombolai	222.0±0.2 ^{BB}	246.5±0.4 ^{BC}	276.8±1.8 ^{CC}	306.5±0.3 ^{DC}

In column similar letters values no significant difference $\alpha < 0.05$.

Table 3. Variations in conductivity of walnuts at 25°C in different localities of district Swat.

S.No	Sites of district Swat	Conductivity (S/m)			
		15 minutes	30 minutes	45 minutes	60 minutes
1	Malam Jaba	228.5±0.8 ^{DD}	250.3±0.3 ^{BD}	280.9±1.2 ^{CD}	310.3±0.9 ^{DD}
2	Baghderai	224.5±0.2 ^{CC}	241.6±0.3 ^{BA}	272.7±1.4 ^{CA}	308.2±0.1 ^{DC}
3	Yakhtangy	227.3±0.6 ^{DD}	243.6±0.7 ^{BB}	279.3±1.2 ^{CD}	306.2±0.8 ^{DC}
4	Nalcot	227.6±0.6 ^{DD}	241.6±0.7 ^{BA}	277.1±1.0 ^{CC}	301.2±0.3 ^{DA}
5	Tegako	223.4±0.7 ^{BB}	240.7±0.3 ^{BA}	271.6±1.3 ^{CA}	305.2±0.6 ^{DB}
6	Chuprial	228.4±0.3 ^{DD}	246.7±0.1 ^{BC}	276.5±1.2 ^{CC}	301.0±0.8 ^{DA}
7	Milaga	226.5±0.7 ^{DD}	240.7±0.3 ^{BA}	271.2±1.7 ^{CA}	308.2±0.4 ^{DA}
8	Bihar	222.2±0.3 ^{BB}	240.5±0.3 ^{BA}	278.2±1.8 ^{CC}	304.3±0.1 ^{DB}
9	Bar Thana	226.2±0.7 ^{DD}	247.9±0.3 ^{BC}	273.6±1.4 ^{CB}	308.7±0.6 ^{DC}
10	Kalam	221.9±0.1 ^{AA}	274.4±0.7 ^{BC}	279.2±1.1 ^{CD}	308.1±0.6 ^{DC}
11	Ghaki Banda	225.3±0.3 ^{CC}	244.2±0.3 ^{BB}	276.1±1.9 ^{CC}	301.2±0.1 ^{DA}
12	Shakar Dara	220.1±0.2 ^{AA}	242.8±0.6 ^{BA}	272.2±1.5 ^{CA}	302.6±0.2 ^{DA}
13	Qalagy	227.5±0.4 ^{DD}	241.4±0.7 ^{BA}	275.2±1.5 ^{CB}	302.6±0.3 ^{DA}
14	Bazkhela	225.3±0.7 ^{CC}	242.8±0.8 ^{BA}	271.5±1.0 ^{CA}	303.2±0.4 ^{DB}
15	Rangila	219.5±0.5 ^{AA}	238.5±0.2 ^{BA}	268.6±0.3 ^{CA}	300.2±0.3 ^{DA}
16	Shawar	225.5±0.1 ^{CC}	246.3±0.4 ^{BC}	273.3±1.2 ^{CB}	307.1±0.9 ^{DC}
17	Sar Bala	222.7±0.2 ^{BB}	248.1±0.2 ^{BC}	272.8±1.8 ^{CA}	305.5±0.2 ^{DB}
18	Peochar	223.5±0.8 ^{BB}	249.0±0.5 ^{BD}	274.4±1.3 ^{CB}	309.8±0.3 ^{DD}
19	Totanu Banda	227.1±0.9 ^{DD}	242.2±0.1 ^{BA}	274.6±0.2 ^{CB}	305.2±0.6 ^{DB}
20	Baidara	222.9±0.4 ^{BB}	244.7±0.9 ^{BB}	270.4±1.3 ^{CA}	304.2±0.5 ^{DB}
21	Lalko	226.2±0.4 ^{DD}	242.5±0.4 ^{BA}	273.6±1.3 ^{CB}	306.3±0.2 ^{DC}
22	Murghozar	222.2±0.7 ^{BB}	241.1±0.4 ^{BA}	272.5±1.6 ^{CA}	306.2±0.2 ^{DC}

In column similar letters values no significant difference $\alpha < 0.05$.

Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar locations respectively (Table 2 & 3).

In districts Dir conductivity of walnuts was noted by conductivity meter after 60 minutes as 302.7±0.5, 308.1±0.4, 309.4±0.2, 302.2±0.4, 302.1±0.5, 312.2±0.3, 304.2±0.1, 206.5±0.3, 300.7±0.9, 304.4±0.2, 309.6±0.8, 306.5±0.4, 307.0±0.4, 308.2±0.4, 305.1±0.7, 301.3±0.1, 310.5±0.7, 296.4±0.6, 298.8±0.2, 303.5±0.7, 304.1±0.7 and 306.5±0.3 S/m, in their locations Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar, Kasky Toormang, Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai locations respectively whereas in district Swat it was noted as 310.3±0.9, 308.2±0.1, 306.2±0.8, 301.2±0.3, 305.2±0.6, 301.0±0.8, 308.2±0.4, 304.3±0.1, 308.7±0.6, 308.1±0.6, 301.2±0.1, 302.6±0.2, 302.6±0.3, 303.2±0.4, 300.2±0.3, 307.1±0.9, 305.5±0.2, 309.8±0.3, 305.2±0.6, 304.2±0.5, 306.3±0.2 and 306.2±0.2 S/m, in the Malam Jaba, Baghderai, Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar locations respectively (Table 2 & 3).

In our study we concluded that conductivity increases with an increase in the time. Walnuts at high altitudes contain lower temperatures which have higher conductivity (Kalam and Laram) in both the districts investigated regions whereas the low altitude which contains higher temperatures has low conductivity (Rangila and Balo Rabat). Significantly variance was existed in all the research places (Table 2 & 3).

3.3. Walnut radical scavenging activity

Radical scavenging activity of walnuts in both investigated district Swat and Dir were noted at diverse elevations.

Radical scavenging activity of Dir walnuts was observed as 45.47±1.6, 49.31±0.4, 47.88±1.6, 44.13±1.4, 48.77±03, 51.73±1.4, 47.83±0.5, 46.23±1.7, 49.87±1.2, 50.34±0.4, 49.63±1.5, 48.11±0.2, 49.48±1.1, 47.23±1.4, 48.53±0.2, 45.74±1.5, 49.26±1.3, 43.62±1.2, 47.22±0.3, 45.27±1.3, 46.27±0.2 and 50.22±0.3% in their locations Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar, Kasky

Toormang, Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai locations respectively whereas in Swat it was noted as 52.18±0.4, 45.25±0.2, 47.84±0.2, 48.67±0.3, 46.22±0.1, 49.67±1.3, 47.12±1.7, 46.23±1.4, 49.71±0.1, 46.42±0.7, 48.66±1.1, 45.22±1.2, 49.37±0.6, 48.68±1.5, 43.32±1.5, 48.81±0.7, 47.35±1.7, 46.42±0.7, 50.12±0.4, 50.34±0.4, 44.13±1.2 and 49.22±0.3%, in the Malam Jaba, Baghderai, Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar respectively (Table 4).

It was noted that RSA was more in walnuts at low temperature and high-altitude places viz. Malam Jaba and Laram and less at low altitude and high temperature places such as Rangila and Balo Rabat in both District Swat and Dir. Significant difference was existed in all the studied localities (Table 4).

3.4. Walnut elemental composition

Elemental composition (Fe, Zn, Cu, Mg and Ca) of walnuts in both investigated regions of districts Swat and Dir were noted at various elevations.

Zinc (Zn): Zinc (Zn) of Dir walnuts were noted in their locations viz. Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar, Kasky Toormang, Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai locations as 0.537±0.13, 0.613±0.12, 0.533±0.20, 0.512±0.16, 0.632±0.14, 0.510±0.17, 0.618±0.21, 0.654±0.19, 0.611±0.12, 0.532±0.19, 0.542±0.17, 0.672±0.17, 0.587±0.14, 0.542±0.19, 0.631±0.13, 0.523±0.18, 0.534±0.39, 0.674±0.23, 0.631±0.14, 0.521±0.12, 0.632±0.19 and 0.565±0.31 mg/L respectively. Whereas in district Swat it was noted in the Malam Jaba, Baghderai, Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar locations as 0.406±0.10, 0.427±0.34, 0.423±0.24, 0.413±0.01, 0.511±0.42, 0.681±0.25, 0.589±0.01, 0.616±0.03, 0.574±0.17, 0.545±0.17, 0.434±0.41, 0.412±0.12, 0.545±0.15, 0.583±0.23, 0.685±0.15, 0.576±0.01, 0.673±0.19, 0.417±0.12, 0.409±0.26, 0.569±0.34, 0.426±0.13 and 0.543±0.12 mg/L respectively (Table 5 & 6).

Copper (Cu): Copper (Cu) of district Dir nuts were noted in their localities viz. Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar,

Table 4. Variations in RSA of nuts at district Swat and Dir.

S.No	Sites of Dir district	RSA%	Sites of Swat district	RSA%
1	Abi Shah	45.47±1.6 ^{A}	Malam Jaba	52.18±0.4 ^{C}
2	Asilo Rabat	49.31±0.4 ^{C}	Baghderai	45.25±0.2 ^{A}
3	Khanpur	47.88±1.6 ^{B}	Yakhtangy	47.84±0.2 ^{B}
4	Siagaonai	44.13±1.4 ^{A}	Nalcot	48.67±0.3 ^{B}
5	Babyawar	48.77±.03 ^{B}	Tegako	46.22±0.1 ^{A}
6	Laram	51.73±1.4 ^{C}	Chuprial	49.67±1.3 ^{C}
7	Barimky	47.83±0.5 ^{B}	Milaga	47.12±1.7 ^{B}
8	Usherai Dara	46.23±1.7 ^{A}	Bihar	46.23±1.4 ^{A}
9	New Kaly	49.87±1.2 ^{C}	Bar Thana	49.71±0.1 ^{C}
10	Jabar	50.34±0.4 ^{C}	Kalam	46.42±0.7 ^{A}
11	Barawal	49.63±1.5 ^{C}	Ghaki Banda	48.66 ±1.1 ^{B}
12	Sia Wargar	48.11±0.2 ^{B}	Shakar Dara	45.22±1.2 ^{A}
13	Kasky Toormang	49.48±1.1 ^{C}	Qalagy	49.37±0.6 ^{C}
14	Sheringal	47.23±1.4 ^{B}	Bazkhela	48.68±1.5 ^{B}
15	Zeyam	48.53±0.2 ^{B}	Rangila	43.32±1.5 ^{A}
16	Parakot	45.74±1.5 ^{A}	Shawar	48.81±0.7 ^{B}
17	Rabat Dara	49.26±1.3 ^{C}	Sar Bala	47.35±1.7 ^{B}
18	Balo Rabat	43.62±1.2 ^{A}	Peochar	46.42±0.7 ^{A}
19	Dog Dara	47.22±0.3 ^{B}	Totanu Banda	50.12±0.4 ^{C}
20	Chokyatan	45.27±1.3 ^{A}	Baidara	50.34±0.4 ^{C}
21	Buth Qillah	46.27±0.2 ^{A}	Lalko	44.13±1.2 ^{A}
22	Bombolai	50.22±0.3 ^{C}	Murghozar	49.22±0.3 ^{C}

In column similar letters values no significant difference $\alpha < 0.05$.

Table 5. Elemental composition of walnuts in Dir sites.

S.No	Sites of district Dir	Elemental composition (mg/L)				
		Zn	Cu	Mg	Ca	Fe
1	Abi Shah	0.537±0.13 AA	0.649±0.27 C	16.1±0.330 DC	26.1±0.334 CB	0.418±0.032 BC
2	Asilo Rabat	0.613±0.12 AB	0.479±0.23 A	11.1±0.010 DA	24.4±0.332 CA	0.312±0.032 BB
3	Khanpur	0.533±0.20 AA	0.446±0.96 A	11.1±0.130 DA	28.5±0.337 CB	0.234±0.038 BA
4	Siagaonai	0.512±0.16 AA	0.583±0.11 B	12.4±0.021 DB	26.5±0.198 CB	0.326±0.020 BB
5	Babyawar	0.632±0.14 AB	0.588±0.85 B	13.4±0.021 DB	21.5±0.186 CA	0.236±0.027 BA
6	Laram	0.510±0.17 AA	0.698±0.82 C	17.6±0.015 DC	28.7±0.234 CB	0.422±0.020 BC
7	Barimky	0.618±0.21 AB	0.543±0.43 B	11.3±0.210 DA	21.2±0.436 CA	0.244±0.013 BA
8	Usherai Dara	0.654±0.19 AB	0.682±0.37 C	12.5±0.023 DB	23.7±0.212 CA	0.363±0.032 BB
9	New Kaly	0.611±0.12 AB	0.532±0.68 B	13.4±0.210 DB	26.5±0.132 CB	0.372±0.036 BB
10	Jabar	0.532±0.19 AA	0.589±0.13 B	13.4±0.022 DB	23.3±0.212 CA	0.313±0.038 BB
11	Barawal	0.542±0.17 AA	0.587±0.72 B	13.4±0.010 DB	21.2±0.224 CA	0.224±0.022 BA
12	Sia Wargar	0.672±0.17 AB	0.534±0.74 B	12.2±0.010 DB	23.7±0.233 CA	0.409±0.021 BC
13	Kasky Toormang	0.587±0.14 AA	0.608±0.56 C	14.2±0.020 DC	22.9±0.232 CA	0.315±0.024 BB
14	Sheringal	0.542±0.19 AA	0.622±0.67 C	10.6±0.040 DA	22.1±0.332 CA	0.392±0.012 BB
15	Zeyam	0.631±0.13 AB	0.685±0.54 C	13.2 ± 0.020 DB	23.2±0.212 CA	0.333±0.035 BB
16	Parakot	0.523±0.18 AA	0.586±0.62 B	14.5 ± 0.013 DC	21.4±0.190 CA	0.241±0.013 BA
17	Rabat Dara	0.534±0.39 AA	0.654±0.23 C	14.7 ± 0.120 DC	21.3±0.336 CA	0.213±0.044 BA
18	Balo Rabat	0.674±0.23 AB	0.442±0.38 A	10.1 ± 0.120 DA	21.1±0.139 CA	0.212±0.018 BA
19	Dog Dara	0.631±0.14 AB	0.484±0.46 A	14.6 ± 0.014 DC	27.3±0.233 CB	0.372±0.029 BB
20	Chokyatan	0.521±0.12 AA	0.481±0.92 A	12.3 ± 0.009 DB	24.2±0.223 CA	0.321±0.033 BB
21	Buth Qillah	0.632 ± 0.19 AB	0.583±0.69 B	13.4±0.010 DB	21.5±0.238 CA	0.234±0.032 BA
22	Bombolai	0.565 ± 0.31 AA	0.463±0.56 A	13.2±0.030 DB	23.4±0.137 CA	0.245±0.022 BA

In column similar letters values no significant difference $\alpha < 0.05$.

Table 6. Variations in elemental composition of walnuts in district Swat.

S.No	Sites of district Swat	Elemental composition (mg/L)				
		Zn	Cu	Mg	Ca	Fe
1	Malam Jaba	0.406±0.10 AA	0.543±0.65 B	14.7±0.030 DB	38.1±0.247 CD	0.348±0.32 BB
2	Baghderai	0.427±0.34 AA	0.623±0.03 C	11.4±0.026 DA	24.6±0.412 CA	0.345±0.34 BB
3	Yakhtangy	0.423±0.24 AA	0.515±0.23 B	13.3±0.021 DB	25.1±0.223 CA	0.312±0.32 BB
4	Nalcot	0.413±0.01 AA	0.522±0.09 B	11.7±0.032 DA	28.4±0.432 CB	0.322±0.04 BB
5	Tegako	0.511±0.42 AB	0.534±0.01 B	13.2±0.032 DB	25.3±0.411 CA	0.322±0.03 BB
6	Chuprial	0.681±0.25 AC	0.412±0.12 A	12.1±0.034 DB	36.3±0.221 CD	0.338±0.03 BB
7	Milaga	0.589±0.01 AB	0.498±0.07 A	12.7±0.021 DB	31.2±0.442 CC	0.223±0.08 BA
8	Bihar	0.616±0.03 AC	0.448±0.16 A	11.3±0.028 DA	32.2±0.344 CC	0.318±0.01 BB
9	Bar Thana	0.574±0.17 AB	0.434±0.10 A	13.2±0.021 DB	21.0±0.322 CA	0.223±0.03 BA
10	Kalam	0.545±0.17 AB	0.433±0.02 A	11.4±0.025 DA	33.2±0.432 CC	0.273±0.13 BA
11	Ghaki Banda	0.434±0.41 AA	0.456±0.25 A	11.4±0.032 DA	32.2±0.202 CC	0.337±0.45 BB
12	Shakar Dara	0.412±0.12 AA	0.687±0.06 C	12.5±0.030 DB	30.7±0.247 CB	0.226±0.02 BA
13	Qalagy	0.545±0.15 AB	0.406±0.12 A	12.9±0.026 DB	23.4±0.249 CA	0.243±0.15 BA
14	Bazkhela	0.583±0.23 AB	0.546±0.23 B	11.5±0.033 DA	34.4±0.222 CC	0.238±0.15 BA
15	Rangila	0.685±0.15 AC	0.592±0.06 B	10.2±0.030 DA	20.0±0.313 CA	0.209±0.13 BA
16	Shawar	0.576±0.01 AB	0.696±0.05 C	12.4± 0.030 DB	33.1±0.218CC	0.211±0.02 BA
17	Sar Bala	0.673±0.19 AC	0.565±0.45 B	11.6±0.031 DA	37.2±0.432 CD	0.327±0.25 BB
18	Peochar	0.417±0.12 AA	0.475±0.15 A	12.1±0.032 DB	27.2±0.349 CB	0.312±0.04 BB
19	Totanu Banda	0.409±0.26 AA	0.445±0.03 A	13.3±0.021 DB	32.2±0.208 CC	0.223±0.08 BA
20	Baidara	0.569±0.34 AB	0.556±0.40 B	13.3±0.025 DB	23.2±0.413 CA	0.321±0.06 BB
21	Lalko	0.426±0.13 AA	0.509±0.08 B	13.2±0.032 DB	21.1±0.218 CA	0.254±0.23 BA
22	Murghozar	0.543±0.12 AB	0.612±0.12 C	12.2±0.025 DB	24.1±0.441 CA	0.325±0.05 BB

In column similar letters values no significant difference $\alpha < 0.05$.

Kasky Toormang, Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai locations as 0.649±0.27, 0.479±0.23, 0.446±0.96, 0.583±0.11, 0.588±0.85, 0.698±0.82, 0.543±0.43, 0.682±0.37, 0.532±0.68, 0.589±0.13, 0.587±0.72, 0.534±0.74, 0.608±0.56, 0.622±0.67, 0.685±0.54, 0.586±0.62, 0.654±0.23, 0.442±0.38, 0.484±0.46, 0.481±0.92, 0.583±0.69 and 0.463±0.56 mg/L respectively, whereas in district Swat it was noted in the Malam Jaba, Baghderai, Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar locations as 0.543±0.65, 0.623±0.03, 0.515±0.23, 0.522±0.09, 0.534±0.01, 0.412±0.12, 0.498±0.07, 0.448±0.16, 0.434±0.10, 0.433±0.02, 0.456±0.25, 0.687±0.06, 0.406±0.12, 0.546±0.23, 0.592±0.06, 0.696±0.05, 0.565±0.45, 0.475±0.15, 0.445±0.03, 0.556±0.40, 0.509±0.08 and 0.612±0.12 mg/L respectively (Table 5 & 6).

Iron (Fe): Iron (Fe) of district Dir nuts were noted in their locations viz. Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar, Kasky Toormang, Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai locations as 0.418±0.032, 0.312±0.032, 0.234±0.038, 0.326±0.020, 0.236±0.027, 0.422±0.020, 0.244±0.013, 0.363±0.032, 0.372±0.036, 0.313±0.038, 0.224±0.022, 0.409±0.021, 0.315±0.024, 0.392±0.012, 0.333±0.035, 0.241±0.013, 0.213±0.044, 0.212±0.018, 0.372±0.029, 0.321±0.033, 0.234±0.032 and 0.245±0.022 mg/L respectively, whereas in District Swat it was noted in the Malam Jaba, Baghderai, Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar locations as 0.348±0.32, 0.345±0.34, 0.312±0.32, 0.322±0.04, 0.322±0.03, 0.338±0.03, 0.223±0.08, 0.318±0.01, 0.223±0.03, 0.273±0.13, 0.337±0.45, 0.226±0.02, 0.243±0.15, 0.238±0.15, 0.209±0.13, 0.211±0.02, 0.327±0.25, 0.312±0.04, 0.223±0.08, 0.321±0.06, 0.254±0.23 and 0.325±0.05 mg/L, respectively (Table 5 & 6).

Magnesium (Mg): Magnesium (Mg) of Dir nuts were noted in their locations viz. Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar, Kasky Toormang, Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai locations as 16.1±0.330, 11.1±0.010, 11.1±0.130, 12.4±0.021, 13.4±0.021, 17.6±0.015, 11.3±0.210, 12.5±0.023, 13.4±0.210, 13.4±0.022, 13.4±0.010, 12.2±0.010, 14.2±0.020, 10.6±0.040, 13.2±0.020, 14.5±0.013, 14.7±0.120, 10.1±0.120, 14.6±0.014, 12.3±0.009, 13.4±0.010 and 13.2±0.030 mg/L respectively, whereas in district Swat it was noted in the Malam Jaba, Baghderai, Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar locations as 14.7±0.030, 11.4±0.026, 13.3±0.021, 11.7±0.032, 13.2±0.032, 12.1±0.034, 12.7±0.021, 11.3±0.028, 13.2±0.021, 11.4±0.025, 11.4±0.032, 12.5±0.030, 12.9±0.026, 11.5±0.033, 10.2±0.030, 12.4±0.030, 11.6±0.031, 12.1±0.032, 13.3±0.021, 13.3±0.025, 13.2±0.032 and 12.2±0.025 mg/L respectively (Table 5 & 6).

Calcium (Ca): Calcium of district Dir nuts were noted in their locations viz. Abi Shah, Asilo Rabat, Khanpur, Siagaonai, Babyawar, Laram, Barimky, Usherai Dara, New Kaly, Jabar, Barawal, Sia Wargar, Kasky Toormang, Sheringal, Zeyam, Parakot, Rabat Dara, Balo Rabat, Dog Dara, Chokyatan, Buth Qillah and Bombolai locations as 26.1±0.334, 24.4±0.332, 28.5±0.337, 26.5±0.198, 21.5±0.186, 28.7±0.234, 21.2± 0.436, 23.7±0.212, 26.5±0.132, 23.3±0.212, 21.2±0.224, 23.7±0.233, 22.9±0.232, 22.1±0.332, 23.2±0.212, 21.4±0.190, 21.3±0.336, 21.1±0.139, 27.3±0.233, 24.2±0.223, 21.5±0.238 and 23.4±0.137 mg/L, respectively whereas in district Swat it was noted in the Malam Jaba, Baghderai, Yakhtangy, Nalcot, Tegako, Chuprial, Milaga, Bihar, Bar Thana, Kalam, Ghaki Banda, Shakar Dara, Qalagy, Bazkhela, Rangila, Shawar, Sar Bala, Peochar, Totanu Banda, Baidara, Lalko and Murghozar locations as 38.1±0.247, 24.6±0.412, 25.1±0.223, 28.4±0.432, 25.3±0.411, 36.3±0.221, 31.2±0.442, 32.2±0.344, 21.0±0.322, 33.2±0.432, 32.2±0.202, 30.7±0.247, 23.4±0.249, 34.4±0.222, 20.0±0.313, 33.1±0.218, 37.2±0.432, 27.2±0.349, 32.2±0.208, 23.2±0.413, 21.1±0.218 and 24.1±0.441 mg/L respectively (Tables 5 & 6).

In our results it was noted that calcium (Ca), iron (Fe), copper (Cu) and Magnesium were more in walnuts at low temperature and high altitude places viz. Malam Jaba and Laram and less at low altitude and high temperature places like Rangila and Balo Rabat whereas zinc were less at low temperature and high elevation viz. Malam Jaba and Laram and more at low altitude and high temperature places like Rangila and Balo Rabat in both district Swat and Dir. Significant differences were existed in all the studied localities (Table 5 & 6).

4. Discussion

The biochemicals parameters like radical scavenging activity, elemental composition, total phenolics contents and conductivity of walnuts were examined at various sites of district Swat and Dir at various elevations.

4.1. Walnuts total phenolics contents

Total phenolics contents of particular walnuts in both investigated regions of district Swat and Dir were noted at various altitudinal sites.

In district Swat more amount of TPC was investigated at Malam Jaba which was 227.8±0.4 mg/100g whereas in Dir it was noted in the Laram that were 226.2±0.5 mg/100g (Table 3). Malam Jaba and Laram annual rainfall were more that was 1660 mm and 1870 mm. The minimum and maximum temperature was less in Malam Jaba and Laram. The altitude of Malam Jaba and Laram was investigated as 2804 m and 2590.8 m respectively.

The intensity of light was maximum in that places which were recorded as 155.45±2.2 Cd and 150±1.6 Cd, whereas the temperature of soil was low detected as 19±0.35 ºC and 19±0.42 ºC. The soil was acidic having 6.90±0.16 and 6.88±0.25 pH values and have more EC was detected as 0.60±0.18 mS/cm and 0.61±0.18 mS/cm. The minimum and maximum temperature in Laram was investigated as 4.0 ºC and 24.1 ºC whereas the minimum and maximum temperature in Malam Jaba was detected as 4.2 ºC and 23.2 ºC respectively (Table 1).

In district Swat less amount of TPC was detected in Rangila that was 211.3±0.3 mg/100g whereas in Dir it was noted in Balo Rabat that was 211.2± 0.6 mg/100g (Table 5 & 6). The soil was less acidic having 6.10±0.27 and 6.11±0.15 pH values and have less electrical conductivity was recorded as 0.20±0.17 and 0.22±0.16 mS/cm. Annual rainfall of Rangila and Balo Rabat is less and 1360 mm and 1333 mm, while minimum and maximum temperature were high in Balo Rabat, was 9.8 ºC and 33.9 ºC and in Rangila that was 13.7 ºC and 33.7 ºC respectively. The altitude of Rangila and Balo Rabat was 778 m and 1062m respectively. Light intensity was lower in these places which were 93±2.4 Cd and 85.56±1.5 Cd, whereas the temperature of soil was more 28±0.22 ºC and 27±0.47 ºC (Table 1 & 3).

In both districts Swat and Dir altitude and temperature indicated a significant positive correlation with TPC whereas soil pH, precipitation, and soil Ec show a weak negative correlation. Light intensity and temperature of soil indicated a weak positive correlation with the TPC in both districts (Table 7).

It was determined that there was a great effect of temperature and altitude whereas the minor effect of light intensity, soil EC, soil pH, and rainfall on the TPC. It was also determined that walnuts of the high altitude that contain lower temperature have higher TPC (Kalam and Laram) in both districts of investigated regions whereas low altitude which contains higher temperature has less TPC (Rangila and Balo Rabat). The similar result was reported by Nerg et al. (1994) that environmental differences diverged the total phenolics contents in 3 regions of different latitudes from Finland. Muradoglu et al. (2010) investigated those nutritive contents of the walnuts vary from a cultivar to the cultivar that can be affected by their cultivators, genotypes, different ecosystems, and different soil conditions. Blomhoff et al. (2006) documented that nuts have maximum quantities of antioxidants, polyphenols, phenolic compounds and tocopherols that are situated in the nut's pellicles. Oliveira et al. (2008) documented those phenolic compounds and natural antioxidants were found in walnuts that were essentials because it reduced the risk of degenerative infections and recover their health. Ghasemi et al. (2011) documented that various climatic and geographical conditions in various areas like lowest daily temperature and highest altitude in a geographic region Abali have various bioactive compounds containing phenolics content and walnuts bioactivities. Omwamba and Hu (2009) investigated that in the highest temperature regions their total phenolics contents reduced due to their degradation.

4.2. Walnuts conductivity

Walnut's conductivity was recorded by a conductivity meter. The conductivity of walnuts in both investigated regions of district Swat and Dir were noted at various altitudinal sites.

In district Swat maximum conductivity up to 60 min after the treatment was noted in Malam Jaba which was

Table 7. Correlation between climatic factors and chemical parameters of walnuts.

Climatic Factors	Districts	Co-efficient correlation							
		RSA	Conductivity	TPC	Elemental composition				
					Zn	Cu	Mg	Ca	Fe
Precipitation	Swat	-.205	-.192	-.106	-.181	.179	.187	-.186	.023
	Dir	-.264	-.105	-.141	-.160	.127	.105	-.148	.062
Temperature	Swat	.542	-.036	.493*	.007	-.211	-.122	.258	-.296
	Dir	.558	-.009	.450*	.030	-.213	-.126	.252	-.247
Soil pH	Swat	-.283	.156	-.297	-.107	.120	-.236	.086	-.133
	Dir	-.225	.146	-.264	-.137	.142	-.286	.008	-.196
Altitude	Swat	.623	-.056	.431*	.046	.212	.115	.283	.280
	Dir	.642	-.028	.478*	.024	.225	.186	.268	.251
Soil temperature	Swat	.158	.215	.216	-.260	-.258	-.166	.163	-.136
	Dir	.120	.257	.226	-.240	-.229	-.197	.177	-.103
Light intensity	Swat	.126	-.184	.353	.023	-.120	.223	-.281	.242
	Dir	.199	-.120	.374	.016	-.174	.274	-.271	.237
Soil EC	Swat	-.138	-.209	-.392	-.289	.078	-.323	.190	.093
	Dir	-.175	-.241	-.316	-.211	.006	-.351	.113	.068

* Correlations are significant at 0.05 level (2-tailed).

310.3±0.9 S/m whereas in Dir it was investigated in Laram that was 312.2±0.3 S/m (Table 3 & 4). In district Swat minimum conductivity up to 60 min after the treatment was noted in Rangila which was 300.2±0.3 S/m whereas in Dir it was investigated in Laram which was 296.4±0.6 S/m (Table 3 & 4).

In both districts Swat and Dir precipitations, altitude, temperature, soil Ec and light intensity indicated a weak negative correlation with the conductivity, whereas soil temperature and soil pH showed a weak positive correlation (Table 3).

It was decided that there is less effect of altitude, light intensity, temperature, rainfall, soil EC and soil pH on the conductivity. It was also observed that at higher altitudes the walnut cotyledons were soft due to which electrolytes were released maximum as compared to the low altitude which contains hard cotyledons. In walnuts conductivity increases with an increase in time due to the existence of vascular opening by which their high quantity of electrolytes discharges and bulky nut size yields maximum electrical conductivity. Our results agreed with the previous study of Sivritepe et al. (2015) and Bonner (1968) who documented that the increased amount of conductivity might be due to the electrolyte that was beneath and within the walnut pericarp. Mark et al. (1990) and Matthews and Powell (2006) as well as McDonald Junior and Wilson (1980) documented that electrolyte total quantity leaked by nuts in soaking period gives us the assessment of electrical conductivity. The results also presented resemblances with the Pandey (1992) who investigated that soft nut possess a poor membrane system that results in maximum electrolyte losing and therefore indicate higher electrical conductivity.

4.3. Radical scavenging activity

The walnuts RSA in both investigated regions district Swat and Dir were noted at various altitudinal sites.

Maximum radical scavenging activity in district Swat was investigated in Malam Jaba which was 52.18±0.4% whereas in Dir it was observed in Laram which was 51.73±1.4% (Table 3). Similarly, minimum radical scavenging activity in District Swat was investigated in Rangila was 43.32±1.5% whereas in District Swat it was observed in Balo Rabat which was 43.62±1.2% (Table 3). In both districts Swat and Dir altitude and temperature indicated a positive correlation whereas light intensity, soil temperature and altitude indicated a weak positive correlation with the RSA. Soil Ec, Soil pH, precipitation and indicated weak negative correlation with the RSA (Table 4).

It was determined that the RSA was more in walnuts at low temperature and high altitudinal places such as Malam Jaba and Laram was less at low altitude and high-temperature places such as Rangila and Balo Rabat in both districts Swat and Dir.

From our results it was determined that there is much effect of temperature and altitude whereas less effect of the soil pH, light intensity, soil EC, and rainfall on the RSA. Our results showed similarities to the Ghasemi et al. (2011) that various climatic and geographical conditions in various areas such as high altitude and low temperature have more bioactive compounds and RSA in the nuts. On the other hand, RSA at higher altitudes is as low as compared to the results reported by Carvalho et al. (2010). Meanwhile Muradoglu et al. (2010) investigated those nutritive substances of nuts vary from a cultivar to another cultivar that can be affected by different ecology, cultivators, genotypes and various soil conditions. Oliveira et al. (2008) investigated that phenolics compounds and antioxidants are present in the walnuts that were essential because they reduce the risk of degenerative infections and recover our health problems. Xu et al. (2017) documented that a decrease of the antioxidant activity may take place due to the phenolic compounds degradation as they heated to a higher temperature of low altitude sites.

4.4. Walnut elemental composition

Walnuts elemental composition (Fe, Zn, Cu, Mg, and Ca) of the investigated localities of district Swat and Dir were noted at diverse altitudinal regions.

In Dir it was noted that iron (Fe), copper (Cu), magnesium (Mg) and calcium was maximum that were 0.422±0.020, 0.698±0.82, 28.7±0.234, and 17.6±0.015 mg/L respectively, whereas zinc was lower which was 0.510±0.17 mg/L in the Laram. Similarly in district Swat iron, copper, magnesium, and calcium were maximum that were 0.348±0.32, 0.543±0.65, 38.1±0.247 and14.7±0.030 mg/L respectively, whereas zinc was lower viz. 0.406±0.10 mg/L in Malam Jaba site (Table 3 & 4). In Dir it was observed that iron, copper, calcium, and magnesium were minimum that were 0.212±0.018, 0.442±0.38, 21.1±0.139 and 10.1±0.120 mg/L respectively, whereas zinc was maximum that were 0.674±0.23 mg/L in Balo Rabat. Similarly in district Swat iron, copper, magnesium and calcium was less that were 0.209±0.13, 0.592±0.06, 20.0±0.313 and 10.2±0.030 mg/L respectively, whereas zinc was more that was 0.685±0.15 mg/L in Rangila (Table 3 & 4). In both regions of district Swat and Dir the copper concentration indicated a weak negative correlation with the Soil and air temperature, light intensity whereas it presented a weak positive correlation with soil pH, altitude, precipitation and soil Ec. The zinc concentration indicated a weak negative correlation with soil Ec, soil pH, precipitation and soil temperature whereas it indicated a weak positive correlation with light intensity, altitude and temperature. The iron concentration indicated a weak negative correlation with the soil pH, temperature and soil temperature whereas it indicated a weak positive correlation with altitude, precipitation, soil Ec and light intensity. The calcium concentration indicated a weak negative correlation with light intensity and precipitation whereas it indicated a weak positive correlation with altitude, temperature, soil pH, soil Ec and soil temperature. The concentration of magnesium indicated a weak negative correlation with the soil pH, temperature, soil Ec and soil temperature whereas it indicated weak positive correlation with light intensity, altitude and precipitation (Table 4).

Coefficient correlation between all the environmental features i.e. altitude, temperature, precipitation, light intensity, soil pH, soil EC and temperature of soil with all

the parameters of the elemental analysis i.e. zinc, copper, iron, magnesium and calcium were noted in (Table 3).

It was concluded that there is a small effect of light intensity, altitude and temperature, soil EC, rainfall, and soil pH on the elemental composition (Zn, Cu, Mg, Fe and Ca). It was also noted that iron, copper, magnesium, and calcium was more in walnuts at high altitude and lower temperature places viz. Malam Jaba and Sheringal and less at low altitude and high temperature places like Rangila and Balo Rabat whereas, zinc was less at low temperature and high-altitude places viz. Malam Jaba and Sheringal and more at low altitude and high temperature places like Rangila and Balo Rabat in both district Swat and Dir. Our results agreed with Cosmulescu et al. (2009) stated that minerals composition of 9 *Juglans regia* L. cultivars at various climatic and geographical environments instigated from Romania. Cosmulescu et al. (2009) investigated that nuts macronutrient had a rich source of vitamins, minerals like magnesium, potassium, iron, and phosphorus. Siahnouri et al. (2013) investigated that calcium, zinc, sodium and copper were present in proper percentages. Jeszka-Skowron et al. (2017) investigated macronutrients that were essential for proper body growth and development for human beings. Abdel-Aziz et al. (2016) reported that micronutrients maintain the immune system, heart health, function of bone and nervous system functions. In contrast the examined values of magnesium were nearly closer to the values investigated by (Irfan et al., 2018f; Ravai, 1992) whereas calcium, copper, zinc and iron indicated some differences.

5. Conclusion

In current study the effects of different climatic drivers on the chemical composition of *Juglans regia* L. nuts were investigated. In climatic investigations it was concluded that higher altitude regions have a low temperature of the soil, high light intensity, more precipitation, high EC have less acidic soil with more rainfall, low maximum annual temperature whereas the less altitudinal areas with less light intensity, low precipitation, more soil temperature have less acidic soil with less EC and regions with great maximum annual temperature and low rainfall. In the chemical investigations it was determined that walnuts of greater altitude have lower temperature with higher radical scavenging activity, total phenolics contents and conductivity in both districts' localities, while less altitude with maximum temperature has less total phenolics contents. The calcium (Ca), iron (Fe), magnesium (Mg), and copper (Cu) was maximum in the walnuts of low temperature and higher altitude locations and less at higher temperature and lower altitude locations while, element zinc was minimum at low temperature and high altitude sites and maximum at higher temperature and low altitude locations in both district Swat and Dir. Altitude and temperature resulted moderate correlation with total phenolic contents (TPC) and radical scavenging activity (RSA) while soil temperature, precipitation, soil Ec, soil pH and light intensity indicated a weak correlation with the walnuts chemical traits. Walnuts chemical composition was changeable that depends on their diversities, soil conditions, climatic conditions, ecology and genotypes.

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