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Impact of alternate bearing on proximate composition of *Olea ferruginea* Royle fruits collected from Zhob, Balochistan, Pakistan

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Abstract

Olives are the only species of the family Oleaceae with edible fruits and thus, these fruits due to their properties as food and oil have found a significant value. These plants however exhibit a pattern of alternate bearing which affect fruit yield during consecutive years. The present study, therefore, was designed to compare the nutritional status of *Olea ferruginea* Royle fruits in terms of proximate and elemental composition collected during on-year and off-year of fruit-bearing. Proximate (moisture, ash content, crude protein, crude fiber, crude fat, organic matter, total carbohydrate) composition was quantified and compared for alternate bearing. Results of the proximate analysis showed a relatively higher percentage of moisture content, organic matter, Crude protein and crude fat in on-year samples whereas Ash content, Crude fiber and total carbohydrate content were documented in higher amounts in off-year samples. Analysis of variance however depicted a significant difference ($p \leq 0.05$) between percentages of the carbohydrate content in consecutive year samples. These results emphasized the fact that lower yield during off-year may reinforce the accumulation of certain elements in olive fruits and thus, may be considered a good source of elemental supplements and mineral fertilizer. However, overall findings agreed to the *O. ferruginea* fruits may serve as a good source of nutrition and thus, sustainable conservation of this wild olive specie is highly recommended.

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Introduction

Olive trees with approximately six hundred species are the only members of the family Oleaceae with edible fruits (Raman and Shukla, 2017). *Olea ferruginea*, is locally known as Indian olive or Kahoo (Ashraf *et al.,* 2016). It is one of the most important medicinal and edible plants commonly spread in the diverse areas of Pakistan, such as Swat, Azad Kashmir, Waziristan, Dir, Chitral and the western mountains of Balochistan (Ahmed *et al.,* 2009). There are about 30 to 40 species of the genus Olea are largely distributed in Asia, Africa, and the Mediterranean Basin (Besnard *et al.,* 2002). Different parts of *O. ferruginea* tree are traditionally used for various purposes such as the leaves being used for the treatment of bleeding gums, cough, skin problems, and toothache. Among other parts, the bark is used for fever while dried fruit has been used as an antidiabetic medicine. The oil has been used for several purposes including pain relief and swollen muscles (Hashmi *et al.,* 2014). Apart from these traditional uses the fruits of *O. ferruginea* are used as a source of food in different forms and also used as an appetizer and emmenagogues. Fruits are also a source of oil which efficiently serve as a source of oleic acid and is used to treat scabies, typhoid, eye burning, jaundice, biliousness, toothache and teeth caries (Ahmad *et al.*, 2009; Zabihullah *et al.,* 2006).

The olive tree, however, shows an unexceptional behavior called Alternate bearing which affects fruit yield during consecutive years. Alternative bearing is a phenomenon where there is a high-yield crop in one year (on-year) followed by a low-yield crop in a subsequent year (off-year). Due to the alternate bearing pattern of plant species, overall yield property may get affected (Lavee, 2007). This may occur on fruit trees of different types that have shown alternate bearing properties. The phenomena of the alternate bearing are dramatic in the olive tree, it has been observed that the olive yield shows a biennale (two-year) developing cycle (Monselise and Goldschmidt, 1982). In olives, the biennial behavior inhibits the floral induction of seed-developing fruits. In the olive tree, the alternating bearing process is largely determined by a load of flowers if the tree produces a large number of flowers in one year then in the next year very few numbers of the flower are seen.

The biennial trees such as olives, can cause serious problems in the agricultural propagation of fruits and also the breeding period (Levin and Lavee, 2005). According to Kour *et al.*, (2018), the main factors responsible for alternate bearing are carbohydrate storage, flower site limitation and endogenous plant growth hormones. Apart from factors causing alternate bearing, the phenomenon may also affect the quality of fruits during consecutive years. According to Turktas *et al.*, (2013), the alternate bearing of olive shows a strategic mechanism to save the nutritional reserves for the survival of biotic and abiotic stress in the environment and is also significant for the vegetative growth and also face the micronutrient and macronutrient deficiency in a harsh climate. It is now suggested that an “on” year in the olive tree later reduces the energy reserves of olives with the highest energy growth and development and accumulation of fruits and oils.

O. ferruginea Royale is a commercially underutilized species of naturally occurring olives of Zhob. *O. ferruginea* has small-sized edible fruits which are locally used in the form of pickles in daily routine. These fruits are also utilized as appetizers, antidiabetics and emmenagogues. This evergreen widespread tree species, however, exhibit an alternate bearing pattern that affects fruit yield during consecutive years and thus, the present research aimed to establish the effects of alternate bearing on the nutritional status of *O. ferruginea* fruits. To attain this prime goal study was conducted to evaluate the proximate and elemental composition of olive fruits collected during two consecutive years. These findings may help formers and olive growers to decide adequate quantities and ratios of different fertilizers during consecutive years of biennial bearing for cultivation whereas in wild olives nutritional prospects may help as a nutraceutical agent to acquire mineral nutrition depending upon the number of minerals available in fruits of alternate years.

Materials and methods

Study area

Zhob is a district in the northwest of the Balochistan province of Pakistan. The population of Zhob district is estimated at 310,544 in 2017. The total area of Zhob district is 20,297km² (7,837 sq ml). The main language of district Zhob is Pashto. Zhob has a semi-arid climate, its rainfall being high enough to avoid the arid climate category found at lower elevations. Zhob district receives monsoon rainfall. Zhob is known for the natural production of olive trees. The agro-ecological zone in which olive trees occupy 126,719 hectares. It is substantial for cultivation and fine recognized for its logically happening olive wooded area residents. Olea and its numerous constituents are valued for its efficient nutrients and the bioactive nutrient components which stimulate strength (Ghanbari *et al.,* 2012). The *Olea ferruginea* as fruit is eatable, stored, used for tastes, against diabetics and also has emmenagogue stuff. Olives fruits oil is effective in oleic acid, established as a treatment for jaundice, typhoid, scabies, teeth cares, scabies, vomiting and toothache (Zabihullah *et al.,* 2006). Garden-fresh fruitlet of Olive in the midsummer period are calm, and dry and are also recommended for diabetics in wintertime for decreasing glucose levels in the blood (Ahmad *et al.,* 2009).

Data collection and processing

In this study, Fruit samples of *olea ferruginea* Royle were collected for two consecutive years in order to get representative samples of on-year and off-year (alternate bearing) from Zhob district of Balochistan. Samples were collected by opting for the technique of Simple Random Sampling (SRS). During each year sampling was collected and, washed carefully with tap water and distilled water thrice in order to remove any dust and contamination. Fruits samples then, were shed dried and preserved in zip-lock bags prior to further analysis.

Analytical procedure

To evaluate the nutritional worth of fruit samples certain analytical procedures were performed.

Preparation of fruit samples

To perform laboratory analysis, as per requirements of the selected protocol dried fruit samples were powdered with the help of a ceramic pestle and mortar to avoid the sample from metal contamination.

Proximate analysis

This technique helps to elaborate the quantities of many nutrients in fruit samples.

Moisture content

The moisture content of fruit samples was determined by taking 2 grams of plant samples in separate Petri dishes and placing them in the electronic oven at 105°C for 4-6 hours and then transferring them to a desiccator for 30 minutes to cool down. Then the sample is weighted to find the moisture content (%) by using the formula.

%Moisture = X/ wt of sample ×100

Ash content

At least one to 2 gm of plant samples were combusted in the Muffle Furnace at 550-600° C for a period of 8 hours and ash contents of fruit samples were estimated (ref) by using of the following formula:

Ash % = Weight of Ash/ Weight of samples x 100

Crude protein

The micro kjeldahl technique was used to determine the crude protein in the fruit samples (A.O.A.C, 1984).

This procedure involves three steps i.e., the process of Digestion Distillation and Titration procedure will start from the digestion of 1 gram of sulfuric acid in each sample will be added to each digestive tube., digestion will be persuaded by the strain of the digested samples when nitrogen is added to a 4% boric acid solution from each specimen. The standardized H_2SO_4 titration against 0.02 will eventually be titrated with the help of a semi-automatic titrator.

Crude Protein % = (ml H_2SO_4 - blank) × N ×100 × 6.25 × 14.01/ Sample weight × 1000

Crude fiber

Using AOAC technique (1990) crude fiber was extracted from the samples and used to determine fat-free content or organic residue. The material was poured into a beaker and1.25% H_2SO_4 was added to heat 200ml. The solution was boiled for about 30mins, and hot water was added to the solution for maintaining a constant amount of acid, the mixture was then, filtered as it heats up. Bucker flask funnel draped with Whatman filter. With the help of boiling water, the residue was washed several times and transferred back into the beaker. Then add 1.25% Na_2SO_4 (200ml) per heat and heat for another 30 minutes after which the residue was dried at 650°C at least for 24 hours and weighed. The residue in the crucible was placed in a muffle furnace (400-600°C) and burned for 4hrs, and desiccators were used for cooling the resultant ash and weighed by using the following formula:

% Crude fiber = W2-W1/ Wt of the sample ×100

Crude fat

Two to three grams of sample was filled in filter paper (extraction thimble) and placed in Soxhelt apparatus (Zarnowski and Suzuki 2004) in an extraction chamber. A dried and clean bottom flask of 250 ml filled with Petroleum ether. The bottom flask was connected to an extraction tube containing a thimble and the apparatus was run for 5 to 6 hours. The extraction of solvent in the bottom flask was evaporated by using a water bath then reweighted the crude fat (%) was calculated by using the following formula (AOAC 2000).

%Crude fat = (Wt of flask + extract –tera wt of flask × 100)/ Wt of sample.

Organic matter

Organic matter is composed of compounds with oxygen, carbon, nitrogen and hydrogen. It was calculated by applying the following formula (Galyen, 1985).

Organic Matter % (Dry matter basis) = 100–ash

Total carbohydrate

Carbohydrates contents were calculated by subtracting the sum of the weights of crude proteins, crude fibers, ash and moisture content from 100. Carbohydrates (%) =100 – (protein + crude fiber +ash + moisture contents).

Statistical analysis

The analysis was performed in triplets. Standard deviation and mean were calculated from the given values.

The statistical significance between the samples for proximate analysis was calculated by analysis of variance (ANOVA).

Results and discussion

Wild edible plants may help to provide an amazing amount of nutrition to the surrounding local communities and at a very low cost due to their availability and accessibility. These nutritional attributes of wild plants are a direct relation to promoting the growth and development of consumers and are usually measured by percentages of protein, carbohydrates and other elements present (Waziri & Saleh, 2015). *Olea ferruginea* Royle is one of those trees which a pattern of alternate bearing and according to *Kour et al.,* (2018), the main factors responsible for alternate bearing are flower-site limitation, endogenous plant growth hormones and carbohydrate storage.

This pattern not only affects quantity but may also interfere with the quality of fruit. Therefore, in the present work; an attempt was made to highlight the effects of alternate bearing on the nutritional status of *Olea ferruginea* Royle fruits collected from Zhob district of Balochistan Province. To evaluate the nutritional status of fruits, the results were quantified into two categories of Proximate and elemental analysis.

Table 1. Proximate analysis of *O. ferruginea* fruit samples collected during alternate years of fruit-bearing from the study area.

Sr no	Plant Specie	Alternate Years of fruit bearing	Parameters (%)						
			Moisture (%)	Ash (%)	Crude fat (%)	Crude fibers (%)	Crude proteins (%)	Organic matter (%)	Carbohydrates (%)
1	*O. ferruginea*	A	8.40 ± 0.61^{a}	2.40± 0.61^{a}	14.0 ± 1.00^{a}	33.33 ± 9.91^{a}	14.370 ± 5.25^{a}	97.6 ± 0.61^{a}	4.7 ± 0.61^{a}
2		B	7.80 ± 1.21^{a}	3.0 ± 0.61^{a}	10.0 ± 1.10^{a}	40.0 ± 5.00^{a}	12.770 ± 0.58^{a}	97.0 ± 2.00^{a}	8.4 ± 0.61^{b}

A= on year B= off year Mean ± Standard Deviation, N=3 Mean values followed by different superscripts within a column differ significantly at P≤ 0.05.

Proximate analysis

The results of proximate analysis help to associate the quality of the sample by providing logical and basic information (Bashir *et al.,* 2017). These results were recorded in percentages intended for olive fruit samples (Table 1). The moisture content listed in the most detailed data in *Olea ferruginea* on-year samples was 8.4% while minimal concentration was detected in *Olea ferruginea* off-year samples was 7.8 %. These findings were relatively low when compared with the moisture content percentage recorded by other researchers in fruit samples of other wild olives (Gulfraz *et al.,* 2009, Rafaqat *et al.*, 2020).

Lower moisture content may be because smaller fruit size of *Olea ferruginea* in the present work. However, results were non-significant (P≤0.05) for moisture content for consecutive years of fruit-bearing.

Table 2. Factorial analysis of variance (ANOVA) for proximate analysis *O. ferruginea* fruit samples collected during alternate years of fruit-bearing from the study area.

D.F	Replicates		Moisture (%)		Ash (%)		Fat (%)		Fibers (%)		Protein (%)		Organic matter (%)		Carbohydrates (%)	
		Alternative years	F	P	F	P	F	P	F	P	F	P	F	P	F	P
1	3		0.4	0.59	0.85	0.45	16	0.05	5.54	0.14	0.31	0.63	0.17	0.72	111	0

Among other parameters, the total amount of minerals in a sample can be represented by inorganic residues quantified as ash material (Keta-JN *et al.,* 2018). However, in the case of edible plants, it is preferred that ash content should not be more than 2.5% because lower ash values indicate low mineral quantity yet high energy values (Nwaogu *et al.,* 2009). In the overall results, the ash content was higher in *Olea ferruginea* off-year samples (3.0%) and was reported low in samples collected during on-year (2.4%). These values indicated that off-years not only exhibited lower yield but fruits were also found to be under desired energy levels compared to on-year samples. Relatively lower percentages were measured in other wild olive fruit samples (Gulfraz *et al.,* 2009; Rafaqat *et al.*, 2020). Further analysis showed a non-significant difference (*p*≤0.05) between olive fruit samples for ash values.

Unlike ash content, the percentage of crude protein was found to be relatively high in *Olea ferruginea* on-year samples (14.37%) as compared to off-year samples (12.77%). These results of the crude protein when compared to available literature, showed a relatively higher percentage (Gulfraz *et al.,* 2009 ; Rafaqat *et al.*, 2020). Although, the present work reported a good percentage of crude protein data deciphered a non-significant difference (P≤0.05) between on-year and off-year samples.

High values of crude fat are significantly important because unusual energy is stored in the form of raw material which can be used by body cells after utilization (Odedire and Babayemi, 2008). Among

Olea ferruginea samples alternate bearing patterns of fruits depicted a difference yet non-significant (P≤0.05) in crude fat content as well. It was found that *Olea ferruginea* on-year samples had more fat content (14.0%) than off-year samples (10.0%). Comparatively, higher amounts were measured in other wild olive fruits (Gulfraz *et al.,* 2009, Rafaqat *et al.*, 2020).

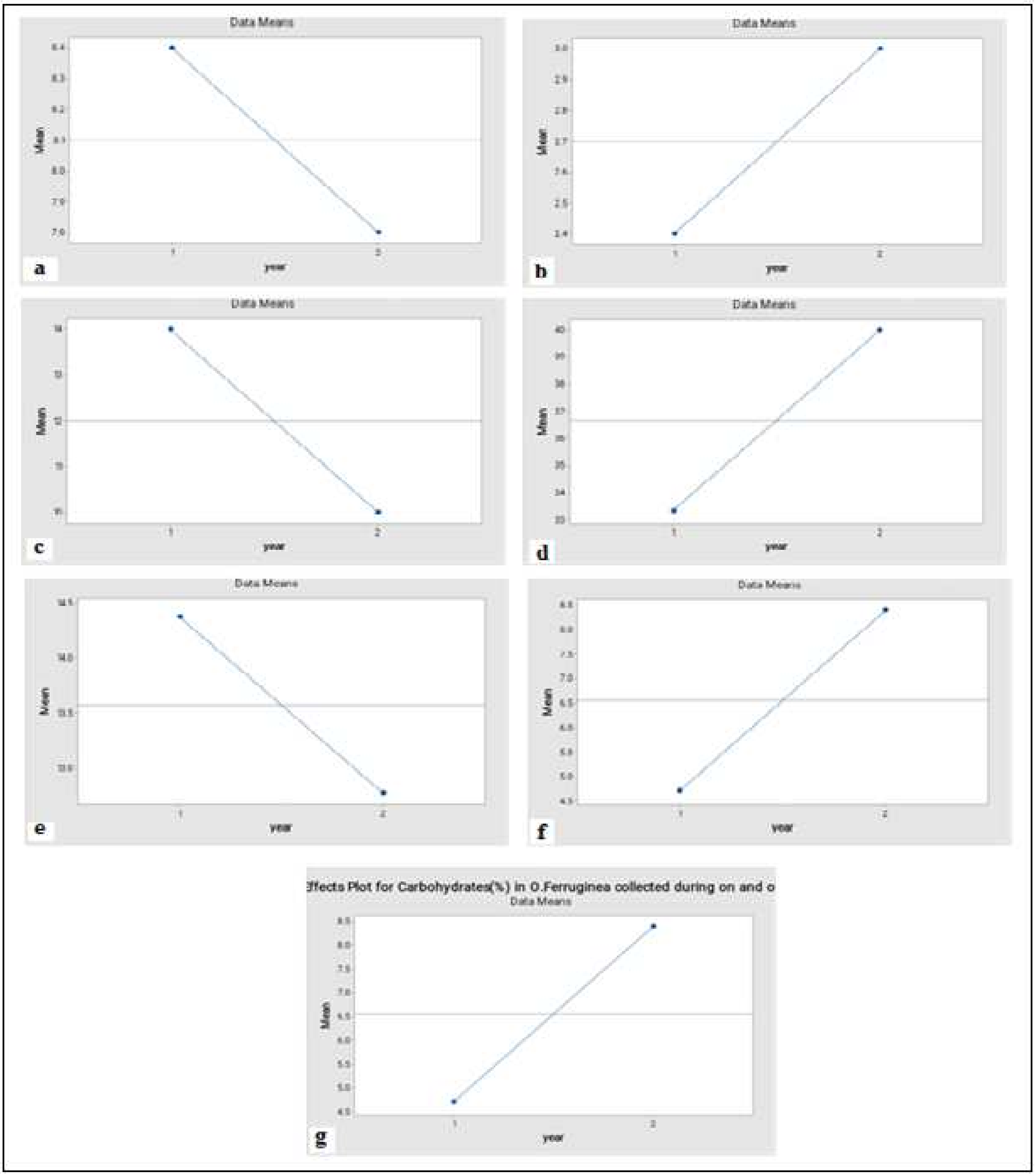

Fig. 1. Main effect plots for a) moisture content b) Ash content c) crude protein d) crude fiber content e) crude fat f) total carbohydrate g) organic matter percentages in *Olea ferruginea* on- year (1) and off-year (2) fruit samples.

The excess value of the crude fiber in the food may enhance the mineral products in plants and depict lower levels of total nutritional consumption (Imran *et al.*,2007). Crude fiber plays a valuable role in plants and cause a reduction in total nutritional consumption and also help in controlling high blood pressure, colon and breast cancer and heart disorder (McBurney *et al.*, 2004). In the present study, a higher quantity of crude fiber was present in *Olea ferruginea* off-year samples (40%) while lower amounts were found in on-year samples (33%). The difference was however non-significant ($p \leq 0.05$). Relative different concentrations of crude fiber were reported by other authors in wild edible plants (Gulfraz *et al.,* 2009; Rafaqat *et al*., 2020).

The organic matter concentration was higher in *olea ferruginea* on-year (97.6%) whereas a lower value was reported in *olea ferruginea* off-year samples (97.0%). The obtained values however revealed a non-significant difference at ($p \leq 0.05$).

Other data revealed a variation in total carbohydrate percentage as well. The total carbohydrate content was found higher in *olea ferruginea* off year (8.4%) while lower in *olea ferruginea* on year (4.7%). These findings agreed with the fact that the growing of fruits in a large numbers may produce carbohydrate reserves in the tree. When carbohydrates are stored in plants the amount differs in "On" and "Off" years. The relation between biennial bearing and carbohydrate metabolization was also reported by Barranco *et al.* (2010). The obtained data when compared with the literature, showed Lower as well as high carbohydrate percentages in other wild olive fruit samples (Gulfraz *et al.,* 2009). Unlike other proximate parameters the difference between carbohydrate percentage measured from on-year and off-year samples was significant ($p \leq 0.05$).

These results indicated a higher nutritional value in terms of energy in on-year samples whereas high mineral content in off-year samples but energy levels are compensated by higher carbohydrate percentage in off-year samples. The overall data further unveiled the nutritional compensation of off-year olive fruits by the accumulation of high mineral content.

Conclusion

Since last decade, olive consumption as a food in the form of fruits and oil has increased. People are now more aware of the quality of food. Among several cultivated olive cultivars of Pakistan, *O. ferruginea* Royale is one commercially underutilized species of naturally occurring olives found in Zhob area of Balochistan. Although, *O. ferruginea* has small-sized edible fruits communities living nearby are mostly dependent on them for getting their nutritional needs. These fruits are locally consumed in the form of pickles in a daily routine diet. However, its availability is constant as this evergreen widespread tree species exhibits alternate bearing patterns which affect fruit yield during consecutive years and so is its quality. The present research, therefore, was conducted with the prime goal to evaluate the effects of alternate bearing on the nutritional prospects of olive fruits collected during two consecutive years.

Overall results of proximate analysis highlighted that alternate bearing not only affects plant yield but also alters its quality at least in terms of nutrition. Based on the results of the present work it can be concluded that these plant species are a rich source of nutrition and thus, need to be conserved on sustainable terms. Furth more, research endeavors are suggested based on advanced biotech methods to find out opportunities in the propagation and management of *O.ferruginea*

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