Ethno-biological and nutraceutical evaluations of selected grape cultivars from Quetta, Pishin and Bannu regions of Pakistan

A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Philosophy in Plant Sciences

By

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APPROVAL CERTIFICATE

This is to certify that the dissertation/thesis titled "**Ethno-biological and nutraceutical evaluations of selected grape cultivars from Quetta, Pishin and Bannu regions of Pakistan**" submitted by **Abdul Rehman** is accepted in its present form by the Department of Plant Sciences, Quaid-i-Azam University Islamabad Pakistan, as satisfying the dissertation requirement for the degree of M. Phil in Plant Sciences/[illegible].

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Declaration

I hereby declare that no part of this thesis has been previously submitted to this or any other University as part of the requirement for a higher degree. The contents of this thesis are results of my own work unless otherwise acknowledged in the text or by reference. The research work presented in this thesis was carried out by me in the Plant Ecology and Conservation laboratory, Department of Plant Sciences, Quaid-i-Azam University Islamabad.

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Abstract

Grape is one of the 70 species of the genus vitis belonging to the family Vitaceae. Grapes are grown all over the world on a large scale. In Pakistan it is mainly grown in Balochistan and Dry areas of Potohar. Almost 98% production of the grapes come from Balochistan, the rest are from Khyber Pakhtunkhwa and Northern Areas with little production from Punjab. A questionnaire survey was done to find the ethno-biological values of grapes in Quetta, Pishin and Bannu regions of Pakistan. Different home gardens were surveyed for this purpose and soil was collected from each of these places. Experiment plot was set in the Botanical Garden of University of Science and Technology Bannu, where cuttings collected from Quetta and also local cuttings from District Bannu of different cultivars were planted. The nutritional analyses were also done for 9 cultivars. The results of ethno-biology indicated that the cultivar Sundarkhani was highly preferred in the region. The production of Sundarkhani was also very high than others. The income from grapes was also significant. Natural fertilizers were reported to be used in the study area for plants. The people showed their willingness towards the cultivation of grapes in future. The soil was very suitable for the grapes cultivation, i.e., average pH value were 6.24 which is required for grapes growth. The study from experimental field at University of Science and Technology Bannu proved that the highest survival percentage was observed in a local cultivar which was well adapted with the environment. For example the highest number of leaves were reported from some local cultivars tagged as local lines. The highest mean value of leaf length was 7.575 cm. The greater mean number of nodes were reported from cultivar local line 1 which were 13.4 in number. The nutritional analysis of grapes showed that the pH value of grape fruits lie in a range of 3.2 to 4.1, while the Electrical conductivity value was observed to be 1210 ppm. The values for reducing and non-reducing sugar were 33.33% and 55.49%, respectively. The concentration of vitamin C recorded was 3.61 mg/100g. The varieties Sundarkhani and Kishmishi were considered best for cultivation. The daily basic needs for fruits can be best fulfilled by growing grapes in the region. The mean values of soil and fruit pH were relatively close with one another from Balochistan and Bannu, which indicates the suitability of the environment for grapes cultivation.

INTRODUCTION

1.1 Background

The Vitaceae family contains the genus *vitis* and the so called *Vitis vinifera*. *Vitis vinifera* is the most cultivated out of 70 species of the genus *vitis*. According to (Alleweldt and Possingham 1988) and (He 1999), the genus *vitis* originated from East Asia, Asia Minor, Central and North America and South Europe. Wine, a fermented product of the grapes has been a chief way to consume grapes, since the start of its cultivation (McGovern et al. 1996). The residues of wine have been recognizedin Iran around 7000 years old jars (McGovern et al. 1996). However the fresh grapes are also consumed in a large quantity where they grow wild. Grape is one of the most cultivated fruit crop in the world with its production rate of 69 million tons in 2006 (Gallai et al. 2009). Usha (2010), described that grapes contain vitamins, anti-oxidants, fibers, minerals and vitamin C in high concentration. Kishmish and Monaka are the dried forms of grapes which are consumed at high rate.

1.2 Botanical description

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Vitales

Family: Vitaceae

Genus: *Vitis*

Species: *V. vinifera*

Binomial name; *Vitis vinifera*. L.

The grapes are perennial woody vines with its fruit categorized as berries. The location of inflorescence is at tendril but sometimes it might be axillary. The berry colour can be green, black, golden, red, pink, purple and rarely white whereas the taste can be Muscat, fruity or spicy depending upon varieties (Uddin et al. 2011). The growth stages of grapes are vegetative growth, bud break, fruiting, ripening and the leaf fall in the autumn and then comes the dormancy period during winter (POUDEL et al. 2010). When the bud bursts then the fruit cluster matures after 5-7 months (Golob et al. 2002). The European *Vitis vinifera* (L) grape is hermaphrodite while the species of North American are monoecious.

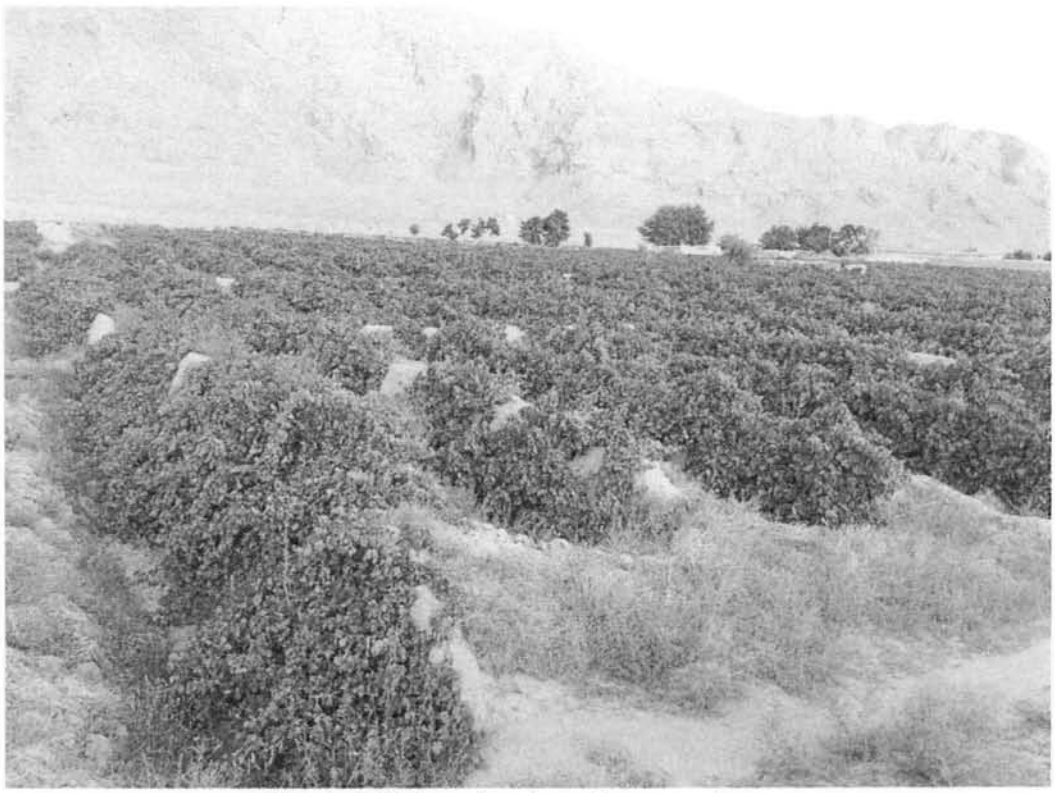

Fig. 1. 1 Picture showing a Grape garden.

1.3 Climatic conditions for Grapes

Grapes are best grown in all types of climates and soils where the production of other deciduous fruits is restricted. Grapes, wine grapes and raisins are best grown in areas having strong varying climatic conditions. Temperate region is best for *Vitis vinifera* L. cultivation whereas the hotspot area for its cultivation lies between 30°N and 50°N

latitude and 30°S and 40°S longitude. The factors that limit the growth of grapes are lack of water, extreme heat and inadequate winter chilling (Mullins et al. 1992). Grapes are largely grown in comparatively warm temperate regions. According to (Golob et al. 2002), to make the varieties quality best for drying, wine and other purposes a right time should be selected for the right harvesting of varieties. Among the grape varieties *V. vinifera* is the main variety for the production of fresh fruit, wine and drying. Caucasus mountainous area is thought to be the origination place for this species. In North America, some grape species are used for rootstock, some for juicing and some interspecific crosses for wine (Jackson and Palmer 1999). Specific conditions are required for production of grape like temperature, specific mesoclimatic properties viz precipitation, solar radiation and evaporation (Jones 2007). Wind speed less than 4 m/s, 25°C -30°C temperature and 60-70% humidity is needed for maximum vine photosynthetic activity (Jones 2007).

1.4 Grape varieties

There are number of varieties of grapes grown in Pakistan and other countries. In Upland areas of the province Balochistan, different varieties of grape plants are grown. The most leading and popular varieties are Sundarkhani, kishmishi, Haita, Shakhali and Sahibi which are grown on high level in Quetta, Pishin, Mastung, Zhob, Killa Abdullah and Kalat districts of the province. The other varieties include King Ruby, Early Muscat, Askari, Black Rose, Flame Seedless, Cardinal, Fresno seedling, Loose Perlette, Emerald, Thomson seedless and Exotic (Uddin et al. 2011). In Punjab the following varieties are found Marsh seedless, Red blush, Shamber and Duncan.

1.5 Distribution of Grapes

1.5.1 Grapes in world

It is believed that the grapes cultivation is originated from Armenia near Caspian and Black seas of Russia, from there it came to Afghanistan and Iran in eastward and to Europe in the westward. In 2009 it was predicted that the grapes production would be about 68 million metric tons. The grapes were mainly produced in China, France, Turkey, Argentina, Italy, United states, Spain, Chile and India (Gallai et al. 2009). About one quarter of this product is exported. The main exporter countries were Italy, Chile, France, Spain, United states, Australia, South Africa, Portugal and Germany. According to survey conducted by FAO (2002), a total of 2.73 million metric tons (MT)

of table grapes were exported worldwide while a total of 0.6 million MT of raisins were exported around the globe. Due to the unique taste and quality, the grapes are cultivated on wide range around the world. In 2005, the area covered by the grapes and its production rate was 7,488,196 hectare and 66,901,419 tons, respectively, which ranked second in the production of fruits (FAO, 2002).

1.5.2 Grapes in Pakistan

The climatic condition of Pakistan varies from temperate to tropical where fruits of different types are grown. A total of 0.833 million hectares area is covered by fruits where the production is 6.011 million tons. During a survey by GoP (2007), Balochistan contributes 30% and 14% by area and production of fruits respectively. Grape is also a fruit crop grown in Pakistan in some specific climatic zones. The cultivation of grape fruit mainly occurs in the upland areas of Balochistan and northern hilly areas of KPK and Punjab. The main areas for the cultivation of grape fruits in Balochistan are Qalat and Quetta divisions where 72,800 metric tons of grapes are produced on an area of 8.4 thousand hectares. Khyber Pakhtunkhwa shares 1,359 tons of grapes production grown on 133 hectares of area. According to a survey by (GoP, 2006), 98% of the total grape production of the country occurs in Balochistan (Table 1.1).

Table 1. 1 Area, production and yield of grapes in Pakistan and Balochistan 1991-2007.

Years	Area (hectares)		Production (tons)		Productivity	
	Pakistan	Balochistan	Pakistan	Balochistan	Pakistan	Balochistan
1990-91	3.2	3.1	32.8	31.8	10150	10258
1991-92	3.5	3.4	35.5	34.3	10143	10088
1992-93	3.8	3.7	37.6	36.3	9895	9811
1993-94	8.1	8.0	40.3	39.0	4975	4875
1994-95	8.2	8.1	42.9	41.5	5232	5124

1995-96	8.4	8.3	72.0	70.7	8571	8518
1996-97	8.5	8.4	74.1	72.8	8718	8667
1997-98	8.7	8.6	74.3	73.0	8540	8488
1998-99	8.9	8.7	75.8	73.8	8517	8483
1999-00	10.4	10.2	40.3	38.2	3875	3745
2000-01	12.5	12.3	51.1	48.8	4088	3968
2001-02	12.7	12.5	52.6	50.3	4142	4024
2002-03	12.7	12.5	51.8	49.5	4079	3960
2003-04	12.8	12.6	50.8	48.4	3969	3841
2004-05	13.0	12.8	49.1	47.7	3777	3727
2005-06	13.0	12.8	48.8	47.5	3754	3711
2006-07	13.8	13.6	46.5	45.2	3370	3324

Source: Agricultural Statistics of Pakistan, 2006-07

1.6 Grapes Morphology

1.6.1 Morphology of Fruit

Each individual berry of grape fruit is attached to the rachis and branches through a cap stem having vascular bundles. A multi-layered pericarp with four seeds (commonly) are developed in each berry while there are many cultivars which have no individual seed in them. Pericarp has three main parts that are endocarp, mesocarp (pulp) and exocarp (skin). Major part of berry weight is covered by the pulp while the vacuolated cells contain sugars in high level and many soluble compounds. According to (Winkler 1974)

oil (10-20%), tannins (5-8%) and phyto-hormones are found in cells of grape berry. Plastids are present in the pericarp during berry development which are then changed morphologically to lipid like globules ((Hardie et al. 1996)). Hypodermis (107–246 μm) and epidermis (6.5–10 μm) are two layers which are found in the exocarp (Alleweldt et al. 1981). A well complete epidermis is protected by a 3 μm thick cuticle which has 0.5μm outer waxy layer that contains a non-functional stomata at maturity (Casado and Heredia 2001). The skin thickness and toughness is different amongst different varieties. There is a direct link between the thickness of skin and the resistance to physical stress. Thus, we can say that an injury to grapes is correlated to thickness and toughness of skin, the more resistant is the skin the less will be the injury. Furthermore, many compounds are present in the skin which play an important role in flavor, aroma and taste of the grapes (Winkler 1974). Water loss in grapes mainly occur through cuticular transport despite of the presence of stomata.

1.6.2 Development of Berry

Many researchers such as Kanellis and Roubellakis-Angelakis (1993), (Coombe 1992), (Alleweldt 1977) and (Ollat et al. 2002) defined the berry development to occur in three stages based on research. In stage I there is fast division of cell which is followed by cell enlargement. Stage II is known as lag phase in which there is slow growth and chlorophyll is lost while the embryo gets full sized and highest level of acidity is also observed. Stage III is veraison that is known for berries softness, rapid growth, amino acids and sugar increases and anthocyanin accumulation with some enzyme's activity in colored cultivars. While in this stage decrease in respiration rates, acidity, ammonia and chlorophyll is observed.

1.7 Grapes Physiology

1.7.1 Acids

Acidity plays a vital role in grapes either they are used as fresh or used in wine making. The level of acid balance attracts a consumer towards the getting of grape juice and table grapes which make it sweeter (Winkler 1974). It also points out the fruit from which wines can be made. A grape having low level of sugar will be so bitter which results in cheap quality wine (Ruffner 1982). Areas having warm climate are

characterized by grapes having high acidity and low pH levels. According to (Winkler 1974), the bluish and dull color grapes have high pH and low acidity level, while that of red and brilliance colored grapes have high pH value and low acidity level.

A total of 90% acids in grapes are constituted by malic acids and tartaric acids, whereas the ratio between these two acids may be different in different grape cultivars. The pattern of accumulation is specific of both the acids when they accumulate before veraison (Ruffner 1982). It is observed that the tartaric acid is stored as free acid in vacuole and as insoluble calcium tartrates. According to new findings by (DeBOLT et al. 2004), tartaric acid has a precursor that is ascorbic acid. In meta bolism of grape malic acid works as an active intermediate. Acids level goes down at veraison stage specifically that of malic acid which declines rapidly due to respiration through oxidative phosphorylation. It is found by Kanellis and Roubellakis-Angelakis (1993), that under warm conditions the acidity level decreases quickly.

1.7.2 Aromatic complexes

Many different compounds present in the skin of grapes contribute to give rise to a good aroma. There are few cultivars which have a very specific aroma like *V.labrusca* and *V.rotundifolia* same as that of Muscat *V.vinifera* have but not all the cultivars of this species have a good aroma. Damascenone and monoterpenes are two isoprenoid secondary metabolites which are aromatic in nature (Jackson, 2000). It is reporte d that the production of these two secondary metabolites is associated with lipid-like globules formation in the plastids which are found in pericarp (Hardie et al. 1996), while there are some other precursors of aroma that are present in glycosylated form (Williams et al. 1995).

1.7.3 Phenolic compounds

Tannins (proanthocyanidins), a dominant class of phenolics are found in grape berries which are principal elements of acidity in red wines (Williams et al. 1995); (Cheynier et al. 1997). There are some other important phenolics which are benzoic acids, flavonols, anthocyanins and cinnamic acids (Deloire et al. 2005). More hydroxycinnamic tartrates are found in berry skin than its flesh while procyanidins and flavan-3-ols are present in large quantity in latter. Phenolics are found in high amount in seeds from which a substantial proportion of wine tannins are formed and it contribute

meaningfully to the oxidative browning of the grape juice. Scientists pay significant attentions to the chemical properties of grapevine including polyphenols, flavonoids (flavanols, anthocyanins and flavonols) and nonflavonoids (phenolic acid derivatives, stilbenes), and also to the pharmacological and biological activities (Vitrac et al. 2004). The purple and red colors of specific grape cultivars arise due to the presence of Anthocyanins and plays an important role in wine and table grapes.

1.7.4 Minerals and Sugars

Sugars accumulation in the grape fruit is an utmost quality change which indicates the degree of its ripening. The alcohol present in wine is the result of change of this sugar during the process of wine making and also in dry and fresh fruit. The carbon imported from anthesis to veraison is apportioned alike between seed growth, pericarp and respiration whereas import of carbon increases during veraison and stored in the form of glucose, hexose and fructose in the pericarp (Ollat and Gaudillère 1997). A berry pulp has these two key sugars as their carbohydrates. The amount of their presence varies with the cultivars although they may be found in same amounts too. As fructose is more sweeter than glucose so a cultivar having greater quantity of fructose is harvested earlier. Ratio between fructose to glucose increases when the fruit over-matures (Winkler 1974). Xylem works as the water transporter till the veraison stage. Embolism blockage replaces xylem as the stage of ripening reaches (Coombe 1992). During this stage a fivefold increase in carbon import is reported as the phloem is now activated. In few cultivars the berry weight is lost due to back flow of water from berry to parent vine, e.g., Shiraz (Tyerman et al. 2004).

1.8 Hormonal Fluctuations

1.8.1 Ethylene

Grape a non-climacteric fruit is not associated with respiratory burst and it is not prompted by ethylene gas. In fact grapes are characterized by very low level of ethylene, so as a range of pmoles g-1 FW, however it is reported that ethylene is involved in the ripening metabolism as it increase the volume of berry in second growth phase as well as the accumulation of anthocyanin (Tyerman et al. 2004). The berry expansion is controlled by ethylene gas through transcription regulation of many other genes, in

which aquaporins and xyloglucan endot ransglucosylases are critical (Chervin and Deluc 2010). According to (Coombe and Hale 1973; Weaver and Montgomery 1974), abscisic acid and ethylene act synergistically that improve the pre-harvest ripening of fruit. Ethephon (2-chloroethylphosphonic acid), which is an ethylene precursor is applied about veraison that improve colour development in the pigmented cultivars wi th a fall in the level of acids and increase in level of sugars (Weaver and Montgomery 1974); (Weaver and Montgomery 1974). Abscission is stimulated by ethephon and it also improve the removal of berry for the production of wine during harvesting (Szyjewicz et al. 1984).

1.8.2 Gibberellic Acids

There is a direct relationship between the number of seeds and size of a mature berry, more the number of seeds larger will be the berry size. The fruit size of a parthenocarpic or stenospermocarpic cultivar is generally small until growth hormones are applied which improves its size. Hormones are generally found in seeds in high amount i.e., gibberellic acid and abscisic acid. Size of the berry and bunch shape of a evolving grape is controlled by spraying the gibberellic acid on it (Lynn and Jensen 1966).

1.8.3 Abscisic Acid

Mature fruit have high amount of Abscisic acid (ABA) and it is believed that it persuade *de novo* production of gluconeogenic enzymes (Palejwala et al. 1985). In the mesocarp of grape berry there are endomembranes which have specific ABA binding sites overlapping with growth phase II and falling off during veraison (Palejwala et al. 1985). According to (Stoll et al. 2000) the partial drying of root zone is responded by abscisic acid.

1.8.4 Brassinosteroids and Jasmonates

Many compounds have been reported as high latent grape hormones, in which the brassinosteroids are produced at high level in the start of ripening of berry which then boost-up the developmental process of berry (Symons *et al.*, 2006). There is a great interest to study the role of jasmonate in grape berry. The level of jasmonate present in berry depends upon the number of seeds in that berry and like ethylene they are also involved in accumulation kinetic (Stoll et al. 2000); (Chervin et al. 2004). Accumulation

of stilbene is also stimulated by them with an extra noticeable consequence on leaves (Larronde et al. 2003).

1.9 Grapes Drying

The preservation of grapes by drying is a major industry in many parts of the world where grapes are grown. Methods of drying like shade drying, open sun drying and mechanical drying results in products like sultanas, raisins and currants. After wine the second utmost imperative produce is raisin of grape vine (Salunkhe and Kadam 1995). The most effective and best drying method is the solar drying in comparison with mechanical and traditional drying, especially in areas where there is good sunshine when harvesting season is going on (Pangavhane and Sawhney 2002) There are various kinds of solar dryers for drying of grapes (El-Sebaii et al. 2002). A solar dryer contains flat plate solar air heater which is connected with drying chamber is good for grape growers, but it has large initial costs and supplementary charges due to which many of the small scaled farmers are not able to use such type of grape dryer.

1.9.1 Varieties

Approximately all the *V. vinifera* cultivars are used for drying purposes (Jackson and Palmer 1999). The currants are the products of dark seedless Zante type grape which are used commercially (Barrett et al. 2004). In Australia, sultanas are made from Sultana grapes and Thompson's seedless while in California, Raisins are made from Thompson's seedless. Raisin word is used in Australia while in America and Europe they are known by the name Muscat raisins, whereas these types of raisins are much sweeter than others. The berries of Muscat grapes are large in size and have seeds which after drying may be removed if desired (Ashurst 2005).

1.9.2 Harvesting

Normally hand-picked grapes are used for drying purposes because berries are damaged by machine harvesting. Mechanically canes could be trimmed while bunches are attached still and are dried by hanging them on vine (Jackson and Palmer 1999).

1.9.3 Technology for Drying

Grapes having high content of sugar i.e., 20-24° Brix are best for drying purposes. Grapes may be pre-treated to boost up the process of drying while in Iran, California

and USSR they are naturally dried. According to Fuller *et al.*, (2009), solar drier is much efficient than natural drying as in solar drying 70% to 15% moisture is reduced. The toughness and thickness of the skin plays a key role in making of raisins which has impact on water loss (Winkler 1974). Leaves, capstem and stem pieces are removed by winnowing the raisins. Raisins after washing and grading are packed in different quantities ranging from few grams to 12.5 kg which are then used in different manufacturing industries. Potassium carbonate solution (2.5–4.5%) is used to dip the grapes in it before drying. There are some other solutions which are used in dipping process such as citric acid, sodium hydroxide or mixtures like an alkaline or oil-in water emulsion. The drying time can be reduced by merging the microwave pre-treatment with an alkali dipping (Kostaropoulos and Saravacos 1995). According to (Rojchev and Botiyanski 1998), waxy bloom's structure is changed by the dip which becomes more leaky to water. As a result, the infrared rays can attack the dipped grapes easily which speed up the heating process and fastens the process of drying by several weeks. Dip used such as that of citric acid and NaOH are observed to reduce pectins in cell wall (Femenia et al. 1998). Alkaline treatment is generally used for Muscat grapes as they are large in size. The untreated grapes are handled carefully to make safe their bloom from damaging. As a result the dried fruits are commercially sold out at stores i.e., health food stores (Barrett et al. 2004). Different pre-treatments are used for optimizing the quality of grapes and to make them safe for future use (Gowda 2000).

1.9.4 Making Product Useful

In commercial market and local people there is a high demand of light colored dried grapes. Polyphenol oxidase (PPO) is found in the skin of dried grapes through which the level of browning is determined and low level of PPO in grapes generally dry to lighter color as compared to others (Rathien & Robinson 1992). In dried fruits browning is increased by low (<21° Brix) or high (>23° Brix) level of sugar. Product will be darker when the berries are visible to sun as compared to that of protected from sun (Uhlig and Clingeleffer 1998). A golden color fruit is obtained in some countries where raisin grapes and sultanas are treated with sulphur dioxide. Fumigation chambers are made where g fruits are stored. Burning of Sulphur is done in chamber and the fruit is treated with the gas. Up to 2000mg/kg residues are tolerated. To avoid clumping and stickness during packing of products a light layer of mineral oil is applied to fruits.

1.9.5 Mycotoxins and Pests

In dried products, Aflatoxin and Ochratoxin A are found and the concentrations of which can be measured by different methods (Bacigalupo *et al.* 1994; MacDonald *et al.* 1999). Pests and many diseases can attack the dried grape products easily if they are not preserved chemically by applying insecticidal compounds or good packaging. There are certain fungi like *Cladosporium*, *Aspergillus, Erotium, Penicillium and Alternaria* species and Pests (insects and mites) which attack the berry of grape before, during and after drying. Insecticides spray play a key role in controlling these pests even after harvesting (Buchanan et al. 1984), however, chemicals should be used carefully as pesticide deposits can be increased fourfolds by drying (Cabras et al. 1998). *Plodia* specie can be controlled effectively by a granulosis virus using a biocontrol method (Vail *et al.* 1991).

1.10 Table grapes and their post-harvest technology

Table grapes play a key role as fresh fruit products. Its high value quality and taste make the consumers to pay a best for its quality consumption. China is the leading country in producing table grapes around the globe. According to a survey conducted by USDA (2007), China with a net production of 6.5 million MT produced threefold grapes of the second largest country Turkey. (Perl et al. 1998), described that consumers prefer the use of seedless cultivars while there are some other countries where seeded varieties are consumed mostly.

1.10.1 Varieties

In Italy the Italia (Muscat) is the major table grape cultivar with an annual production of 7 lacs tons, while Chasselas is the major table grape cultivar in France (Vidaud *et al.* 1993). Flame seedless and Thompson seedless are the two key cultivars grown in California which shares 90% to the total table grapes of USA. There are some other varieties such as Superior Seedless, Perlette, Flame Seedless, Ruby Seedless, Autumn Royal and Crimson Seedless that make the table grapes a valuable and high economical product. In the specific season of mid-to-late there is high production rate of the cultivar Red globe which is imperative for export.

1.10.2 Maturity and Eminence catalogues

Table grapes are less active physiologically and they are non-climacteric by nature. Sugar content, which is determined as total soluble solids (TSS), is the core maturity index (Perl et al. 1998). The requirements for color maturity are less for all other cultivars except the white grapes. For quality table grapes there are some requirements that are flavor, thin skin, good texture, appearance, large size and free of decay. Blooming also play a key role in producing excellent table grapes. Maturity requirements depend upon the growing area, cultivar and market, however in major markets the standards are balanced gradually. For example the economic commission for Europe has its specific table grapes standards (UNECE 2003). According to EU standards the definition of table grapes is that, it is produced from *Vitis vinifera* cultivar. The standards of EU categorize the cultivars into field grown and greenhouse grown cultivars. The berries are also categorized into large and small berries. There are classes made for berry expression which determines the size, shape, colour and other characters of berry.

1.10.3 Packing in Shed

The fruit is picked up by the picker and kept in lugs. For transporting the crates are gathered at one place in the shed of vines. The crates are then circulated in packers for selection, trimming and packaging in fruits. Whereas trimming, selection, first quality grapes and color differentiation could be done in field.

Fig. 1. 2 Picture demonstrating the shed packing of grapes.

1.10.4 Packing in the field

Avenue packing is the very common type of packing. The picker removes the imperfect berries and attain a good bunch. These bunches are then carefully placed in filed crates, prepared from plastic or wood. These crates are then brought to a place where all the crates are placed and gathered. To save the grape fruits from further injuries they can be packed in shipping cartons directly. It is more difficult to keep quality supervision on the packing stands than to keep on a shed packing (Golob et al. 2002).

Fig. 1. 3 Picture showing the Field packing of grapes fruit.

1.10.5 Packaging

Usually there are two grades of packing by packers, bunches of high quality which are generally meant for export, will be packed in zipper bags of polyethylene. The other one is normal packing which are forwarded to the local market and for open use by the consumers. The damage of fruits is reduced with the use of cluster plastic bags when marketing the fruits (Luvisi *et al.,* 1995). The bags are punctured for the proper ventilation which decreases the occurrence of microbial decay. After that these individual fruit bunches are packed into crates of different varieties. Beside this extra filters are placed in these cartons which reduce physical damage of fruits but the side effect is that, absorbent resources can boost up the weight loss (Mencarelli et al. 1994).

1.10.6 Storage

Generally, the temperature of storage room for grape fruit is −1 to 0°C where the flow of air is moderate, i.e., 20 to 40 cube feet/minute and the RH value ranges between 90-

95 percent, for one ton of grapes. By maintaining these three factors at the above mentioned levels, the stem of grape fruit can be protected from loss of water. The stored products should be monitored frequently to avoid fruit rotting, physiological destruction, drying of stem and SO2 injury.

1.10.7 Cooling

After harvesting the grapes are air dried very soon to avoid the destruction of berries and rachises due to the loss of water. When the palletisation is over, pallets are forwarded to fumigation chamber to treat them with Sulphur dioxide or to the forced - air cooler for fumigation process. Cooling and SO2 application should be done very quick, within 6 to 12 hours after harvesting. After completion of cooling with forced air, pallets are forwarded to storage room for transport.

1.10.8 Transportation

The local transportation of grapes is done generally in refrigerated trucks. Those grape fruits which are to be transported outside the country are mainly transported via sea consignments or they can be transported through trucks. Air freight can also be used provided that the worth is acceptable. The temperature of fruit pulp is to be maintained between -0.5°C to 0°C (Luvisi *et al.* 1995).

1.11 Diseases in Grapes

Many diseases attack on the Grapes and certain diseases are developed by moist environment i.e., botrytis, dead arm, black rot, anthracnose, bunch rot, powdery mildew and downy mildew. Bacteria do not cause severe diseases in grapes as compared to other disease-causing agents. There are some viral diseases like poor color development, stunting, reduced yield, chlorosis, delayed fruit and deformity which affects the grape plants badly (Mukhtar Uddin et al., 2011). Other agents that cause harm to grapevines and their fruits are grape mealy bugs, omnivorous leaf roller, spider mites, nematodes, grape bud beetle, grape leaf hopper, grape leaf folder and phylloxera (Janick and Moore 1975). Termites also destroy the grapevines as they attack on main trunk of grape plant. (El-Sebaii et al. 2002)and (Baginsky et al. 2003), reported that many species of the plant parasitic nematodes (PPN) cause commercial impairment to

grapevines and among them most common species are Meloidogyne ethiopica, Tylenchulus semipenetrans, Mesocriconema xenoplax and Xiphinema index. Production is affected by fungal diseases dramatically. Few serious diseases in wet weather include downy mildew (*Plasmopora viticola*), Phomopsis (*Phomopsis viticola*), grey mould (*Botrytis cinerea*) and anthracnose (*Elsinoë ampelina*). Another disease known as Powdery mildew (*Uncinula necator syn. Erysiphe necator*) is observed to occur both in dry and wet regions. Among bacterial diseases Crown gall (*Agrobacterium* spp.) is the most serious while a number of viruses can damage the vine badly (Jackson and Palmer 1999); (Salunkhe and Kadam 1995).

Fig. 1. 4 Picture expressing disease on grapes leaves.

1.11.1 Diseases and Control

1.11.1.1 Causing Agents

Botrytis cinerea causes the disease Botrytis rot or grey mould in the table grapes which is the principal source of post-harvest losses (Salunkhe and Kadam 1995) (Pearson and Goheen 1988). The minimum temperature for this fungus to grow is −0.5°C and it spread from berry to berry during transportation and storage although a uniform temperature and pre-cooling are maintained. This disease can be recognized by

characteristic 'slipskin' disorder, and later on the berries are decayed and are enclosed in a white mycelium. According to (de Kock 1991), the main source of post-harvest berry destruction is conidial infection which occurs during or after the veraison stage. It is suggested that the main reason behind the loss in berry confrontation is the less concentration of proanthocyanidin and fall in the production of antimicrobial stilbenes in maturing berry (Hill et al. 1981); (Creasy and Coffee 1988)). Infection is also caused by cracking of berry in some specific cultivars. There are some of the disea ses caused in table grapes after harvesting namely, Alternaria rot (*Alternaria alternata*), Cladosporium rot (*Cladosporium herbarum*), Rhizopus rot (*Rhizopus oryzae; R. stolonifer*), ripe rot (*Botryosphaeria ribis),* bitter rot (*Greenaria uvicola*), Coniella rot (*Coniella diplodiella*), mould rot, (*Penicillium* spp), Phompsis rot (*Phomopsis viticola*), blue anthracnose (*Elsione ampelina*, *Glomerella cingulata*) and black rot (*Guignardia bidwelii*) (Snowden 2008).

1.11.1.2 Disease control

Fungal attacks can be minimized by the safe removal of crop remainings through proper cleaning. Some fungal infections can be controlled by spraying the fungicide sprays before harvesting (Snowden 2008). Research suggests that the harvesting should not be carried out after 3 days of rain. The infected berries should be removed during trimming to avoid more losses to grape bunches. Transmi ssion of bacterial and some viral diseases can be prevented by the sterilization of harvesting tools. After harvesting of grapes the berries should be cool as soon as possible as it minimizes the injuries.

1.12 Study Areas

1.12.1 Study area Pishin

1.12.1.1 Geography

Pishin is important district of Balochistan which lies in the north-west of Balochistan. The Pishin name derives from "Pushang" which mean that how the city was design in Persian. The District was bounded by Killa Saifullah in the east, Afghanistan in the west, Killa Abdullah in the north while southern range is surrounded by Ziarat and Quetta District.

1.12.1.2 Location

The district is stretched between 30°.33′ N to 31°.20′ N and 66°.79′ E to 67°.85′E (IUCN, 2011). The total area of the district is 7819 sq.km. Its altitudinal range start from 1370 to 1680 masl.

1.12.1.3 Population

The total population of the area is 736,481in which 51.48%is male while 48.52% are female. In which the urban population is 143,142 and the rural population is 593,339. The sex ratio is 106.09 population growth rate is 3.58% per year. A year wise population data are given below in the table 1.2 (Census PBS,2017)

Table 1. 2 Census Wise Population (In '000').

1961		1972	1981	1998	2010(Projected)
Total Pop.	125	249	122	367	559
Male Pop.	63(50%)	126(51%)	n/a	196(53%)	299(53%)
Female Pop.	62(50%)	123(49%)	n/a	171(47%)	260(47%)
Urban Pop.	n/a	n/a	15(12%)	23(6%)	35(6%)
Rural Pop.	n/a	n/a	107%(88%)	344(94%)	524(94%)
Sex ratio(Males per 100 females)	102	102	102	115	115
Number of households (in '000')	n/a	n/a	22	35	n/a

1.12.1.4 Literacy ratio

According to PSLM, the literacy rate are high in Pishin district as compared to other district. Its rank is 7th in the literacy rate because in the 15+ population the literacy rate is 50% while in the 10+ population the literacy rate is 56%. The district is on no. 5 in male literacy rate while the female literacy rate are less because in 15+ population is 32% while in 10+ population its 32% . The given table (Table 1.3) shows male and female literacy rate of different years.

Table 1. 3 Literacy Rates in the District.

Literacy rates						
	15+			15+		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
2005	73%	39%	58%	71%	33%	54%
2007	67%	21%	47%	63%	16%	42%
2009	74%	30%	55%	72%	24%	51%
2011	71%	10%	42%	72%	8%	41%
2013	74%	32%	56%	72%	23%	50%

Source: Various PSLMs

1.12.1.5 Climatic conditions

The climatic condition of the area are variable, summer are warm and winter are cold. Mostly the rainfall are uneven because the monsoon currents are lays outside. The January and February the snow covering months. July are the hottest month in which the minimum temperature is 3°C and the maximum temperature is 10°C. Storms mostly occur in winter seasons. Most of the rainfall occur from December to April.

Table 1. 4 Expressing the Rainfall (mm) and Temperature (°C).

Month	Rainfall	Maximum temp.	Minimum temp.
January	58.8	11	-2
February	46.3	13	-1
March	68.5	18	4
April	38.8	24	9
May	5.4	31	14
June	0.3	35	18
July	19.4	36	21

August	23.9	34	19
September	6.8	32	13
October	2.7	25	5
November	3.5	21	2
December	33.8	14	-1
Mean	25.7	25	8
Total	308	-	-

(RMC Karachi, 2008)

1.12.1.6 Transport

Modernization and socialization of in the area transportation an roads, and telecommunication paly a significant role. These three represent the level of the development of the transport and communication. In the zone a road of 100sq.km is available for public and private transport. The facility of internet and telephone connection is present on per hundred thousand individuals.

1.12.1.7 Languages

Pashto is the native language of the district but rarely the Persian is also spoken. In pishin the Tareen tribe speak the language which is spoken in Quetta, Dukki and Gulistan which are similar to Kandahar type.

1.12.1.8 Tehsils and Union Councils

District pishin consist of 5 tehsils and 54 union councils. The detail of the tehsils and union councils are given in the table below;

Table 1. 5 Tehsils and Union councils of the district Pishin.

Tehsil	**Union council**
Barshore	Bagh, Barshore, Behram Khan, Bela, Ghaizh, Injani, Kach Hassanzai, Kaza Viala, Kut, Mandozai, Walma, Ziarat.
Karezat	Balozai, Bostan, Dilsora, Khanozai, Khushab, Lumran, Mughutian, Rod Mulazai, Yaru
Huramzai	Alizai, Gangalzai, Hajian Shakarzai, Manzari, Huramzai

Pishin	Ajram Shadizai, Batezai, Bazar Kohna, Dab Khanzai, Karbala, Malezai, Malikyar, Manzaki, Muchan, Pishin Bazar, Saranan, Shahdizai

Source: UNOCHA, 2017

1.12.1.9 Agriculture and Horticulture

The total agricultural area of district Pishin is 185,280 hectares, that is about 23.5% of total area of District Pishin. District Pishin lies in tropical agro-ecological region (Agricultural Statistics of Pakistan, 2008-0). The total cultivated area of the Pishin district are 155,189 hectares. The main crops of the area are wheat, barley, onion, tobacco and potato. The major fruits of the district are apricot, grapes, apple, almonds, peach, plum and cherry.

1.12.1.10 Irrigation

The main irrigation system consists of tube wells, springs and wells. Tube wells are of two types one is private which is used for daily basic needs while other is government type which is used for irrigation of land.

1.12.1.11 Sport

Sport is the part of a vigorous society. The main purpose of sport is to keep human beings energetic and active. Wrestling is the most common game which is totally different from that of professional wrestling. Ghazae is the local name given to wrestling. On the occasion of religious and social gathering this game is played. The people also play volleyball, smashing ball, cricket and football.

1.12.2 Study area Quetta

1.12.2.1 Geography

Shalkot is the former name given to quetta. Little London was used as the nickname of quetta before the earthquake of 1935. It is 1650 m high from the mean level of sea, and it is restricted by mountain ranges called as Murdar whose top reaches to 3134 m (Sami et al. 2012).

1.12.2.2 Location

The district Quetta is situated in the Northern Balochistan where it is close to Pak-Afghan border. The latitude of quetta is 29°48' to 30°25' N while the longitude is 66°13'

to 67°17' E. Quetta is surrounded by different hills named as Takatoo, Zarghun, Chiltan and Murdar (Nazir et al.). The total area of Quetta is 2,653 km^2.

1.12.2.3 Population

The population of Quetta according to Census 1941 was 65,000 which reached to 84,000 in 1951 Census and showed a percentage increase of 29.2%. Gradually the population reached to 107,000 in 1961 with percentage increase of 27.4%. In 1 972 Census, population of the city was 158,000 with percentage increase of 47.7% and 286,000 was reported in 1981 with percentage increase of 81.0%. Similarly in Census of 1998 the population of Quetta was reported to be 565,137 with 97.6% of increase. And then in the last Census which was conducted in 2017, the population of the city was 2,275,699 and the percentage was reached to a peak of 77.2%.

1.12.2.4 Literacy Ratio

The data recorded shows that Quetta is having high literacy rate when compared with other districts. Literacy rate is 66% observed in 15+ population while in 10+ population it is 70%. The district has been ranked 1st when talking about literacy rate. The female literacy rate is also high as compared to other districts which is 46% in 15+ population while in that 10+ population it is 52%.

Table 1. 6 Literacy rates of the district.

Literacy rates						
	10+			**15+**		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
2005	80%	47%	65%	79%	41%	62%
2007	75%	44%	62%	74%	39%	59%
2009	80%	51%	67%	77%	46%	63%
2011	84%	40%	64%	83%	36%	61%
2013	85%	52%	70%	83%	46%	66%

Source: Various PSLMs

1.12.2.5 Climatic conditions

The district quetta falls in sub-tropical climatic zone having variances in winter and summer temperatures. May to September is summer season which is full of diversity in regard to temperature. Winter season starts from october and ends in late march with the severe cold. Four seasons occur in quetta which are spring, summer, autumn and winter. The mean temperature is 64°F annually. According to (Sami 2009), the mean winter temperature is 40°F while the mean summer temperature is recorded to be 78°F.

Table 1. 7 Annual rainfall(mm) since 1982.

Year	Rainfall	Year	Rainfall
1982	949.8 mm	2003	219 mm
1991	360 mm	2004	105.9 mm
1992	349 mm	2005	310.5 mm
1993	190 mm	2006	223 mm
1994	225 mm	2007	284 mm
1995	290 mm	2008	138 mm
1996	155 mm	2009	317 mm
1997	305 mm	2010	104 mm
1998	152 mm	2011	437.6 mm
1999	150 mm	2012	265.7 mm
2000	60 mm	2013	316.7 mm
2001	80 mm	-	-
2002	115 mm	-	-

Pakistan Meteorological Department

1.12.2.6 Languages

Quetta is the city with a mixture of cultures, in which Baloch, Pashtoon, Hazara, Punjabi and Saraiki are the settled casts. Variety of languages are found in quetta of which Pashto and Balochi are the dominant languages, others include Persian, Panjabi, Saraiki and Urdu.

1.12.2.7 Sports

Majority of the people of study area play Cricket and Football, which are quite popular games. Badminton, taekwondo, hockey are also played at minor level.

1.12.2.8 Irrigation system

The main source of irrigation of the Quetta is tube-well system. In rare cases irrigation by canal and sewerage system is also reported. Rain is also a source of irrigation in many areas.

1.13 Aims and Objectives

The current study was carried out to find the ethno-biological studies as well as the nutritional values of *Vitis vinifera* cultivars in Quetta, Pishin and Bannu areas of Pakistan.

- To explore the indigenous knowledge about grapes through questionnaire survey
- To find out the nutritional values of grape fruits from Quetta and Pishin, Balochistan
- To find out the suitable soil for the cultivation of grape varieties
- To find the suitable grape cultivars for cultivation in FR Bannu

MATERIALS AND METHODS

2.1 Selection of the study Area

The literature was studied to find out previous studies on this area for Ethno-biological and nutraceutical studies of Grapes in Quetta, Pishin and Bannu regions of Pakistan. No such study was conducted before, therefore these areas were selected for the current study.

2.2 Materials

During conduction of survey a number of different materials were used such as Questionnaire, Polythene bags, Tags, Digging tool, Measuring tape, Camera and Umbrella.

2.3 Details of gardens surveyed

A total of 45 grape gardens were surveyed in quetta and pishin, details of which are mentioned in mentioned in table 2.1.

Table 2. 1 Table showing details of the grape gardens.

S.No.	Village/Garden	Area covered (Hectare)
1	Chaki Shahwani	6
2	Chaki Shahwani	6
3	Saryab road	4.5
4	Saryab road	4.5
5	Saryab road	5

6	Kirani	2.5
7	Kirani	2
8	Kachi Baig	3.5
9	Kachi Baig	3
10	Kali Azizabad	5.5
11	Kali Azizabad	5
12	Kali Azizabad	6
13	Kali Sharifabad	1.5
14	Kali Sharifabad	2
15	Nawah Kali	5
16	Nawah Kali	3
17	Kali Qambrani	1.25
18	Kali Qambrani	2
19	Hazarganji	5
20	Hazarganji	3
21	South Saryab	4
22	South Saryab	4
23	South Saryab	5
24	Bypass	4.5
25	Bypass	4

26	Bypass	2.5
27	Bypass	2
28	Poly Technic College	1.5
29	Poly Technic College	1
30	Poly Technic College	1
31	Quetta Pathak	1.5
32	Quetta Pathak	2
33	Mastung road	4.5
34	Mastung road	4
35	Surkhab road Research center	5
36	Surkhab road Research center	5
37	Eidgah	2
38	Eidgah	4
39	Band Khushdil	3
40	Band Khushdil	4
41	Qabristan Kali	4
42	Qabristan Kali	5
43	Surkhab road	3
44	Surkhab road	2
45	Surkhab road	1

2.4 Questionnaire data

The data was collected using questionnaire method from different farmers in Quetta and Pishin districts of Baluchistan and Bannu district of Khyber Pakhtunkhwa for the identification of suitable grape varieties and comparisons during February-July 2018. A total of 45 questionnaires were recorded in Baluchistan and 15 in FR region of Bannu FATA. The farmers for survey were selected randomly at different places in studied area. All the questions asked were related to beginning of grapes plantation till the ripening of fruits. Fig. 2.1 demonstrates the questionnaire survey.

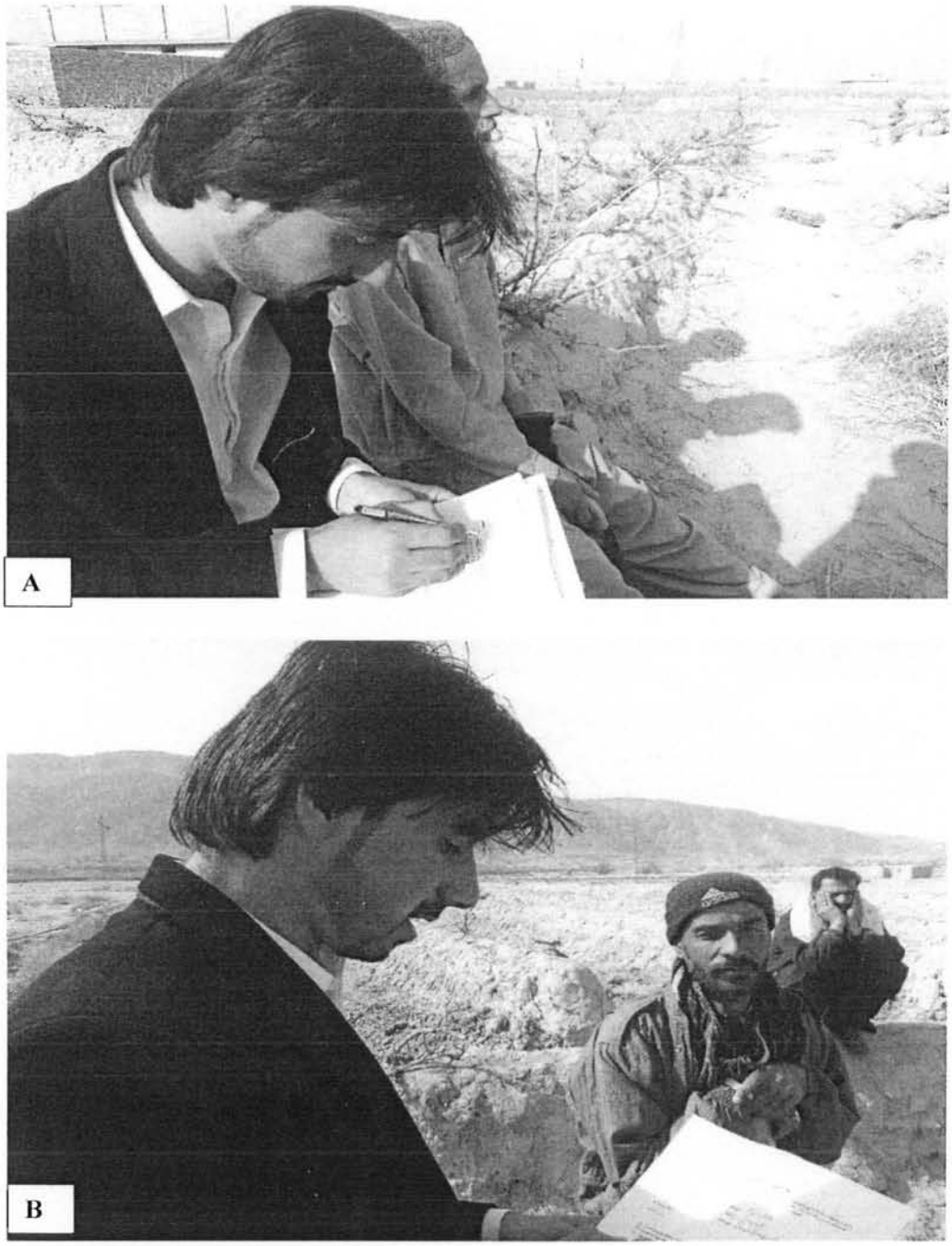

Fig. 2. 1 A and B illustrating the questionnaire survey in the study area.

2.5 Soil collection and analyses

Soil samples were collected from various sites at the depth of 6 inches from the surface of the ground, samples were tagged and air dried. Various physio-chemical tests such

as pH. E.C, TDS and various metals concentration was screened out in the Plant Ecology and Conservation Lab, Department of Plant Sciences, and Atomic Absorption Spectrophotometer was done in the Lab, Department of Biochemistry, Quaid-i-Azam University, Islamabad. These metals were comprised of Nickle, Chromium, Sodium, Potassium, Zinc, Iron, Copper, Manganese, Calcium, Magnesium, Cadmium and Lead.

2.5.1 Determination of soil pH

Negative log of hydrogen ion concentration was determined with the help of pH meter. The following process was followed:

To determine pH and EC of the soil 23g of soil sample was taken from each site. The sample was weighed via digital balance. The soil sample was dissolved in 120ml distilled water in a conical flask. The solution was shacked for 45 minutes via shaker at 70rpm. Shaking was done thoroughly to dissolve all the nutrients in the soil. Then a filter paper was fixed in a funnel and putted in a beaker then the mixture was poured for filtration till all the liquid part was filtered in the beaker. Then pH of the solution was measured via pH meter and noted in the excel sheets for further analysis.

2.5.2 Electrical Conductivity (EC)

For EC determination 23g of Soil was weighed in a bottle and thoroughly dissolved in 120 ml distilled water. The solution was shacked via shaker for 45 minutes Shaking was done thoroughly so that all the nutrients were mixed completely. Then a filter paper was fixed in a funnel and putted in a beaker and the mixture was poured for filtration till all the liquid part was filtered in the beaker. Then the solution in the beaker was kept for measuring EC by dipping the Electrode in the solution and noting the reading on the screen of the EC meter.

2.5.3 Total Dissolved Solids (TDS)

The total dissolved solids of the soil were measured by using the following formula;

Total dissolved solids= Electrical conductivity x 0.700

2.5.4 Heavy metals and nutrient analyses

The heavy metals and other essential nutrients analyses were done of the collected soil samples. First the soil samples were dried via Oven for 30 minutes at 65 ^{0}C. After drying

soil samples 0.5 gram of soil was taken in a conical flask. Nitric acid and Perchloric acid were mixed in a ratio of 3:1 and then from this mixture 10ml solution was poured in the conical flask. The prepared solution was then digested on hot plate until the color was changed. After that the digested solution was cooled down and 40ml distilled water was poured in it. The prepared samples were kept in bottles for further tests i.e., Nickle, Chromium, Sodium, Potassium, Zinc, Iron, Copper, Manganese, Calcium, Magnesium, Cadmium and Lead through Atomic Absorption Spectrophotometry (VARIAN, AA240FS) in the general lab of Biochemistry Department, Quaid-i-Azam University, Islamabad.

2.5.4.1 Nickle (Ni)

The concentration of Nickle (Ni) was screened out in samples via atomic absorption spectrophotometer.

2.5.4.2 Chromium (Cr)

The chromium concentration was checked with the atomic absorption spectrophotometer.

2.5.4.3 Sodium (Na)

The amount of sodium particles in the sample were analyzed through the atomic absorption spectrophotometer.

2.5.4.4 Potassium (K)

The presence of potassium was measured using atomic absorption spectrophotometer.

2.5.4.5 Zinc (Zn)

Zinc concentration in soil sample was checked by atomic absorption spectrophotometer.

2.5.4.6 Iron (Fe)

The amount of accumulation of zinc in soil was analyzed by using atomic absorption spectrophotometer.

2.5.4.7 Copper (Cu)

Quantity of copper present in the sample was checked out via atomic absorption spectrophotometer.

2.5.4.8 Manganese (Mn)

Presence of manganese in soil was found through the use of atomic absorption spectrophotometer.

2.5.4.9 Calcium (Ca)

Analyses through atomic absorption spectrophotometer gave the concentration of calcium present in soil sample.

2.5.4.10 Magnesium (Mg)

The quantity in which the magnesium particles were present in soil were checked by the use of atomic absorption spectrophotometer.

2.5.4.11 Cadmium (Cd)

Atomic absorption spectrophotometer analyses gave the concentration of cadmium present in soil sample.

2.5.4.12 Lead (Pb)

The concentration of lead was measured through atomic absorption spectrophotometer. Fig. 2.2 below shows the analysis of all the metals through Atomic Absorption Spectrophotometry.

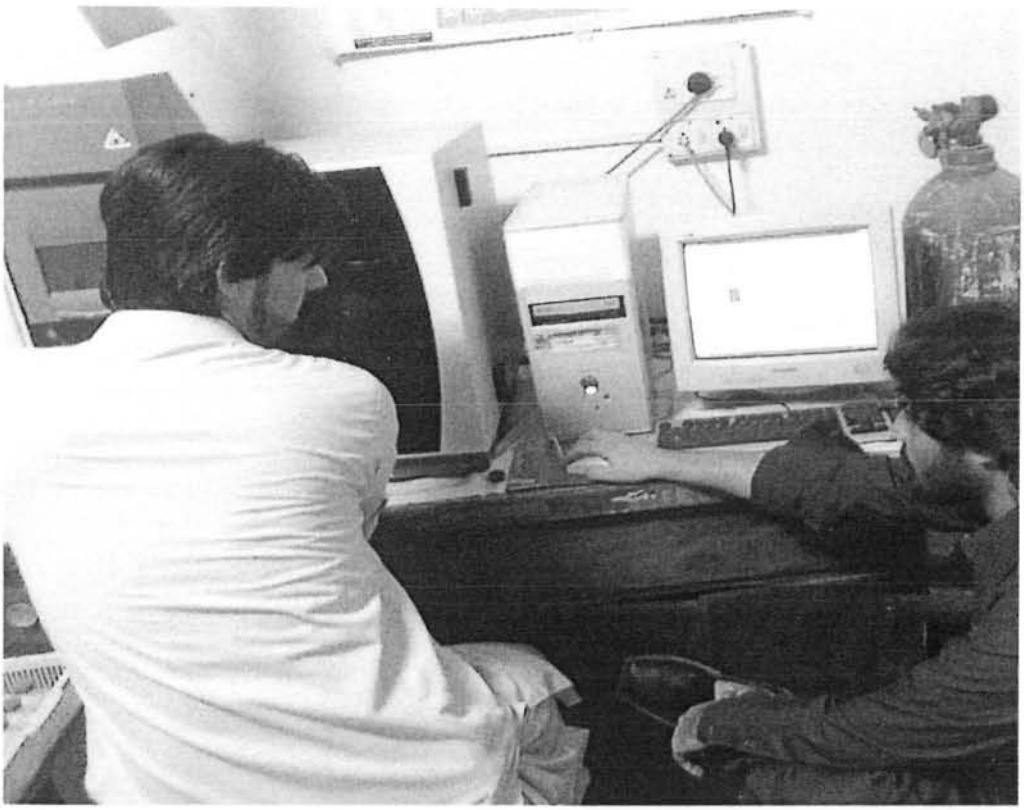

Fig. 2. 2 Metal analysis via Atomic Absorption Spectrophotometer.

2.6 Cultivation of cuttings in the field at FR Bannu

An experiment was conducted at the University of Science and Technology Bannu in the month of March 2018 to point out the most suitable and successful varieties of grapes in the region. A total of 450 grape plants belongs to 18 different varieties were planted.

2.6.1 Selection of the field

On the basis of pre-soil tests suitable field for grapes cultivation experiment was selected in the University of Science and Technology Bannu.

2.6.2 Size of the experimental field

The total area of the field was one 1 kanal which is approximately equal to 5445 square feet. Length of the field was 75 feet while width was 70 feet which makes an area of 5250 sq. feet that is approximately equal to 1 kanal.

2.6.3 Field preparation

The field was levelled and ploughed through tractor. The furrows were made in the field with equal length and width. A total of 20 rows were developed at a distance of 3 feet from each other in the field as shown in fig.2.3.

Fig. 2. 3 Picture illustrates the Field Preparation at FR Bannu.

2.6.4 Plantation date

Cuttings were planted on the 3rd and 4th of March, 2018, at USTB. Fig. 2.4 illustrates the plantation.

Fig. 2. 4 Plantation in the Field at FR Bannu.

2.6.5 Varieties Planted in the field

A total of 450 cuttings of 18 different varieties were planted in the field (Table 2.2) which were collected from Quetta and Pishin districts of Balochistan and local varieties from Bannu district of Khyber Pakhtunkhwa. Total 450 cuttings were planted in the field. The number of cuttings from each variety was different ranging from 5 to 70. The highest number of cuttings planted were that of Shogran and the lowest was that of local line collected from local area of district Bannu.

Table 2. 2 Varieties/lines planted in the field.

S.No.	**Varieties**	**No. of Cuttings**
1	Shogran	70
2	Sundarkhani	50
3	NARC-Black	15
4	Sultania-C	13

5	Thomson seedless	20
6	Haita	20
7	Sra Sabi	15
8	Peon Sabi	30
9	Local line 1	10
10	Local line 2	30
11	Local line 3	35
12	Local line 4	17
13	Local line 5	15
14	Local line 6	50
15	Local line 7	10
16	Local line 8	15
17	Local line 9	10
18	Local line 10	25

2.6.6 Experimental design

Cutting cultivation experiment was designed in a way that 22 cuttings were planted in a row. Total number of rows were 20. The distance between row to row was kept 3 feet and that of plant to plant was also kept 3 feet. The furrows were made from east to west and flow of water was set from east to west.

*2.6.7 **Parameters studied***

2.6.7.1 Survival Percentage of cuttings

When the cuttings were planted their successful subsistence percentage was calculated by the following formula;

2.6.7.2 Number of leaves

The number of leaves were counted at different intervals when the leaves were started such as, 1st, 2nd, 3rd, 4th and 5th leaves stages as shown in Fig. 2.5.

2.6.7.3 Leaf size

The leaf size (length and width) was measured It was measured in replicates in the unit centimeters (cm) with the help of a scale.

2.6.7.4 Number of nodes

The number of nodes were counted on the basis of different dates on which they were appeared. Following are the dates on which the number of nodes were counted; 26/03/2018, 07/04/2018, 17/04/2018, 26/04/2018 and 05/05/2018 where nodes stages were 1st, 2nd, 3rd, 4th and 5th.

2.6.7.5 Internode distance

The distance between two respective nodes were measured with a scale in centimeters. Internode length and internode width were measured.

2.6.7.6 Petiole length

The measurement of petiole length was done using a simple scale in centimeters.

Fig. 2. 5 Different stages of vegetative growth in the field.

2.7 Nutritional analyses of grapes

Different parameters of nutritional analyses were done in the Plant Ecology and Conservation lab as expressed in Fig. 2.6.

2.7.1 Moisture contents

The moisture content was found out by weighing 5 grams of fresh fruit and marked as W1. Then the sample was oven dried at 70°C. After that the fruit was dried completely and weighed again with mark W2. Following formula was applied to check out the moisture contents;

Moisture= W1-W2

2.7.2 Determination of pH

1) Fresh fruit sample were ground in a grinder.

2) After grinding the sample was taken in a beaker.

3) pH reading was measured by dipping the Electrode of pH meter in the sample.

4) Reading on the main screen was noted down until the colour was changed.

2.7.3 Determination of EC

1) Fresh fruit sample were grinded in a grinder.

2) After grinding the sample was taken in a beaker.

3) EC reading was measured by dipping the Electrode of EC meter in the sample.

4) Reading on the main screen was noted down until the colour was changed.

2.7.4 Determination of Total Acidity

1) Juice from the sample fruit was prepared and 10ml was taken in 250ml beaker.

2) 2 drops of Phenolphthalein were added.

3) About 100ml distilled water was added to the solution and then Titrated with 0.1% N NaOH.

4) Reading was noted of the 0.1% N NaOH used till the colour of the sample was changed to brownish.

Acidity of the samples was calculated via following formulae

$$\text{Acidity (\%)} = \frac{\text{CF x N x ml of NaOH used x 100}}{\text{A x B}} \times 100$$

Where

A = the volume of sample (ml) collected for dilution

B = Volume of sample (ml) collected for titration

C.F = Conversion factor for citric acid i.e. 0.064

N = Normality of NaOH

2.7.5 Determination of Ascorbic Acid (Vitamin C)

1) Fresh fruits were grinded via Juicer and a juicy solution was prepared. Ten ml Juicy solution of fresh fruits was taken in a100ml beaker.

2) By adding 90ml, of 0.4% Oxalic acid to the juicy solution (to reach its volume into 100 ml)

3) 100ml of 2,6-dichlorophenol was taken in a burette and fixed in burette stand.

Step IV) in this step 5 ml sample solution were taken in a beaker and 2,6-dichlorophenol was added dropwise in the beaker as indicator.

5) Reading was noted of the 2,6- dichlorophenol used until the colour was changed.

The ascorbic acid was calculated by using the following formula:

$$\text{Ascorbic acid (mg/100g)} = \frac{F \times T \times 100}{S \times D} \times 100$$

$$F = \text{Factor from standardization} = \frac{\text{ml of ascorbic acid solution taken}}{\text{Volume of dye used}}$$

T = ml of dye used for sample

S = ml of dilute sample taken for titration

D = ml of sample taken for dilution

2.7.6 Determination of Reducing Sugars

Lane and Eynon method defined in AOAC (2012) was utilized for determination of reducing sugars.

1) A 10 ml fruit sample was taken in a beaker and distilled water was added and the volume was reached to 100 ml.

2) The mixture was transferred to a burette.

3) Fehling A and Fehling B were taken 5 ml each and were mixed in a beaker.

4) Then this solution of Fehling A and Fehling B were heated until it boiled.

5) After that this solution was dropped drop wise until brick red colour was appeared.

2.7.7 Calculations

5 ml of Fehling A + 5 ml of Fehling B = X ml of 10 % sample solutions = 0.05 g of reducing sugar.

100 ml of 10 % sample solution will contain = $\frac{0.05 \times 100}{X \text{ ml}}$ = Y g of reducing sugar

% reducing sugar in sample = $\frac{Y \times 100}{10}$

2.7.8 Non-Reducing Sugars

Lane and Eynon method as defined in AOAC. (2012) was utilized for determination of non-reducing sugars.

1) From the prepared fruit sample 20 ml was taken in a beaker.

2) 10 ml of 1N. HCl was mixed with the fruit sample.

3) Heated this mixture until the steam was made but did not allowed the formation of bubble in it. And the mixture was left to cool down.

4) In this mixture 10 ml 1N. NaOH was added and the volume was reached to 250 ml by the addition of distilled water.

5) The mixture was shaked thoroughly to mix all the chemicals.

6) Fehling A and Fehling B were taken 5 ml each and mixed with 10 ml distilled water.

7) Heated up this mixture till the bubbles formed and then the prepared sample was dropped (from 250 ml) dropwise till the colour was changed.

8) Then the reading was noted down.

2.7.9 Calculations

Xml of sample solution contain = 0.05 g if reducing sugar.

250 ml of sample contain = $\frac{250 \times 0.05}{X \text{ ml}}$ = Y g of reducing sugar.

This 250 ml of sample solution was prepared from 20 ml of 10 % sample solution.

So, 20 ml of 10 % solution contain = Y g of reducing sugars.

100 ml of 10% solution contain = $\frac{Yx100}{20}$ = P g reducing sugar

Theses 100 ml were prepared from 10 g of the sample.

So, 10 g of sample contain = $\frac{Px100}{10}$ = Q g of total reducing sugar

Q g of reducing sugar= Inverted sugar + Free reducing sugar

Non-reducing sugar = Total reducing sugar – Free reducing sugar.

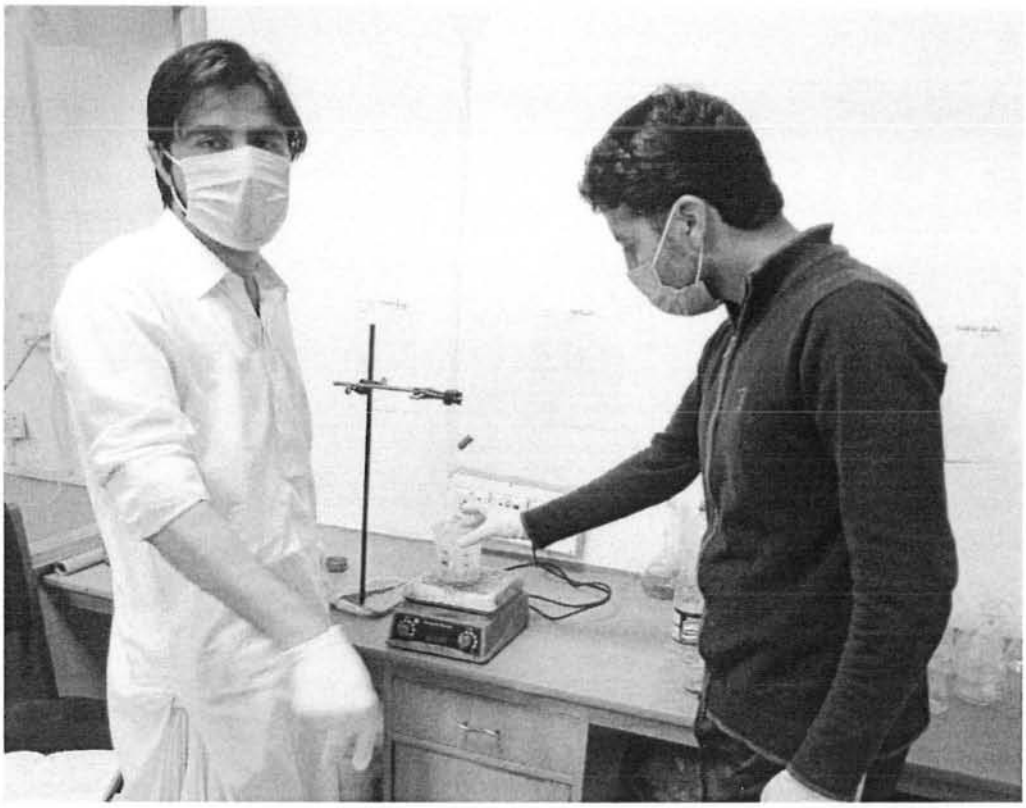

Fig. 2. 6 Nutritional analysis in the Plant Ecology and Conservation lab.

2.8 Statistical analyses through XLSTAT

The fruits related data were analyzed using statistical software XLSTAT version 2019. The Principal Component Analysis (PCA) was also done.

RESULTS

3.1 Interview results

The questionnaire results showed a significant variation in grape varieties, income of the farmers, preference of varieties usage, production and many other aspects. Primary data showed that Quetta is the main hub where many varieties of grapes were domesticated and have high quality taste, maximum production and high market value. A total of 45 questionnaire surveys were conducted in the study area in which 9 different varieties were reported. The most dominant and preferable variety reported and cited 23 times in different grapes gardens was Sundarkhani followed by Kishmishi. The least observed variety was Peon Sabi reported from only one grape garden in the region. Questionnaire survey show that varieties for cultivation in the area were selected on the bases of their taste and economy as well as their high value production.

Overall varieties reported during questionnaire survey were Sundarkhani, Kishmishi, Haita, Sultania-C, NARC-Black, Sra Sabi, Khison, Shogran and Peon Sabi.

3.1.1 Age groups of informants

During the questionnaire survey 45 gardeners of different ages were interviewed. The maximum ages of gardeners were between 25-31 and 39-45 years. The lowest number of gardeners were observed in the age between 60-66. Data was collected from those gardeners who were well trained and expert in their field. In Bannu district a total of 15 individuals were interviewed who planted grape trees in their home gardens. Following is a chart (Fig. 3.1) showing the ages of the surveyed gardeners.

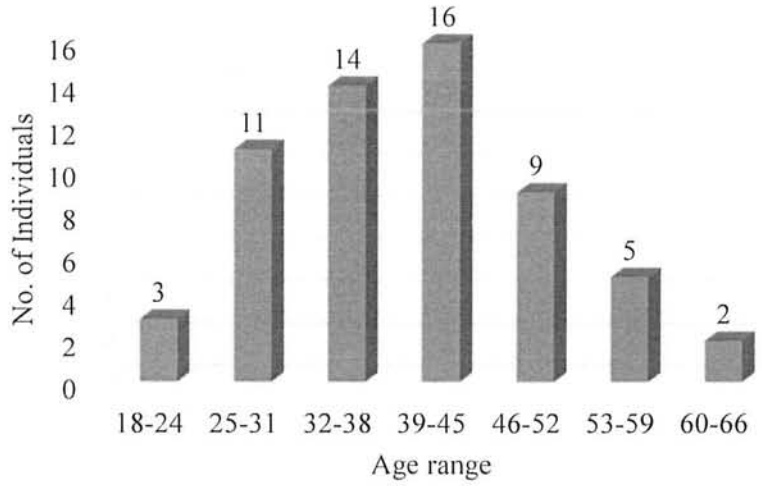

Fig. 3. 1 Graph showing the age groups of informants.

3.1.2 Varieties reported in Quetta and Pishin during survey

A total of 9 varieties were reported during surveys in the districts of Quetta and Pishin. The most popular and most cultivated variety was Sundarkhani followed by Kishmishi and the rarely domesticated variety observed was Peon Sabi reported from only one garden as mentioned (Fig.3.2). Sundarkhani is preferred in the study area by the people due to its good aroma, sweet taste and high market value.

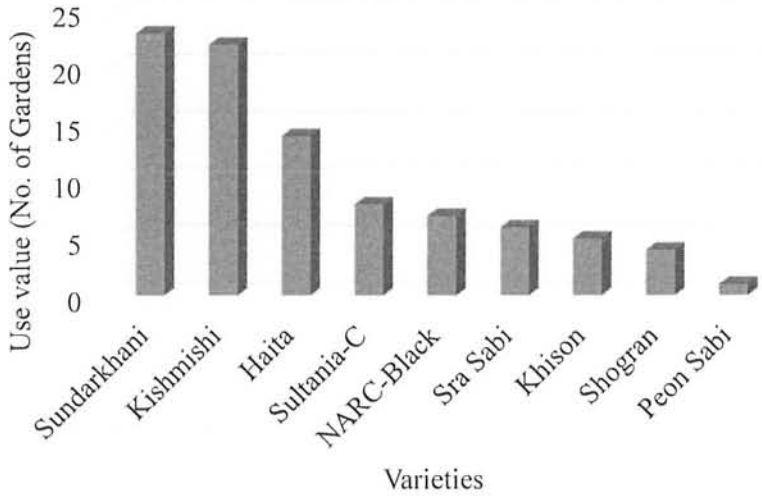

Fig. 3. 2 Graph showing the reported grape varieties.

3.1.3 Preference of grape varieties for plantation

The gardeners as well as local peoples preferred different grape varieties for cultivation and domestication in their commercial fields or home gardens. The most preferred variety was Sundarkhani which was reported in 23 gardens out of 45 gardens while the variety that was least preferred was Peon Sabi which came in observation only one time out of 45 gardens (Fig. 3.3). The reasons for the cultivation of Sundarkhani were sweet and fabulous taste with high product and economy in the market as compared to Peon Sabi that was not observed so.

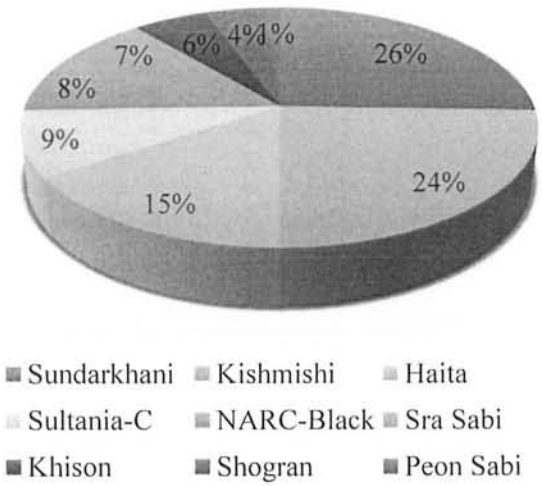

Fig. 3. 3 Graph showing the preference of grape varieties for plantation.

3.1.4 Use of fertilizers in grape gardens

According to current survey it was observed that maximum number of people of the study area preferred to use Natural fertilizers followed by Urea. DAP was reported to be used in a very less quantity (Table 3.1) as compared to Natural fertilizers and Urea in the study area.

Table 3. 1 Use of fertilizers in grape gardens.

S.No	Fertilizer	Use value	Percentage
1	Natural	42	93.3
2	Urea	41	91.1
3	DAP	15	33.3

3.1.5 Use of Fertilizers in Grape Gardens (In Combination)

Gardeners in different sites of the study area mostly use natural manure (cow, buffalo etc., dung) and artificial fertilizers (urea and DAP) in different combinations. The results of the survey showed (Fig. 3.4) that the most applied combination of fertili zers was organic and artificial fertilizers observed in 30 gardens (66.6%) followed by the combination of DAP, urea and natural fertilizers in 8 grape gardens (17.7%). DAP and natural fertilizers combinatiere was observed to be applied in 4 gardens (8.8%) and the combination of DAP with urea was reported from 3 gardens (6.6%).

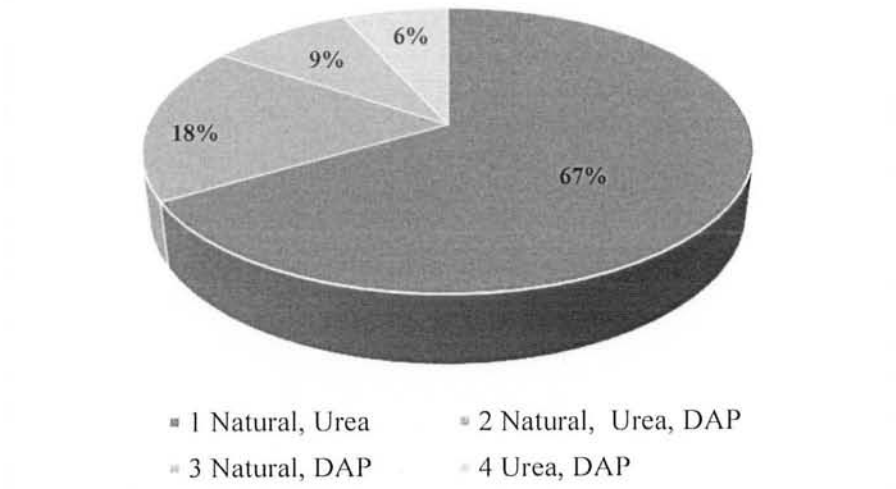

Fig. 3. 4 Graph showing the use of fertilizers in gardens (in combination).

3.1.6 Area VS number of plants

The grape gardens in the study area were grown on different sized areas ranging from 1-6 hectares. The maximum area for grape garden reported in the survey was 6 hectares having 1300 plants while the garden cultivated on an area of 1 hectare with 200 plants was the smallest in size (Fig 3.5).

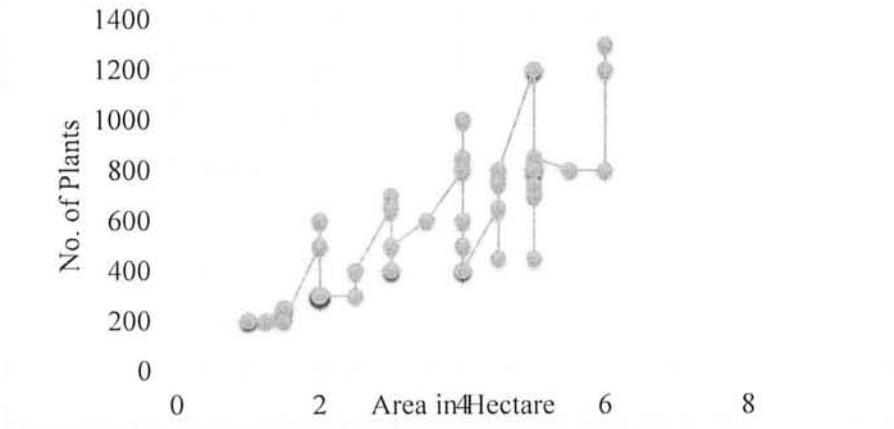

Fig. 3. 5 Illustrate the area covered by the plants (Quetta and Pishin).

3.1.7 Number of plants in home gardens

The survey conducted in District Bannu showed that grape plants were limited only to home gardens, no grape garden was observed in the open fields of Bannu (3.6). The number of plants varied from 1-4 within area from 3-6 meters in houses.

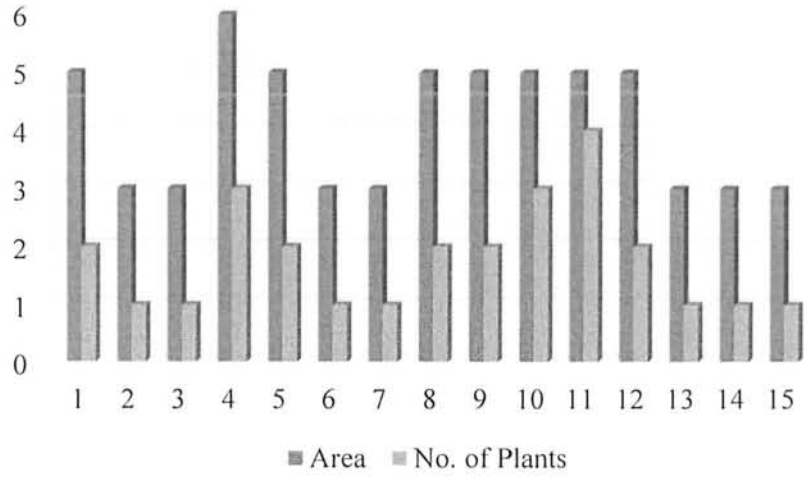

Fig. 3. 6 Illustrate the area covered by the plants (Bannu).

3.1.8 Income from grapes

Grapes being an important economical crop covers most of the economy of the people of the study area. The maximum income per year recorded was 1200000 while the lowest income was 150000 per year (Fig. 3.7). Survey showed that the income of a grape garden depends upon the quality and demands of that specific varieties grown in the garden.

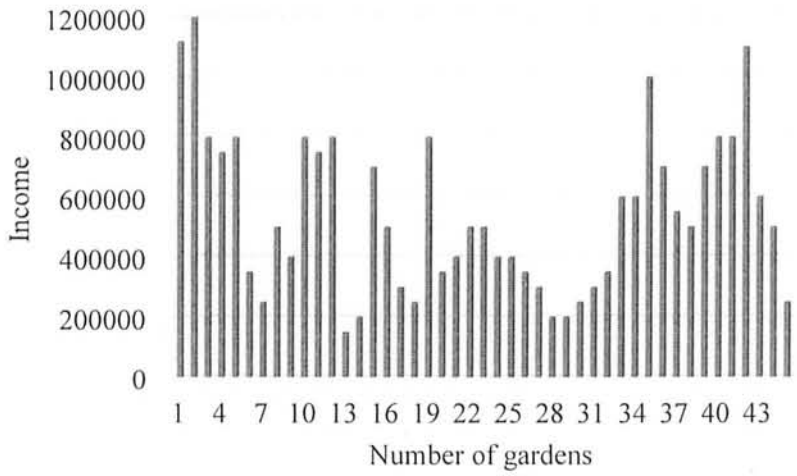

Fig. 3. 7 Graph demonstrates the income per year from each garden.

3.1.9 Varieties wise production

The variety Sundarkhani was reported to have the maximum production i.e., 1840 mounds per year as compared to other varieties (Fig. 3.8). The least production rate was observed in the variety Peon Sabi which was 20 mounds per year.

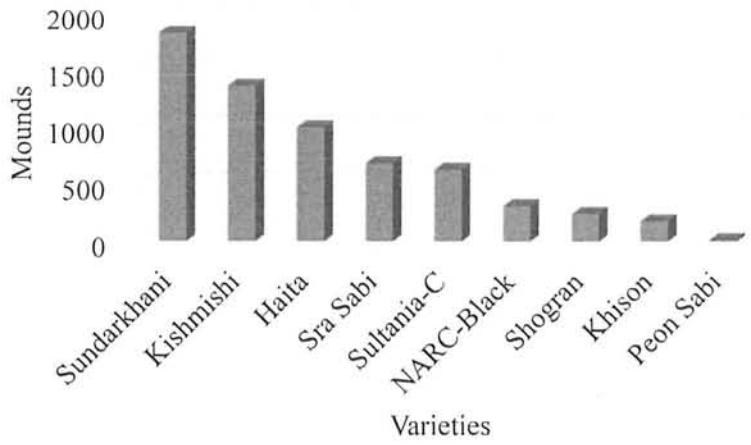

Fig. 3. 8 Graph showing the varieties with their production rate.

3.1.9.1 Production and Income

During the survey a total of 45 gardens were visited in which the production rate was different from each other depending upon size of the area and the varieties grown (Fig. 3.9). The maximum production rate was reported from garden 2 having production rate of 310 mounds with an annual income of 1200000. The lowest total income for a garden reported was 150000 as shown in the graph below.

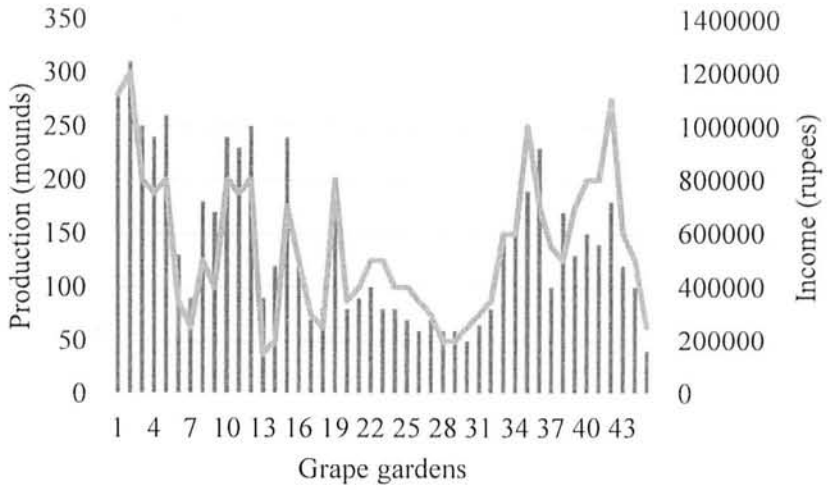

Fig. 3. 9 Illustrates the production rate compared with the income of each garden.

3.1.10 Willingness of Planting Grape Plants

In the questionnaire survey it was also observed that the people were willing to cultivate grape plants in future, where 5 people showed their willingness to cultivate 5000 grape plants in future (Fig. 3.10). The survey pointed out that most of the people were willing to have grape gardens in future for their livelihood and other necessary requirements.

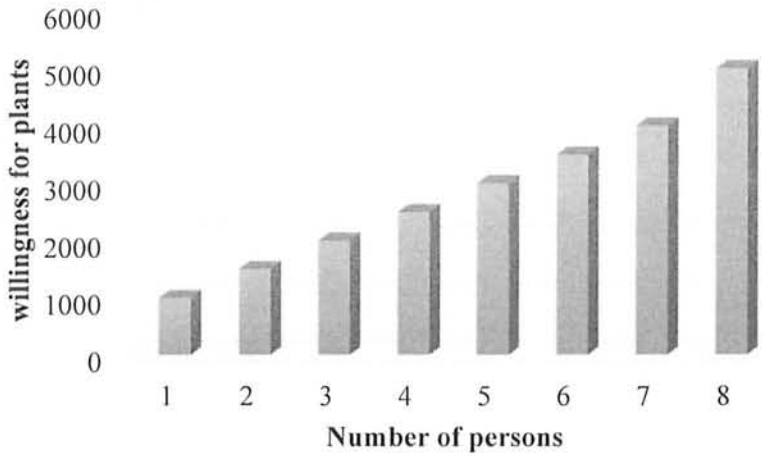

Fig. 3. 10 Graph showing the willingness of people for cultivation of grapes.

3.2 Soil analysis

3.2.1 Average soil metals

The soil analyses were done through Atomic Absorption Spectrophotometery techniques. Same number of metals were measured in all soil samples. The graph below (Fig. 3.11) shows the average metals concentration in the collected soil. Calcium was found with highest amount in soil with a concentration of 363.54 mg/kg, followed by Magnesium 393.87 mg/kg, Potassium 231.31 mg/kg, Lead 121.17 mg/kg, Sodium 89.76 mg/kg, Manganese 83.25 mg/kg, Iron 64.81 mg/kg, Chromium 60.60 mg/kg, Cadmium 43.31 mg/kg, Nickle 31.64 mg/kg, Zinc 7.80 mg/kg and the lowest concentration of metal observed was Copper 4.40 mg/kg.

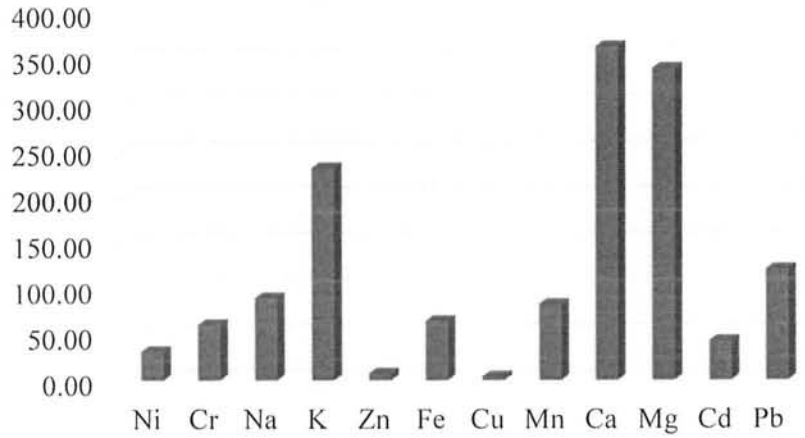

Fig. 3. 11 Graph showing the average metals concentration recorded in the soil samples.

3.2.2 pH values of soil

The negative log of Hydrogen was measured using pH meter. The pH value measured fall in the range of 4.90 to 7.50 as shown (Fig. 3.12). The highest value of pH observed was 7.50 with lowest value of 4.90. The mean value of pH determined for soil samples was 6.24.

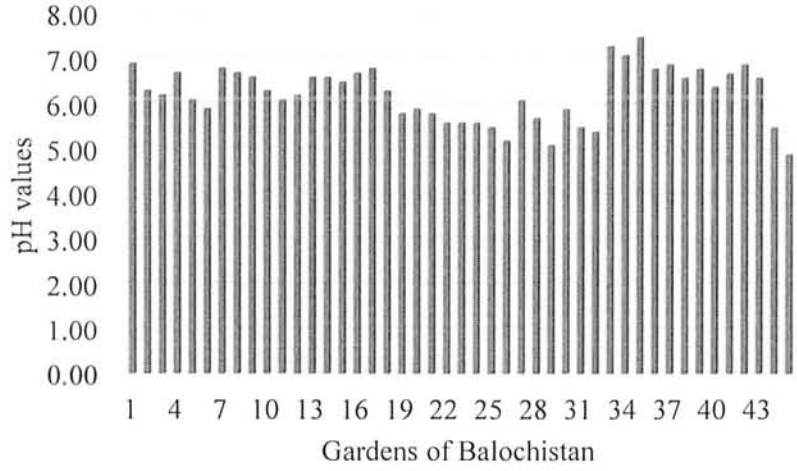

Fig. 3. 12 Graph illustrate the pH value of soil measured.

3.2.3 Electrical conductivity of soil

The Electrical Conductivity was measured using Electrical Conductivity meter. The EC value measured fall in the range of 90 ppm to 370 ppm as shown (Fig. 3.13). The highest value of EC observed was 370 ppm with lowest value of 90 ppm. The mean value of EC was 225.56 ppm.

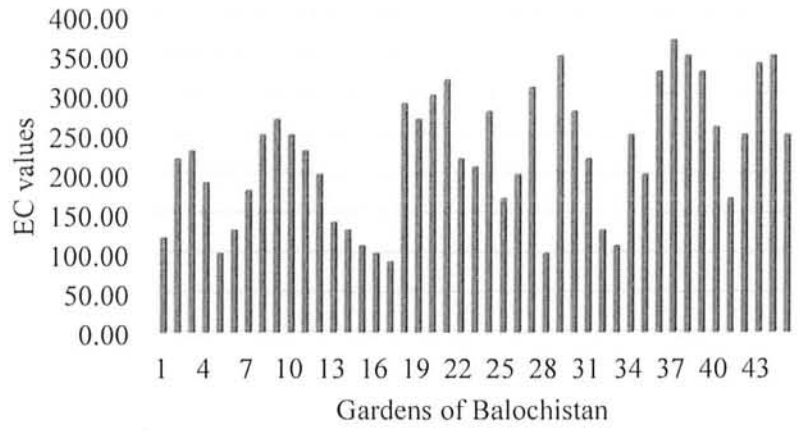

Fig. 3. 13 Graph illustrate the EC value of soil measured.

3.3 Results of field experiment

3.3.1 Survival percentage of cuttings

Survival percentage measures the ratio of plants survived and successfully developed. In this experiment a total of 450 cuttings were planted in which 8 cultivars were known and 10 were unknown that were tagged as local lines. The local lines collected were totally different from each other. The highest survival percentage was shown by a local variety tagged as Local line 1 as shown in (Fig. 3.14), of which 10 cuttings were planted and all of the 10 cuttings were grown successfully. The second highest survival percentage of survival was reported for another local variety tagged as Local line 14, of which 50 cuttings were planted out of which 40 were grown successfully. In local varieties, 4 cultivars were missed totally, they did not survive to grow. Some of the cuttings were missed from each variety, a normal ratio from each variety grown successfully. Survival percentage was measured by the following formula;

$$\text{Survival Percentage} = \frac{\text{No. of cuttings survived}}{\text{Total no. of cuttings}} \times 100$$

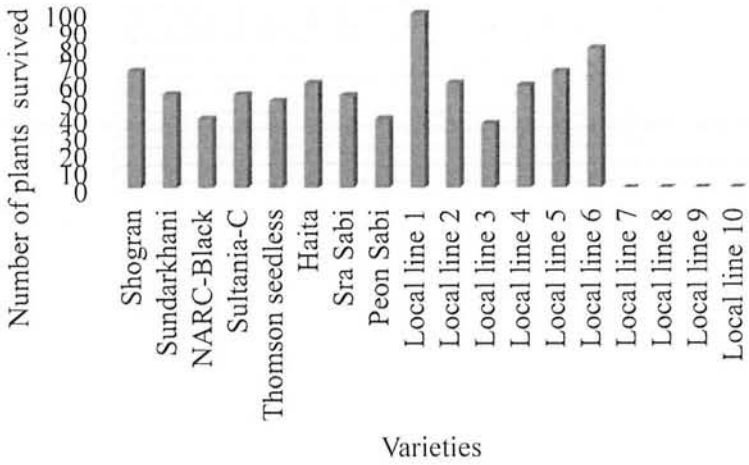

Fig. 3. 14 Graph showing the survival percentage of each grape local line.

3.3.2 Mean number of leaves

Number of leaves for each of the variety were counted during experiment. The mean highest number of leaves were reported from local line 1 where 37.25 leaves were

reported, followed by 36.3 leaves on average from another local line marked as Lo cal line 5. The minimum number of leaves were reported from cultivar Sundarkhani which were 29.84 on average as shown in Fig. 3.15.

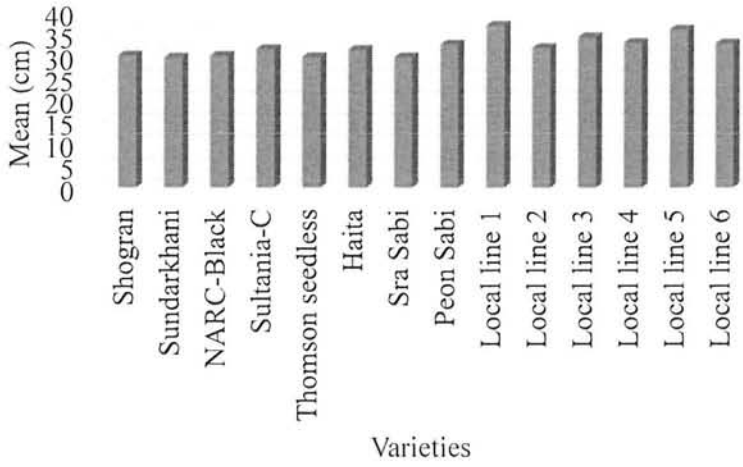

Fig. 3. 15 Graph showing mean number of leaves.

3.3.3 Standard deviation of leaves

Analysis of leaves based on standard deviation showed a much higher variation. The high values of variation were observed in Sra Sabi followed by Local line 4. The minimum deviation value was reported in Local line 5 as shown in Fig. 3.16.

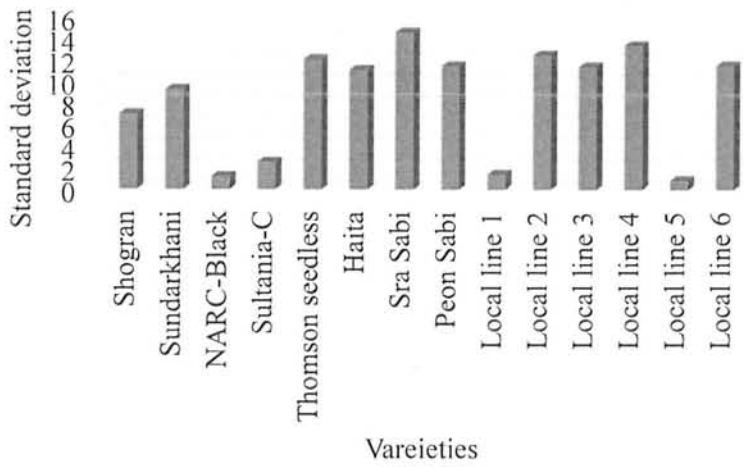

Fig. 3. 16 Graph showing standard deviation of leaves.

3.3.4 Minimum and Maximum Number of Leaves

Number of leaves for each cutting was also documented during the field experiment. The minimum number of leaves were 0 while the maximum number of leaves were 39 reported from few local lines (Fig. 3.17). Minimum and maximum number of leaves reported during the field experiment is given in the figure 3.19.

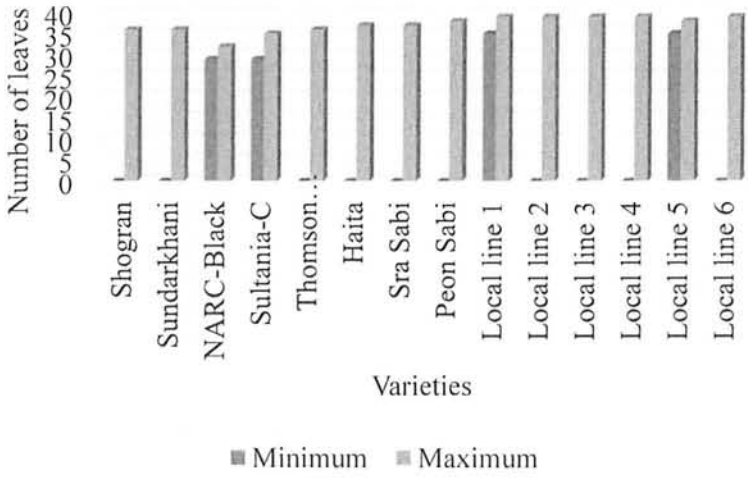

Fig. 3. 17 Graph showing the maximum and minimum number of leaves.

3.3.5 Mean Length and Width of Leaves

The mean length and width of the leaves were measured in centimeter. The cultivar Local line 5 was reported with highest mean length value of 7.575 cm followed by 7.475 cm value of the cultivar Local line 4. The minimum mean length was measured in cultivar NARC-Black which was 5.475 cm. While the highest mean width was 16.11 cm observed in cultivar Haita followed by the cultivar Local line 5 which was 9.6625 cm. The minimum value of mean width observed was 5.3 cm in cultivar Local line 3 (Fig. 3.18).

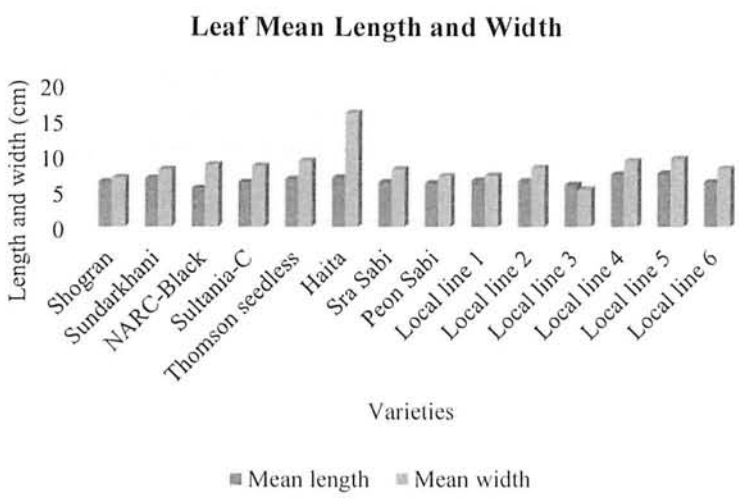

Fig. 3. 18 Graph showing mean length and width of leaves.

3.3.6 Mean length and width of Internode

The average internode length and width of the successful grape cuttings were measured. NARC-Black was reported to have the maximum internode length of 6.475 cm while the minimum internode length observed was 2.2375 cm in a local variety tagged as Local line 5. The average maximum internode width for Sultania-C was reported 8.18 cm to be the dominant while the minimum internode width of 1.264 cm was reported from cultivar Sundarkhani as shown in Fig. 3.19.

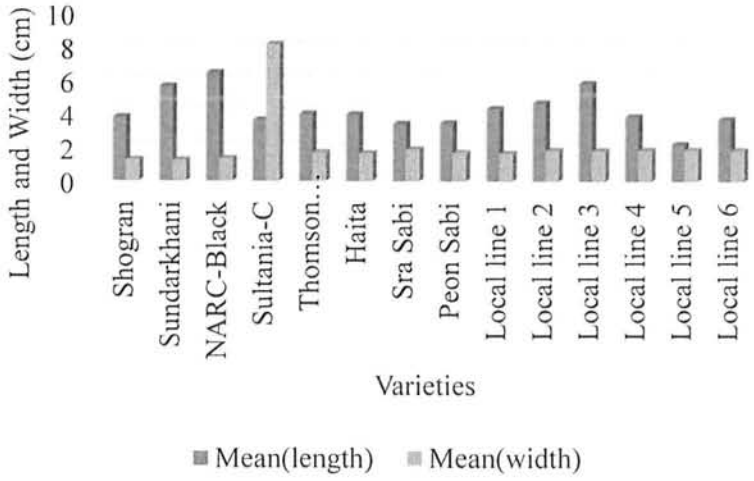

Fig. 3. 19 Graph showing mean length and width of internode.

3.3.7 Mean Length of Petiole

The Mean Petiole length was measured in cm. The maximum mean petiole length was observed in cultivar Haita which was 4.88 cm followed by Thomson seedless whose petiole mean length was 4.7 cm (Fig. 3.20). The minimum petiole mean length was reported that of cultivar Haita which was 2.8 cm. The graph below shows the mean petiole length measured in cm.

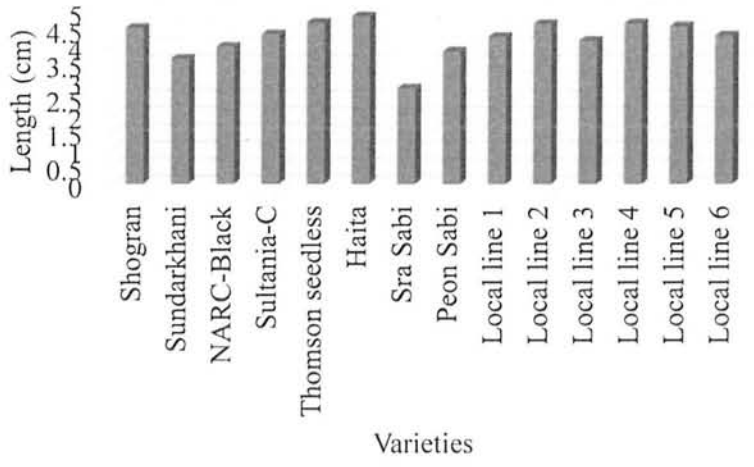

Fig. 3. 20 Graph showing the petiole mean length.

3.4 Nutritional analysis of grapes

3.4.1 pH of the Grape fruit sample

The pH was measured in 9 different grape varieties collected from Quetta and Pishin districts of Balochistan. The pH value observed in these varieties range from 3.2 to 4.1. The highest pH value 4.1 observed in cultivar Laal quetta, followed by the cultivar Sundarkhani whose pH value recorded was 4. The minimum pH value observed in cultivar Toor kishmishi was 3.2. as mentioned in fig 3.21.

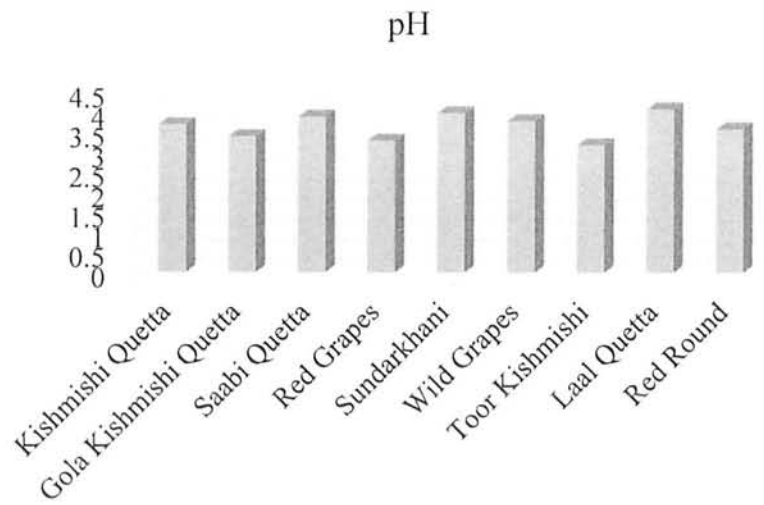

Fig. 3. 21 Graph illustrating the pH values of grape fruits.

3.4.2 Determination of Electrical Conductivity

The Electrical conductivity of 9 grape varieties was determined by using Electrical conductivity meter. The highest EC value determined was that of Laal Quetta followed by Sundarkhani which was 1210 ppm and 1120 ppm respectively. The minimum EC value was 762 ppm which was observed in cultivar Toor Kishmishi as mentioned in Fig. 3.22.

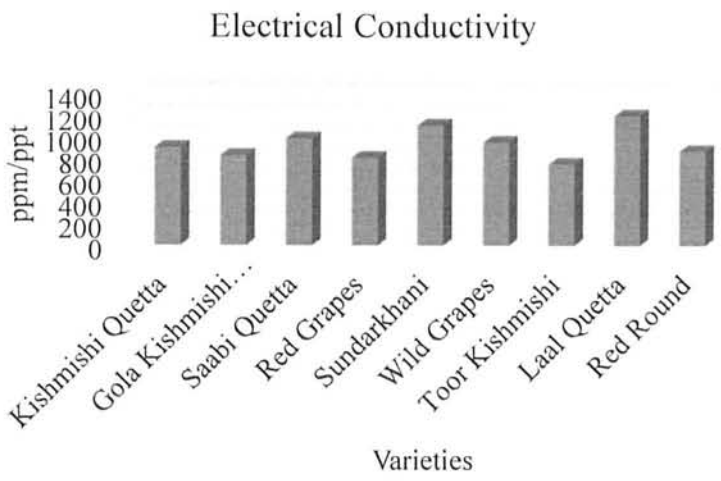

Fig. 3. 22 Graph illustrating the EC values of grape fruits.

3.4.3 Determination of Reducing Sugars and Non-reducing Sugars

The Reducing and Non-reducing sugars were determined through titration method. The highest reducing sugar value was determined in cultivar Red round which was 33.33% followed by 31.25% observed in Sundarkhani. The minimum value of reducing sugar was determined in cultivars Wild grapes and Laal Quetta 11.9% each as shown in Fig. 3.23. While in case of Non-reducing sugar the highest value determined was 55.49% which was observed in cultivar Red grapes followed by the cultivar Gola Kishmishi Quetta observed as 51.81%. The minimum value of Non-reducing sugar was 28.27% observed in cultivar Sundarkhani.

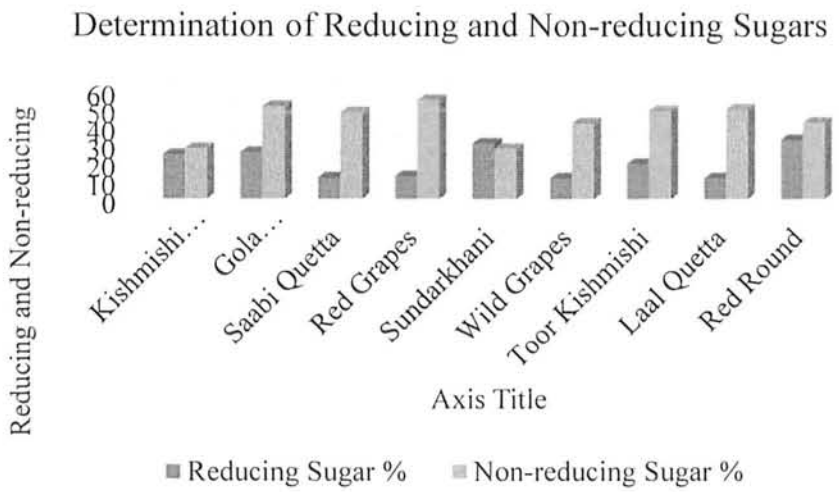

Fig. 3. 23 Graph illustrates the percentage of reducing and non-reducing sugar.

3.4.4 Determination of Vitamin C (Ascorbic Acid)

Vitamin C was determined via titration method. The highest amount of Vitamin C was observed in cultivar Kishmishi Quetta which was 3.61 mg/100g, followed by the cultivar Toor Kishmishi 3.58 mg/100g, while the lowest amount of Vitamin C was recorded in cultivars Gola Kishmishi Quetta, Red grapes, Sundarkhani and Wild grapes whose values were 3.52 mg/100g of each variety as shown in Fig. 3.24.

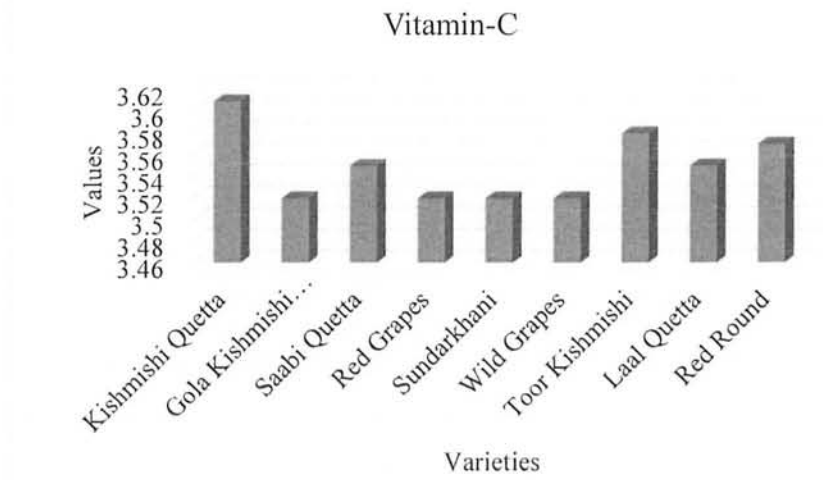

Fig. 3. 24 Graph showing the results of Vitamin C.

3.4.5 Mean value of metals in fruits

The mean value of metals was recorded for all the varieties through Atomic Absorption Spectrophotometer as mentioned in fig. 3.25, in which the highest concentration of Iron was observed for all varieties which was 0.554889 mg/kg, followed by Sodium concentration 0.227856 mg/kg. The remaining metals observed were Magnesium 0.133411 mg/kg, Calcium 0.091333 mg/kg, Copper 0.076778 mg/kg and the lowest mean value was observed for Zinc 0.052989 mg/kg.

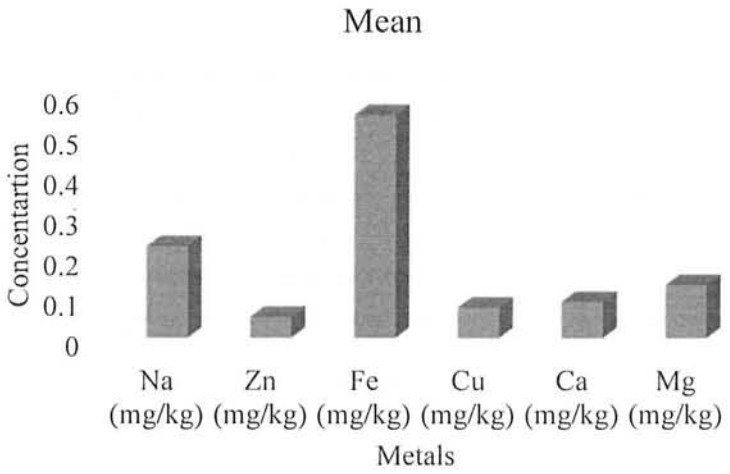

Fig. 3. 25 Illustrates mean values of metals in fruits.

3.4.6 Standard Deviation of Metals in Fruits

Standard deviation for metals concentration in fruits was also measured (Fig. 3.26). The highest value observed was that of Iron 0.178369 mg/kg followed by Sodium concentration 0.168407 mg/kg. The standard deviation of other metals were Calcium 0.120402, Copper 0.101219, and Magnesium 0.031098 mg/kg. The lowest value of standard deviation observed was that of Zinc 0.019691 mg/kg.

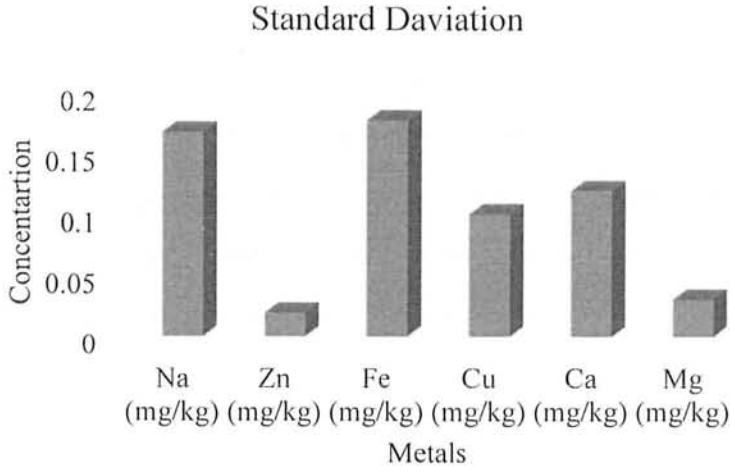

Fig. 3. 26 Graph showing the standard deviation of metals in fruits.

3.5 Nutritional data analysis by XLSTAT

3.5.1 Principle Component Analysis (PCA)

The data in which the observations are designated by numerous inter-correlated quantitative dependent variables are analyzed by principal component analysis. All the necessary informations are extracted from table by PCA and express it in the form of new orthogonal variables that is known as principal component. All the informations are displayed in the form of maps

3.5.1.1 Scree plot

The graph below (Fig. 3.27) illustrates the variation between the varieties. The total variation observed was 48.06%, in which the eigenvalue of factor F1observed was 3.017%. Similarly the eigenvalue of F2 was 2.270%, F3 eigenvalue was 2.111 and the eigenvalue of F4 was 1.297%. The eigenvalues of F5, F6, F7, F8, F9, F10,F11were less than 1%.

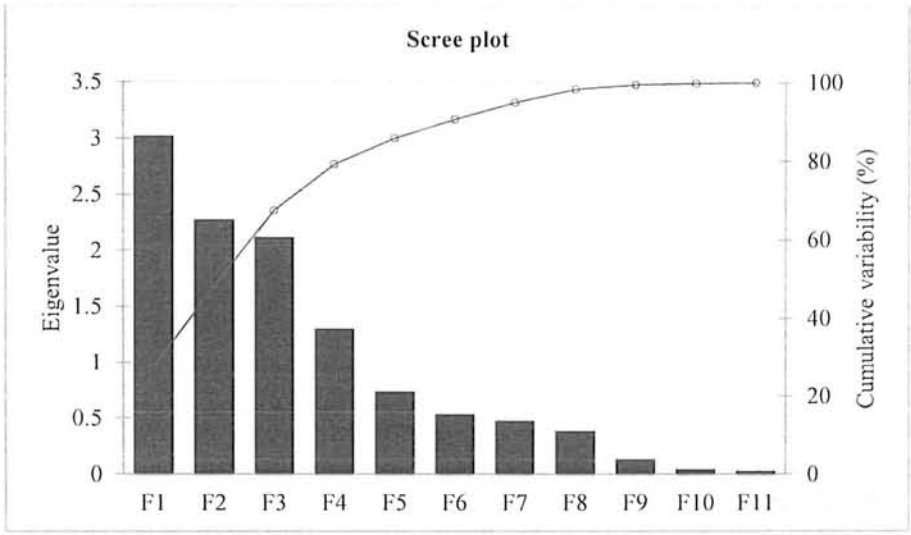

Fig. 3. 27 Graph demonstrating the scree plot.

3.5.1.2 Bi-plot between the nutrients

The bi-plot between the nutrients was observed to be 48.06% where the F1 contributed 27.43% and F2 contributed 20.63%. There was a positive correlation as shown in (Fig. 3.28) between the nutrients within a plot area and they were having negative correlation with the plot area of opposite side.

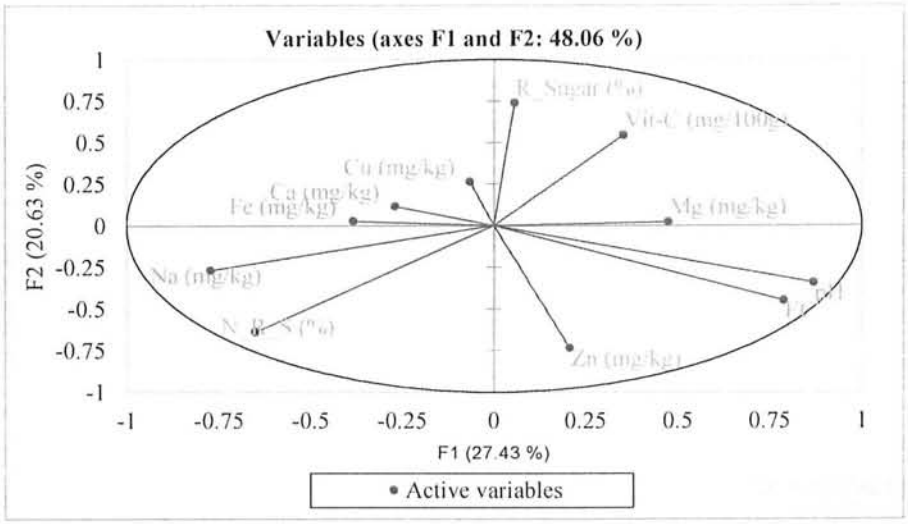

Fig. 3. 28 Graph illustrating the bi-plot between the nutrients.

3.5.1.3 Bi-plot between the varieties

The total variation among the varieties/lines was 48.06% (Fig. 3.29). The percentage of variation contributed by F1 was 27.43% whereas that of F2 was 20.63%. The varieties within a plot area showed positive correlation with each other while negative correlation was observed against the opposite side plot area.

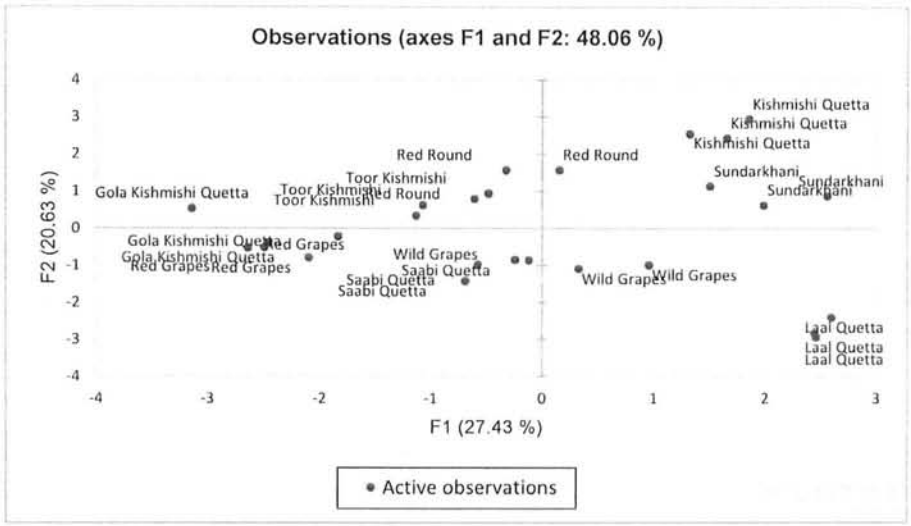

Fig. 3. 29 Graph showing the bi-plot between the varieties.

3.5.1.4 Bi-plot between the nutrients of varieties

The net variation among nutrients of varieties was 48.06% of which F1 was 27.43% and F2 was 20.63% as shown in Fig. 3.30. The varieties with nutrient lying in the same plot have positive correlation with one another. The nutrients of a plot have negative correlation with the opposite plot. Very close correlation in nutrients was observed within a plot. The graph below illustrates the correlation factor among nutrients of varieties.

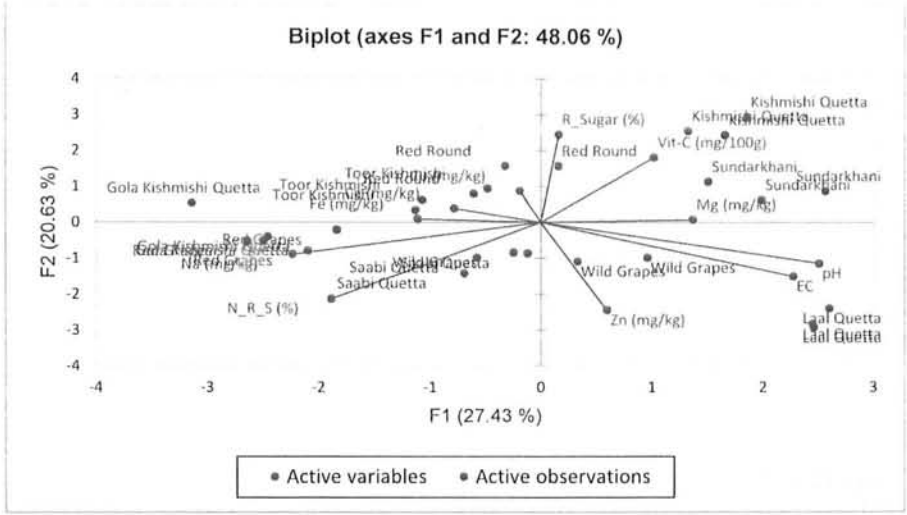

Fig. 3. 30 Graph demonstrating the bi-plot between the nutrients of varieties.

DISCUSSION

The grape plants belong to the family Vitaceae very important according to economic nutritional point view. Many important and essential nutrients, minerals, vitamins, antioxidants, fibers and water are found in a considerable amount grapes fruits (Usha, 2010). The fruits of grape plant are categorized as berries. The berries are golden, pink, purple, black, red, green or rarely white in color. The flavor also varies from sweet to bitter depending upon the variety (Uddin et al. 2011)The suitable climatic conditions are very important for quality of grapes production and their market value. The climatic condition of Pakistan varies from temperate to tropical where different type of fruits is grown (Aujla et al. 2011). Wind speed less than 4 m/s, 25°C -30°C temperature and 60-70% humidity is needed for maximum vine photosynthetic activity (Hunter and Bonnardot 2011). There are many different varieties of grapes grown in the world while in Pakistan the most leading and popular varieties are Sundarkhani, Kishmishi, Haita, Shakhali and Sahibi. The origination point of grapes cultivation believed to be Armenia near the Black and Caspian seas in Russia, from where it spread eastward to Iran and Afghanistan and westward to Europe. Grapes can be consumed in fresh as well as in dry forms, such as raisins etc. Grapes are also used to make wines on a great scale in the world where a major part of production is exported across the globe. The major threats responsible for the loss of grapes fruits are fungal diseases, post-harvest losses due to poor handling, deprived stacking, inappropriate transport system and many others.

4.1 Ethno-biological data

In the present study questionnaire surveys were conducted in Quetta and Pishin districts of Baluchistan and Bannu district of Khyber Pakhtunkhwa. The grape gardens were surveyed in this regard and different informations were collected from gardeners, i.e., varieties cultivated, preference of grape varieties for cultivation, use of fertilizers, area covered by the grapes, number of grape plants, income per year, production of grape fruits, willingness of planting grape plants.

The respondents were in different age groups ranging from 18-66 years. A well questionnaire result can be collected from the expert of that field which comes based on

experience in the field work. The gardeners in this survey were well expert as most of their life was spent in grape gardening. Experience comes through proper practicing in the field for many years, which was observed in this survey. According to Abbas *et al.* (2016), younger generation do not have much ethnobotanical knowledge as compared to elder ones because of their experiences.

Varieties reported in this study were Sundarkhani, Kishmishi, Haita, Sultania-C, NARC, Black, Sra Sabi, Khison, Shogran and Peon Sabi. The cultivar Sundarkhani is found to be the dominant variety in the Quetta and Pishin districts of Balochistan during this survey. The basics behind the preference of this variety is its good quality and taste. It was reported that Sundarkhani has great potential to survive against various diseases and it is very strong against severe heat and cold. The production rate of fruits per plant of Sundarkhani is greater compared to other varieties reported from the study area.

The people of the study area used Natural fertilizers, Urea and DAP as food materials for plants. In which the Natural fertilizers (cow, buffalo) were recorded as dominant in their usages followed by urea. The reason behind the use of natural fertilizer is their good performance observed by the people, easy availability, safe use, no side effects, low cost as compared to others and much effective. The DAP was reported to be used not more commonly. According to informants it has side effects on grape plants and fruits, high prices, not available easily.

In Baluchistan, the Quetta and Qalat divisions are considered to centers for grapes cultivation and cover 8.4 thousand hectares area (GoP, 2006). The maximum area in this survey reported was 6 hectares while minimum of area was 1hectare. The highest number of grape plants reported from a garden was 1300 whereas the lowest number was 200 plants reported during survey. Grapes are fruit crop of great economic value. It is a great source of the livelihood of most of the people of the study area. When the survey was conducting, the income per year of a garden was also recorded which revealed a maximum number of rupees 1200000 while there were some gardens which were small and number of plants were also less so their income per year was also less. A minimum total of rupees 150000 were reported which was the net income of that specific garden. So, the net finding from this can be concluded that more the number of plants in an area more will be the income per year.

The grapes production rate was also reported while conducting the survey. The maximum production rate recorded was 1840 mann from Sundarkhani while a minimum production of 20 mann was reported from cultivar Peon sabi. The main areas

for the cultivation of grapefruits in Baluchistan are Qalat and Quetta divisions where 72,800 metric tons of grapes were produced (GoP, 2006). A survey conducted by (GoP, 2006), Balochistan shares 98% production of grapes to the total net production of the country. For future cultivation of grapes, a question was asked in this regard in the present questionnaire survey. It was observed that the people were willing and interested to cultivate grapes in their lands as much as they have the space. In the survey, cultivating 5000 grape plants was the highest margin expressed by the people surveyed.

4.2 Experiment discussion

4.2.1 Survival Percentage

Survival percentage for the plants cuttings successfully survived were checked in which the cultivar Local line 1 survived successfully, the survival percentage was 100%, of which 10 cuttings were grown and all of them survived. It was followed by the Local line 6 whose survival percentage was 80% of which 50 cuttings were planted and 40 survived successfully. The remaining survived varieties was, Shogran 67%, Local line 5 was 66%, Haita and Local line 2 were 60% each, Local line 4 was 58%, Sundarkhani 54%, Sultania-C and Sra Sabi was 53% each, Thomson seedless was 50%, NARC-Black and Peon Sabi survivals were 40%, while the lowest survival percentage was observed in the Local line 3 whose survival percentage was 37%.

4.2.2 Number of Leaves

The data for the number of leaves was taken regularly. The stats were applied on the leaves and the following factors were measured. The maximum mean value of leaves was observed in Local line 1 which was 37.25 followed by the Local line 5 where mean value of leaves was 36.375, in Local line 3 mean leaves value was 34.63, Local line 4 value was 33.375, local line 6 value was 33.21, Peon Sabi 32.9, 32.1 in Local line 2, Sultania-C 31.8, Haita 31.7, Shogran 30.26, NARC-Black 30.21, Thompson seedless and Sra Sabi were 30 each, while the lowest mean value was recorded in Sundarkhani where mean value fall in 29.84. The variety whose cuttings number survived greater in number produced more leaves than rest of the varieties.

4.2.3 Leaf Size

The mean length and width of the leaves were measured in centimeters. The cultivar Local line 5 had the highest mean length of 7.575 cm followed by 7.475 cm of the

cultivar Local line 4. The minimum mean length was measured in cultivar NARC-Black which was 5.475 cm. While the highest mean width was 16.11 cm observed in cultivar Haita followed by the cultivar Local line 5 which was 9.6625 cm. The minimum value of mean width observed was 5.3 cm in cultivar Local line 3. The reason behind the highest mean length of cultivar is its adaptation with the local environment. As the local lines were collected within the Bannu District, so they are well adapted to their native environment. The temperature of District Bannu is high in summer as compared to Quetta and Pishin Districts of Balochistan. As well as the winters are also cold and harsh. According to (Mullins et al. 1992), the grapes growth is inhibited by the severe heat conditions, lack of water and scanty winter chilling.

4.2.4 Average number of Nodes

The highest average number of nodes reported were 13.4 from a local cultivar tagged as Local line 1 which was followed by the cultivar NARC-Black in which 13 nodes were reported. The mean number of nodes of the remaining cultivars Sultania-C, Shogran, Local line 6, Local line 3, Peon Sabi, Local line 2, Sundarkhani, Thompson seedless, Haita, Local line 4, local line 5 were 12.71 cm, 12.17 cm, 12.12 cm, 12 cm, 12 cm, 11.88 cm, 11.85 cm, 11.7 cm, 11.7 cm, 11.4 cm, 11.4 cm respectively. Whereas the cultivar Sra Sabi produced the lowest number of nodes which were 11 in number. The highest number of nodes were produced in a local cultivar which proves its suitability with the environment. The second highest number of nodes were reported from cultivar NARC-Black which was brought from Quetta. Its second largest number of nodes indicate its adaptations with the climatic conditions of Bannu. So, it can be concluded that NARC-Black can survive best in the climate of District Bannu.

4.2.5 Mean length and width of Internode

The maximum size of internode length was reported from NARC-Black which was 6.475 cm while the maximum size of internode width was observed in cultivar Sultania-C which was 8.18 cm. The minimum length of internode was reported from a local cultivar that was named as Local line 5 having internode length of 2.2375 cm and the minimum internode width was observed in cultivar Sundarkhani which was 1.264 cm. The NARC-Black shows the highest mean length of internode indicating its suitability with the climatic conditions of District Bannu. Sultania-C can also be adapted in this type of climatic condition.

4.2.6 Mean Length of Petiole

The petiole's mean length was also measured during the survey where the highest mean length was observed in cultivar Haita which was 4.88 cm followed by the mean length of 4.7 cm of cultivar Thompson seedless. The lowest mean length was observed in cultivar Sra Sabi which was 2.8 cm. Its lowest value shows the maladaptation of cultivar Sra Sabi with the climate of Bannu. The cultivars Haita and Thompson seedless could be proved best for cultivation in District Bannu as they have the highest mean length of petiole.

4.3 Nutritional Discussion

4.3.1 pH

The maximum negative log of hydrogen was determined in cultivar Laal quetta which was 4.1 while the minimum pH value was observed in cultivar Toor kishmishi whose value fall in 3.2. There was no such difference observed in the pH values of the grape fruits. The pH values observed in all the 9 cultivars were normal in value. The normal values of pH indicate that the varieties are well adapted to their native environment. Less the pH value more the fruit will be bitter (acidic) while more the pH value basic will be the fruit. So, neither acidic nor basic range is acceptable, it should be in a range as observed in the cultivars analyzed. The pH value contributes in the consumption of grape fruits by the consumers.

4.3.2 Electrical Conductivity

The highest range of Electrical conductivity observed was 1210 ppm in cultivar Laal quetta whereas the minimum EC value recorded was 762 ppm in Toor kishmishi. The EC values fall in approximately the same range which indicates the closeness of each variety with one another and with the climatic conditions.

4.3.3 Reducing and Non-reducing sugar

The reducing and non-reducing percentage was observed for all the varieties via titration method. The highest value in terms of reducing sugar reported was 33.33% in cultivar Red round while the highest percentage for non-reducing sugar was reported in cultivar Red grapes which was 55.49%. The minimum percentage for reducing and non-reducing sugar were 11.9% and 28.27% which were observed in cultivars Wild grapes and Sundarkhani respectively. In both the cases (reducing and non-reducing) the content

of non-reducing sugar was higher than that of reducing sugar values. (Droby and Lichter 2007), also reported the same results in which the non-reducing values were higher than reducing sugar values.

4.3.4 Vitamin C

The highest amount of Vitamin C was observed in cultivar Kishmishi quetta which was 3.61 mg/100g while the lowest total of Vitamin C determined was 3.52 mg/100g observed in cultivars wild grapes, red grapes, gola kishmishi quetta and Sundarkhani each. The values were coinciding with each other very closely. According to (Droby and Lichter 2007), drying temperature decreases the content of Vitamin C in dried fruits.

4.3.5 Mean values of Metals

The concentration of metals was determined for all the varieties of collected grape fruits. The maximum concentration observed was that of Iron whose recorded value was 0.554889 mg/kg while the lowest concentration was 0.052989 mg/kg of Zinc. Copper, Magnesium, Sodium and Calcium were 0.076778 mg/kg, 0.133411 mg/kg, 0.227856 mg/kg and 0.091333 mg/kg in concentrations respectively. Alcoholic fermentation occurs with the help of many metals. Through preserving the ionic balance and tolerable pH the metabolism of yeasts is modified by metals like Mg, Ca and Na (Pawel pohl, 2007).

4.3.6 Standard Deviation of Metals

The standard deviation of metals in fruits was determined by statistics. The highest value of standard deviation was recorded in Iron whose value was 0.178369 mg/kg. The standard deviation of remaining metals Magnesium, Calcium, Sodium, Copper were 0.031098 mg/kg, 0.120402 mg/kg, 0.168407 mg/kg, 0.168407 mg/kg respectively. The lowest standard deviation value observed was 0.019691 mg/kg of Zinc. There is no such difference between the values of standard deviation in metals.

Conclusion

The present work was conducted for the ethno-biological and nutraceutical evaluations of some grape cultivars from Quetta, Pishin and Bannu regions of Pakistan. The ethno-biological study showed that the grapes are the most economical and quality fruit crop. In this study a total of 45 questionnaire surveys were conducted in Quetta and Pishin. The highest preferable cultivar reported was Sundarkhani which was reported from 23 grape gardens. The second most demanded cultivar was Kishmishi, observed in 22 grape gardens. These most demanded cultivars were good in taste, production rate and economy. So, the gardeners preferred them more. The cultivar that was least observed in gardens was Peon Sabi, that was reported from only one grape garden. According to informants this variety was not good in taste as well as economically. So, they did not prefer this cultivar. The production rate of cultivars Sundarkhani and Kishmishi were also high as compared to other varieties. The people preferred Natural fertilizers for their grape plants as food. It was observed from the that natural fertilizers have no side effects and are very good for plants as compared to Urea and DAP. People of the study area showed their high willingness for cultivation of grape plants in future. The mean pH value of soil remained 6.24 which is best for the cultivation of grapes. In experiment the mean number of leaves recorded was 37.25 which was observed in a local cultivar tagged as local line 1. The highest value of standard deviation was 14.71 found in Sra Sabi. The maximum number of leaves were 39. The nutritional analysis showed that the pH range was 3.2 to 4.1. The higher value of EC recorded was 1210 ppm. The highest values of reducing and non-reducing sugar were 33.33% and 55.49% respectively. The highest value of vitamin C was observed in Kishmishi quetta which was 3.61 mg/100g. It is concluded that the country is bestowed with such climatic conditions that are well suited for grapes cultivation. Grapes play a key role in the food values, economic values, ornamental purposes, ethno-biological uses and other daily basis needs.

Recommendations

The current research work suggests that;

- Grapes cultivation should be carried out in the FR Bannu and other areas of Ex-FATA;
- To find out more suitable grape cultivars for cultivation in FATA region
- To overcome the losses due to terrorism
- To aid in the economic conditions of the FATA belt through cultivation of grapes
- To aware the local people about the domestication of grapes in the affected areas
- To help the local communities of FATA to come out from severe conditions from last few years.

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Appendix Table 1: Questionnaire carried out during current research project.

I am Abdul Rehman, M.Phil. Scholar at Quaid-i-Azam University, Islamabad (Plant Ecology and Conservation Lab) Department of Plant sciences. I am working on my M.Phil. research, please fill the questionnaire as it will help me conducting a quality research work.

Name: District:

Age: Tehsil:

Gender: Village:

Contact No: CNIC No:

1) Total area of grapes of grapes cultivation_____
2) Number of plants. _____
3) Age of plants (how old)

Number of Plants	Age of plants

4) Varieties

Local Names	Other Names

5) Number of irrigations_____
6) Fertilizers/ per plant/ area
   a) Natural b) Urea c) DAP d) other

Fertilizers Used	Quantity
Natural	
Urea	
DAP	
Others	

7) Production/season

Verities	Kg/tons

8) Time of pruning

Pruning	Time

9) Age of plant for pruning

Plants	Age

10) Time of fruits/thining.....

11) Procedure of pruning/thining

Tools Used	Tick (✓)
Knife	
Scissor	
Cutter	

12) Support

Material Used	Age of Plants

13) Time of harvesting

Plants Varieties	Harvesting Times

14) Packing- materials other cares

a) Krates b) grass c) paper d) others

15) Other post-harvest measures

16) Parts Used

17) Issues facing

(a) Insects (b) Diseases (c) Rodents (d) Weather (e) Weeds/climbers (f) Others

18) Income per year

19) Income increase/decrease/maintain

20) Replacing plant (incase income decreases)

21) Soil sample

22) Weather data/other data

a) GPS b) humidity c) Aspect

23) Pictures (garden, plant, Leaves, Flower, Fruits etc.

24) Would you like to develop garden of vine. Yes / No

25) If yes how many Plants.

Appendix Table 2: Soil physicochemical analysis of all the stations.

S.No.	pH	EC (ppm)	TDS	Ni (mg/Kg)	Cr (mg/Kg)	Na (mg/Kg)	K (mg/Kg)	Zn (mg/Kg)	Fe (mg/Kg)	Cu (mg/Kg)	Mn (mg/Kg)	Ca (mg/Kg)	Mg (mg/Kg)	Cd (mg/Kg)	Pb (mg/Kg)
1	6.9	120	84	0.4	5.7	224.0	273.4	5.1	37.0	8.1	47.4	369.6	439.3	23.4	51.2
2	6.3	220	154	34.7	8.5	230.5	334.2	3.4	64.5	8.6	85.3	403.7	434.2	24.1	193.6
3	6.2	230	161	13.8	12.6	318.2	258.2	14.3	67.5	11.0	91.0	316.5	345.0	16.5	242.4
4	6.7	190	133	55.0	101.0	245.6	315.1	6.6	66.5	15.5	91.7	288.8	356.9	1.2	29.6
5	6.1	100	70	25.9	58.0	159.3	294.9	10.8	56.9	7.2	80.9	353.5	367.3	7.0	72.8
6	5.9	130	91	6.5	23.3	122.0	307.4	7.2	93.0	5.4	79.0	389.1	429.5	2.9	78.4
7	6.8	180	126	25.7	32.2	53.8	196.1	10.2	75.7	7.4	78.5	333.8	314.5	6.4	59.2
8	6.7	250	175	79.8	3.2	13.0	277.2	11.9	70.5	5.9	94.2	279.5	332.6	9.7	140.0
9	6.6	270	189	17.2	75.0	9.7	283.7	7.3	68.4	3.8	94.4	323.0	406.2	10.4	83.2
10	6.3	250	175	51.8	12.1	14.0	284.0	10.8	60.9	2.6	90.2	410.7	449.4	7.0	69.6
11	6.1	230	161	23.8	93.8	108.8	255.2	5.7	77.8	3.4	80.7	282.8	261.9	2.6	168.0
12	6.2	200	140	12.0	71.0	20.6	233.5	7.7	89.8	5.1	77.9	372.0	352.2	52.2	269.6
13	6.6	140	98	77.7	85.0	22.0	81.5	3.9	55.8	2.2	98.8	432.4	329.1	105.6	90.4
14	6.6	130	91	27.6	66.1	37.8	104.2	6.0	54.4	1.0	129.8	424.6	267.5	19.5	79.2
15	6.5	110	77	8.0	31.0	45.1	11.2	0.5	64.0	4.3	77.5	334.6	214.6	63.0	188.0
16	6.7	100	70	89.5	63.7	83.7	101.7	6.2	23.0	1.7	96.6	348.2	330.3	60.1	323.2
17	6.8	90	63	7.6	100.3	79.1	44.5	3.7	11.2	2.2	100.1	295.8	255.6	54.0	180.0
18	6.3	290	203	22.9	84.9	63.5	56.4	2.3	64.3	2.6	86.9	278.2	240.9	28.2	57.6
19	5.8	270	189	7.3	81.2	104.1	251.7	9.5	71.8	3.9	85.7	304.5	351.3	29.4	960.8
20	5.9	300	210	62.2	96.2	93.8	257.9	7.7	46.6	2.6	74.9	294.1	278.6	15.5	27.2
21	5.8	320	224	47.5	82.5	107.9	259.6	13.2	46.9	2.0	93.7	483.4	442.0	6.2	22.4
22	5.6	220	154	17.9	62.5	18.4	253.9	20.3	68.1	7.5	88.5	291.8	312.2	20.4	120.0

23	5.6	210	147	21.0	6.9	2.1	281.4	6.1	40.4	4.3	78.3	360.3	323.8	7.0	8.8
24	5.6	280	196	1.7	59.6	6.6	239.4	7.7	60.5	3.1	79.8	296.4	293.1	-5.8	15.2
25	5.5	170	119	19.9	84.1	17.1	228.9	8.0	44.1	1.7	71.4	364.8	318.9	22.3	102.4
26	5.2	200	140	12.3	82.0	324.6	290.9	17.2	61.0	2.2	89.2	382.9	412.2	13.8	14.4
27	6.1	310	217	86.6	76.2	365.0	247.1	16.2	64.1	2.7	82.3	352.2	401.7	177.4	238.4
28	5.7	100	70	57.4	64.0	17.1	243.7	10.9	95.3	3.0	79.1	429.9	455.3	39.8	292.0
29	5.1	350	245	21.3	73.8	389.5	257.1	3.1	90.2	3.5	73.0	425.2	416.7	139.5	89.6
30	5.9	280	196	57.0	58.7	245.4	236.0	8.4	72.7	4.4	76.9	342.3	321.1	18.9	13.6
31	5.5	220	154	75.6	85.7	155.6	79.4	1.4	58.6	4.3	97.8	636.6	419.7	41.4	83.2
32	5.4	130	91	28.3	78.4	135.5	91.9	1.7	60.1	3.0	94.6	394.8	303.5	163.3	87.2
33	7.3	110	77	33.4	109.0	86.1	18.3	6.7	50.5	3.9	121.0	657.4	380.2	126.4	5.6
34	7.1	250	175	25.5	109.6	1.2	119.4	6.9	78.6	0.7	101.8	361.0	330.3	92.1	81.6
35	7.5	200	140	41.1	78.2	6.7	58.3	12.2	80.5	5.9	89.5	393.6	331.0	67.9	92.0
36	6.8	330	231	18.6	36.0	23.2	71.3	6.7	115.4	5.7	92.1	367.2	334.8	49.0	57.6
37	6.9	370	259	41.2	35.3	2.5	117.8	7.0	66.8	5.4	94.2	511.4	393.3	195.8	56.0
38	6.6	350	245	12.0	10.4	9.7	122.0	7.3	77.0	4.3	92.4	397.3	340.0	54.6	86.4
39	6.8	330	231	0.3	33.5	3.7	261.5	10.2	69.1	3.6	80.0	413.3	423.7	72.7	76.0
40	6.4	260	182	52.5	29.3	24.1	292.7	8.6	55.1	3.9	91.3	430.2	464.9	19.7	244.0
41	6.7	170	119	27.4	119.8	2.9	265.4	8.3	77.0	3.9	82.0	323.9	311.8	39.5	80.8
42	6.9	250	175	13.1	80.2	0.7	172.5	7.0	58.3	6.4	67.5	416.8	395.7	26.9	26.4
43	6.6	340	238	44.2	47.7	2.3	217.0	9.7	89.6	3.2	79.8	481.0	389.9	36.6	136.8
44	5.5	350	245	4.8	89.5	38.8	863.0	0.0	53.3	3.1	5.8	4.2	11.2	23.2	0.8
45	4.9	250	175	13.8	29.3	3.5	898.4	8.7	63.8	1.4	2.5	5.5	10.3	34.5	57.6

Ethno-biological and nutraceutical evaluations of selected grape cultivars from Quetta, Pishin and Bannu regions of Pakistan

by Abdul Rehman

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