SPATIAL VARIATIONS IN NUTRITIONAL AND ELEMENTAL PROFILE OF MAKO (*Solanum nigrum)* COLLECTED FROM DIFFERENT TEHSILS OF DISTRICT MIANWALI, PUNJAB, PAKISTAN

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ABSTRACT: *The survey was conducted to assess the nutritional composition and elemental profile of Solanum nigrum collected from different tehsils (Mianwali, Esakhel, Piplan) of District Mianwali. Highest moisture (28.48%), ash (21.68%) and fat contents (14.23%) were present in tehsil Mianwali. Highest carbohydrate content (25.75%), crude fiber (13.04%) and crude protein content (0.41%) was observed in tehsil Piplan. Highest concentration of Cr (0.16mg/kg), Mg (6.76mg/kg), Mn (0.12mg/kg), Fe (8.19 mg/kg) and Pb (1.85 mg/kg) was present in tehsil Piplan. Highest concentration of Zn (3.52mg/kg) was noted in tehsil Esakhel. Highest concentration of Cd (0.82mg/kg) and Cr (0.25mg/kg) was present in samples collected from tehsil Mianwali. Variation in nutritional composition and elemental profile of Solanum nigrum may be attributed to soil composition (nutrients) and difference of climatic factor prevailing in different tehsils of District Mianwali.*

INTRODUCTION

The main aim of the study is to explore the nutrition and the elemental profile of medicinal plants in District Mianwali and neighboring areas based on medicinal plant resources with particular importance on citations of native knowledge from neighboring Communities. The people of the district have affiliation with bordering plant resources. Due to lack of work on medicinal plants in District Mianwali, this study is very important. Total 450 species were reported in Mianwali 150 species are important for medicinal point of view, 71 as nutritionally, 40 fuelwoods, 17 timbers, 66 fodder, 15 of agricultural implements, 46 vegetables and 15 species were used as fruit production. The study concludes that there is need of time to awake the local communities to guard and protect their plant resources by educating each other for sustainable growth and consumption [1]. The district is surrounded on the north by district of KPK and Attock district of Punjab, on the east by Kohat Districts, on the south by Bhakkar District of Punjab and on the west by Lakki, Karak and Dera Ismail Khan District of KPK again [2].

The Mianwali district consists of three Tehsils such as Mianwali, Piplan and Esakhel. The Mianwali City is the larger town with 140000 populations. Kalabagh is prominent due to the brown salt hills, Indus River, Kalabagh Dam and the Nawab of Kalabagh. The tehsil Piplan is located on south side and tehsil Esakhel toward north-west. It is also the commercial and economic focus of the District.

Hernoli Is a very well-known town sited south part of district and famous for manufacture on grams and peanut. Musakhel is formed due to awan tribe. They continue still a pleasant summer option for tourists because of the cool temperature in summer and vegetation (highest peak at 1,621 meters).

The area is predominantly inhabited by Niazi tribes followed by Shahs, Mughal, Afghan pathans, Baloch, Sheikh, Ghakkars, Mochi, Rajputs, Qureshi, Khaja, Sheikh, Tarkahns, Khattaks, Blochs and Awans (Malik) etc. The majority of these tribes are present in Mianwali who came from Asia and Afghanistan. Cultural and ancestral affiliations are actually strong [3].

The medicinal plants have retained aromatic compounds used in all over the world. They are also important due to their effective efficiency of curing diseases with no side effects [4]. Medicinal plants have contained oxalates that protect them from herbivores, maintained ion balance in cells and structural support [4]. The phytomedicines are also obtain from medicinal plants. These phytomedicines are important due to presence of trace elements i.e. Fe, Ca, Cr, Mg, Cu and K etc. [5]. The glycoprotein consists of weight up to 150 KDa from seeds of *Solanum nigrum*. This plant has also been consisting of protein contents (30.26%) and sugar contents (69.75%) [6]. The protein contents were high amount in fruits of *Solanum nigrum* then seeds. The seeds of this plant are rich carbohydrates. This plant has retained 6.14% ash and 6.45% moisture contents [7].

The Roots of *Solanum nigrum* are used to cure cough, asthma, bronchitis, chest pain, laxative, phlegmatic, antispasmodic, chest pain and diuretic. Fruits of *Solanum nigrum* are used to cure migraine, cough and headache [8] [9]. The seeds of *Solanum nigrum* are used to purify blood and improve blood cholesterol level while roots of *Solanum nigrum* are used as expectorants agents [9]. The *Solanum nigrum* had retained group of organic compounds such as cellulose, carbohydrates, cellulose, proteins, and gums. It serves as a most important energy foundation in diet of grazing animals [10]. Alkaloids were high amount and saponines were also present in fewer amount in this medicinal plant. Alkaloids are used as anesthetic agents where as saponins boost the immune system, dipping the danger of getting liver cancer and lowering blood cholesterol level [11].

The significant range of Mg in leaves of *Solanum nigrum* is 3.7 mg Kg^{-1} [12]. The concentration of Mn in *Solanum nigrum* was (2.67 mg/kg) [13]. The concentration of Cr was equal in stem and roots of medicinal plants in water logged soil. Soil contained more Cr then other elements [14]. The mineral contents in plants were considerably observed lower winter season [15]. The concentration of Zn ranged from 4.23 to419.66mg/100ml and Cu ranged from 13.53 to 121mg/100ml and they were expressively higher when related with the standard optional dietary allowance. these are very useful in protein synthesis, cellular differentiation,

cellular metabolism, memory, learning, replication, protection and sexual purposes [16].

MATERIAL AND METHOD

Area of study

The survey was conducted to assess the nutrition and elemental composition of selected medicinal plants such as *Solanum nigrum* in the three Tehsils of District Mianwali, Punjab, Pakistan. Mianwali is situated between 32°-10° to33°-15° north latitude and 71°-08 to 71°-57° east. Most of the part is the persistence of Potohar Pleatue and Salt Range the Mianwali District is surrounded on the north by Kheber Pukhtun Khawa and Attock District of Punjab, on the east by Kohat Districts, the south by Bhakkar District of Punjab and on the west by Lakki, Karak and Dera Ismail Khan District of Kheber Pukhtun Khawa again. The Climate of the Mianwali District as an entire is severe with extended hot summer and cold dry winters. Summer season from May to September and winter season from October till February. July is the hottest month in Mianwali with standard temperatures of 43 °C while December and January are the coldest months with average minimum temperature 6°C -7°C.

Collection of samples

Plants sample collection

Three Tehsils of District Mianwali were selected for the purpose of sample collection. Leaves and roots of selected medicinal plant (*Solanum nigrum)* were collected from all three tehsils (Mianwali, Esakhel, Piplan) for analysis. Each sample is randomly picked up, wrapped in a particular envelop and labeled.

Proximate Analysis:

For proximate analysis plants samples were grinded into fine powder. AOAC method is used to determine the proximate nutrient composition (Ash, Carbohydrates, Dry Matter, Moisture, Crude Fat, Crude Protein, Crude Fiber) Following experiments were performed in Food Science Department, University of Sargodha, Sargodha [17].

Ash: (%)

1g of dried shoots samples were carbonized with the oxidizing flare until not any fumes formed. The plant samples were then burned at 602°C furnaces to flame all the whole matter.

The given formula was used to determine the ash percentage composition

$$Ash\ \% = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

Moisture Content: (%)

The following formula was used for the determination of moisture contents

First, we were taking the fresh weight of plant samples. Then we transferred these samples into the oven at 600°C for 24 hour. Then these samples were collected and weight noted on electrical balance.

$$\text{Moisture } \% = \frac{Wt\ sample\ before\ drying - Wt\ of\ sample\ after\ drying}{Weight\ sample\ after\ drying} \times 100$$

Fat: (%)

Soxhlet apparatus was used for extraction of fat with the help of petroleum ether (45°C – 60°C). This method was used to eliminate the part that was ether soluble. 1g plants samples were used in this process. It was dehydrated up at 70°C until the stable weight achieved. The following formula was used for the determination of fat composition**.**

The following formula was used for the determination of crude fat contents

$$Crud\ Fat\ \% = \frac{Wt\ of\ flask\ with\ fat - Wt\ of\ empty\ flask}{weight\ of\ sample} \times 100$$

Crude Fibre (%)

Acid digestion method was used to determine the crude fibre composition. I had taken 1g of oven dried sample and Soxhlet apparatus was used to remove the fat and digested the plant sample in 1.26 percent NaOH and H_2SO_4 separately. Then the sample was transfered in china dishes, positioned the sample in oven at 105^{o}C for one day. Then I cleaned the material and content was washed three to four times by means of distilled water. Then ignited the filtrate in muffle furnace at 600^{o}C to take ash and from that ash, crude fibre was measured. The variation between the weights of the plants samples were the contents of crude fibres.

The following formula was used for the determination of crude fibre content

$$Crud\ Fiber\ \% = \frac{Wt\ of\ flask\ with\ fat - Wt\ of\ empty\ flask}{weight\ of\ sample} \times 100$$

Nitrogen was calculated by using the given formula:

$$Nitrogen = \frac{Volume\ used\ of\ \frac{N}{10} H2SO4\ \times 0.0014\ \times 250}{Weight\ of\ sample\ \times 10} \times 100$$

Crude Protein (%)

The Kjeldhal method was used for determination of protein, first total nitrogen was determined by using Kjeldhal apparatus [18].

Following reagents were prepared.

1. 4 % boric acid solution
2. 0.01 N standard sulphuric acid
3. Bromocersol green methyl red indicator

1 g material with 3 g of digestion mixture ($HgSO_4$ + K_2SO_4 at the ratio of 1:9) and concentrated sulphuric acid (20 ml) were taken in a digestion flask and boiled for 1.5 to two hours until substances in it became clear. The material for digestion was then diluted to 20 ml. solution (10 ml) was poured in Kjeldhal flask apparatus to place on Kjeldhal ammonia distillation unit. 40 % NaOH (10 ml) was then mixed to solution and this flask was instantly linked to distillation flask. 4 % Boric acid solution (10 ml) was mixed with 100 ml alcohol mixed indicator in a conical flask. The conical flask was removed as soon as the distillate was around 40-50 ml. The distillation was turned off and chilled for few minutes. It was then titrated with 0.01 N standards H_2SO_4 until the color changed to pink. Blank was also run in the same way.

Nitrogen was calculated by using the given formula:

The protein contents were obtained by multiplying nitrogen to a factor of 6.25.

% Protein = % Nitrogen × 6.25

Carbohydrates:

Carbohydrates concentration were determined. We subtracted fats, ash, protein, fibre and moisture contents from 100.

Carbohydrates = 100 - (Proteins + Fats + Moisture + Ash + Fibre).

Estimation of mineral contents:

Digestion of Plants Samples

The oven dried plants samples were grinded into fine powder and then digested by wet digestion method. 0.5 g sample was taken in digestion flask and 10ml HNO_3 was added in each sample and kept it for overnight. Then the process of digestion was carried out on hot plate by adding 5ml perchloric acid in sample. Repeat the process until sample solution become transparent. Then added distilled water to make the solution up to 100 ml was added to make 50 ml final solution and placed for analysis. Then standard solutions were formulated and with the help of those standards the digested samples were ready for elemental analysis.

For elemental analysis the filtered sample solution was loaded to the atomic absorption spectrophotometer. Standard curve for each metal was prepared by running samples of known strength. The elemental contents of the samples were estimated by using the respective standard curve prepared for each metal according to the method AOAC [19].

Following mineral contents studied using standard methods.

1) Zinc (Zn)
2) Iron (Fe)
3) Magnesium (Mg)
4) Chromium (Cr)
5) Manganese (Mn)
6) Cadmium (Cd)
7) Lead (Pb)

For each metal, 1000 mg/kg concentration stock solution was prepared by the given formula:

$$X = \frac{Molecular\ weight\ of\ salt}{Molecular\ weight\ of\ mineral} \times 0.1$$

X grams of the salt were dissolved in 100 ml distilled H_2O to make 1000 mg/kg solution that was further diluted to 100 mg/kg solution for preparation of solutions for preparation of the standard curve. For calibration of instrument, 1 mg/kg to 10 mg/kg diluted solution was used.

Following formula was used to make dilutions

$C_1V_1 = C_2V_2$; Where , C_1 = concentration of stock solution ; V_1 = volume of stock solution

C_2 = Required concentration; V_2 = Required volume

RESULTS AND DISCUSSION

Results have shown that highest the highest moisture content of shoots (28.48%) was present in tehsil Mianwali (T1), followed by tehsil Esakhel (T3) (28.04%) while the lowest moisture contents were (26.32%) in tehsil Piplan (T2). Results regarding moisture content are in collaboration with findings of Oyeyeme [20]. The proximate composition of *Solanum anguivi* leaves displayed low moisture content (9.58%). The Difference in result is due to difference in plant species.

The highest ash concentration (21.68%) was present in the shoots of tehsil Mianwali (T1), followed by (T2) (14.26%) while the lowest ash concentration (13.21%) was present in tehsil Esakhel (T1). Results regarding ash are in collaboration with the findings of Hameed [21]. Proximate analysis showed that the ash contents in the shoots of *Solanum nigrum* was 17% in southern areas. Our results collaborate with them.

The highest fat concentration (14.23%) was present in the shoots of tehsil Mianwali (T1), followed by tehsil Piplan (T2) (14.08%) while the lowest ash concentration (13.21%) was present in tehsil Esakhel (T3). Results regarding crud fat are in collaboration with the findings of Dhellot [22]. Average fat content varies between 34.5% and 37.5%. The Difference in results is due to many reasons such as difference in soil texture, Seasonal changes, growing conditions and soil nature.

The highest crude protein concentration (0.41%) was present in shoots of tehsil Piplan (T2), followed by tehsil Mianwali (T1) (0.34%) while the lowest crude protein concentration (0.29%) was present in tehsil Esakhel (T3). Results regarding crud protein are in collaboration with the findings of Hoffman [23]. The protein contents in the leaves of *Solanum nigrum (L.)* was 11.6%.

The highest carbohydrate concentration (25.75%) was present in shoots of tehsil Piplan (T2), followed by tehsil Esakhel (23.96%) while the lowest carbohydrates concentration (18.27%) was present in tehsil Mianwali. Results regarding carbohydrates are in collaboration with the findings of Mali and Harsh [24]. Proximate analysis showed that the carbohydrate contents in the leaves of *Solanum nigrum* were ranges from 12.93% to 14.33% respectively. Our results are similar with the results of other scientists.

The highest crude fibre concentration (13.04%) was present in shoots of tehsil Esakhel (T3) Followed by tehsil Piplan (T2) (9.41%) while the lowest crude fibre concentration (9.28%) was present in tehsil Piplan (T2). Results regarding crude fibre are in collaboration with the findings of Hussain [25]. Proximate analysis showed that at the post reproductive stage crude fibres were maximum (28.00%) in leaves of *Solanum surattense*. Our results are different due to difference in cultivars.

Table 1: Proximate Composition of *Solanum nigrum* Collected from three Tehsils of District Mianwali

Tehsils	Ash	Moisture	Carbs	Crude Protein	Crude Fibre	Fat
Mianwali	12.68±0.10	28.48±0.32	18.27±0.39	0.35±0.001	8.95±0.04	10.80±0.25
Piplan	9.14±0.49	26.11±0.18	22.75±0.41	0.37±0.001	8.42±0.27	09.59±0.11
Esakhel	11.54±0.31	26.32±0.50	26.89±0.18	0.29±0.02	9.04±0.03	08.65±0.17

The elemental analysis of the *Solanum nigrum* showed significant variation among different elements. The maximum Mg concentration was observed in case of tehsil Piplan (T2) (6.76mg/kg) followed by tehsil Mianwali (T1) (6.59mg/kg), and the lowest was in Esakhel (T3) (6.22mg/kg).

Results regarding Mg are in collaboration with the findings of Kippler [26]. The significant range of Mg in leaves of

Solanum nigrum is 3.7 mg Kg^{-1}. The deficiency of Mg can affect the functions of many enzymes that are involved in the metabolism of Glucose. The Mg functions in acid base balance, regulation of osmotic up take, muscle Movement ability and Na+/K+ ATPase. Variation in Mg concentration may be due to soil composition, which is different in all tehsils.

The maximum Zn concentration was present in Esakhel (T3) (3.52mg/kg) followed by tehsil piplan (T2) (3.16mg/kg), and the lowest was in tehsil Mianwali (T1) (2.69mg/kg). Results regarding Zn are in collaboration with the findings of Underwood [27]. Micronutrients such as Zn is essential components of different enzymes, functional or regulatory co-factor. Few elements such as Zn, Cu and Fe are essential for plants growth and become toxic at high amount. The 11.03 mg/kg iron is present in the shoot of *Solanum nigrum*. The Difference in results is due to many reasons such as difference in soil texture, Seasonal changes, growing conditions and soil nature.

The maximum Cd concentration was observed in case of tehsil Mianwali (T1) (0.82mg/kg) followed by tehsil Esakhel (T3) (0.81mg/kg), and the lowest was in tehsil Piplan (T2) (0.80mg/kg). Results regarding Cd are in collaboration with the findings of [13]. The concentration of Cd in *Solanum nigrum* was (2.67 mg/kg). Manganese and iron are parts of enzyme elaborate in urea formation, pyruvate Catabolism and the galactotransferase of connective tissue synthesis. Our results are nearly similar with the result of other scientists.

The maximum Mn concentration was observed in case of tehsil Piplan (T2) (0.15 mg/kg) followed by Esakhel (T3) (0.14mg/kg), and the lowest was in tehsil Mianwali (T1) (0.08 mg/kg). Results regarding Mn are in collaboration with the findings of [13]. The concentration of Mn in *Solanum nigrum* was (2.67 mg/kg). Manganese and iron are parts of enzyme elaborate in urea formation, pyruvate Catabolism and the galactotransferase of connective tissue synthesis. The difference in results is due to many reasons such as difference in soil texture, Seasonal changes, growing conditions and soil nature.

The maximum Cr concentration was observed in case of tehsil Mianwali (T1) (0.25mg/kg) followed by Esakhel (T3) (0.16mg/kg), and the lowest was in tehsil Piplan (T2) (0.08mg/kg). Results regarding Cr are in collaboration with the findings of Gesinski and Nowak [28]. The concentration of Cr in *Solanum nigrum* shoot was 1.09 mg/kg. The concentration of Cr was equal in stem and roots of medicinal plants in waterlogged soil. Soil contained more Cr then other elements. Our results are different due to different environmental conditions.

The maximum Fe concentration was observed in case of tehsil Piplan (T2) (8.19 mg/kg) followed by tehsil Mianwali (T1) (5.55 mg/kg) and the lowest was in tehsil Esakhel (T3) (2.78 mg/kg). Results regarding Fe are in collaboration with the findings of Chandra and Chinma [29], [30]. The Fe is present in the center of and cytochromes and Haemoglobin. The iron contents vary from 14.85 to 81.42 g/ml in *Solanum nigrum* and *Mucuna prurience* respectively. These values were considerably higher than the values reported for some selected leafy vegetables in Nigeria. Variation in Cu concentration in all tehsils may be due to soil composition, which is different in all tehsils.

The maximum Pb concentration was observed in case of tehsil Piplan (T2) (1.85 mg/kg) followed by tehsil Mianwali (T1) (0.95 mg/kg) and the lowest was in tehsil Esakhel (T3) (0.87 mg/kg). Results regarding Pb are in collaboration with the findings of Zenk and Pearson [31], [32]. The difference in results is due to many reasons such as difference in soil texture, Seasonal changes, growing conditions and soil nature. Medicinal plants accumulate heavy metals. These elements are as follows i.e. Zn, Hg, Pb and Co. They are noxious and lead to growth reticence, decline in biomass as well as death of plants. Zinc is very useful in protein synthesis, cellular differentiation and replication, protection and sexual purposes. Variation in Cu concentration in all tehsils may be due to soil composition, which is different in all tehsils.

Table 2: elemental profile of *Solanum nigrum* Collected from three Tehsils of District Mianwali

Tehsils	Mg	Zn	Cd	Mn	Fe	Cr	Pb
Mianwali	5.59±0.16	2.69±0.10	0.82±0.03	0.8±0.006	5.55±0.14	0.25±0.08	1.08±0.03
Piplan	6.76±0.01	3.16±0.03	0.81±0.01	0.15±0.001	6.42±0.18	0.08±0.003	0.87±0.01
Esakhel	6.22±0.08	3.52±0.03	0.81±0.02	0.14±0.02	8.04±0.03	0.16±0.001	1.85±0.02

CONCLUSION

Solanum nigrum has good source of nutritional and elemental contents and is important medicinal plant for us due to its curative and therapeutic properties. Fluctuation in nutritional and elemental profile of *Solanum nigrum* at different tehsils may be due to many reasons such as difference in soil fertility, soil texture, Seasonal changes, climate difference, growing conditions or may be due to different varities.

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