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Seasonal Fluctuations of N, P and K in Leaves Influenced Nutrient Requirement During Fruit Development Stages in Different Olive Genotypes

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HIGHLIGHTS

- Overall, N, P, K contents were higher in leaves during flowering and then depleted during fruit development stages.
- Nitrogen content was found higher in Coratina among four olive cultivars
- Leaf phosphorus and potassium content was observed higher in Ottobratica and Leccino cultivars.

Abstract: Olive is grown in semi-arid climatic conditions; however, little is known about mineral changes in olive plant and nutrient requirements during the production period. Hence, the current study was conducted under Pothwar agro-climatic conditions in order to select appropriate stage of macronutrients (N, P, K) application in relation to soil and leaf nutritional status during 2017 and 2018 growing seasons. Soil and leaf analysis were performed at four different phenological stages (i.e. flowering, fruit setting, fruit enlargement and fruit maturity stages). The results revealed that the assessed macronutrient in leaf and soil varied significantly among varieties, phenological stages and growing year. The results revealed also that nitrogen level was found to decrease from fruit set (1.56%) to fruit enlargement stage (1.47%). Leaf and soil N, P and K contents were found higher before the flowering (stage 1) and depleted after fruit harvesting (stage 4), regardless of olive varieties. However, high yielding varieties showed lower nutrients after fruit harvesting (stage 4). Therefore, N content in leaf and soil gradually decreased during fruit growth and development. Whereas, K content in leaf and soil sharply declined from fruit maturity to fruit ripening stage. Overall, the trend of nutrient depletion showed that plants need phosphorus for fruit setting, nitrogen before and after fruit setting, and potash after pit hardening or at oil accumulation stages.

Keywords: Olea europaea; climatic conditions; soil analysis; Kallar Kahar.

INTRODUCTION

In the last decades, the interest in olive tree (Olea europaea L.) has been increased to various new regions in the world due to the increasing importance and demand for olive oil and table olive [1]. *Olea europea* L. more than 2600 cultivars, many of which may be ecotypes (Topi et al., 2021). Olive cultivation rapidly growing in Pakistan due to its pronounced socio-economic importance and health benefits. An estimated 3166 acres have been brought under cultivation in Pothwar since last five years under different provincial projects. Moreover 2800 (280004 plants) acres area has been cultivated by Federal Government only under the olive promotion project [2,3].The importance of olive has been reported 7 times in the Holy Quran Prophet Muhammad (May the peace and blessings of Allah be upon him) revealed its health benefit and promoted its consumption almost 15 centuries ago [4]. Hazrath Abu Hurairah (RA) reported that the Prophet (May the peace and blessings of Allah be upon him) stated, “Eat olive oil and apply it (locally), as it is a cure for seventy diseases, one of them is Leprosy [5]. Olive is a medium to tall sized vigorous tree having enormous health benefits with the capability of regeneration and may live for centuries [6,7]. Olive is very rich source of polyphenols, which aids in many health stimulating activities [8].

Olive, like higher plants needs balanced nutrients both in the form of macro nutrients (C, H, O, N, P, K, S, Ca, Mg,) and micronutrients (Fe, Zn, Mn, Cu, B, Cl) for smooth growth and production. Other nutrients (C, H, and O) can be fulfilled by absorption form air and soil. The lack of nitrogen, phosphorous and potassium in the soil lead to yield reduction [9, 10]. The deficiency (N, P, K, Fe) and toxicity (Na, Cl, or B) levels can be detected by soil analysis which is easy and convenient method. Measurement of soil pH is also a simple exploratory test used to calculate the availability of some nutrients, e.g. Mn and Fe in certain pH level. The precise and accurate way of examining the nutritional status and to calculate the fertilizer requirements of an olive orchard is leaf nutrient concentrations [11,12].

Leaf analysis is an important tool for determining future fertilization requirements. This technique depends upon the standard sampling processes and the analytical results as compared with the standard (critical) values of olives according to Chapman [13]. This method is based on assessing the nutritional status by determining the concentration of nutrients such as nitrogen, phosphorous, potassium, magnesium, iron, manganese, zinc and boron (N, P, K, Ca, Mg, Fe, Mn, Zn, B) in the leaves, expressed as a percentage of dry matter (DM) or mg/kg-1. Nutrient concentration in leaves varies greatly with plant growth stages, weather conditions, position of leaf on tree, plant age and fruit production. Major nutrients such as N, P, and K are crucial in olive cultivation. Nitrogen is required for the biosynthesis of proteins, nucleic acids, and a variety of coenzymes. Phosphorus aids in the process of energy production in plants. Potassium controls stomata opening and impacts carbon dioxide intake and photosynthesis [14]. Annual fluctuation in nutrients which may affect the alternate bearing phenomenon in different fruit trees [15], and their accumulation varied with tree vegetative growth under different weather conditions in olive [16]. Therefore, intensive care is needed for selection of soil and leaf samples during nutritional status measurement [17,18]. It is strongly recommended the construction of balance sheet regarding nutrient for an olive orchard by keeping in mind the plant population per hectare, nutrients depletion round the year at different stages and depletion of nutrient level by runoff or through leaching, different intensities of pruning and cover crops. This sheet can be built, depending upon the application method (broad casting, around individual plant, by drip irrigation system, foliar spraying or direct injection to the tree trunk) by focusing the cost of nutrient and its application [19]. Hence, the basic objective of the study to determine the seasonal depletion of N, P and K, concentration in the soil and leaves of olive tree at different four stages under common fertilizing program adopted in Pothwar, during an annual production cycle.

MATERIAL AND METHODS

Experimental Location

The experiment was conducted in two consecutive growing seasons i.e. season-I (2017) and season-II (2018) at 7-8 years old well established commercial olive orchard “Izhar Farms Pvt. Ltd.” located at Kallar Kahar, Chakwal Northern Punjab, Pakistan (320 46’33 N and 720 4231 E) at 460 m altitude. The climatic data (minimum temperature, maximum temperature and rainfall) were recorded through local orchard-installed weather station (Sensovant, Spain). The average rainfall during the year attained 1189 mm with

average minimum temperature of 4.83 °C during January and maximum temperature of 37.51°C in June. The detail of the climatic data is shown in figure1.

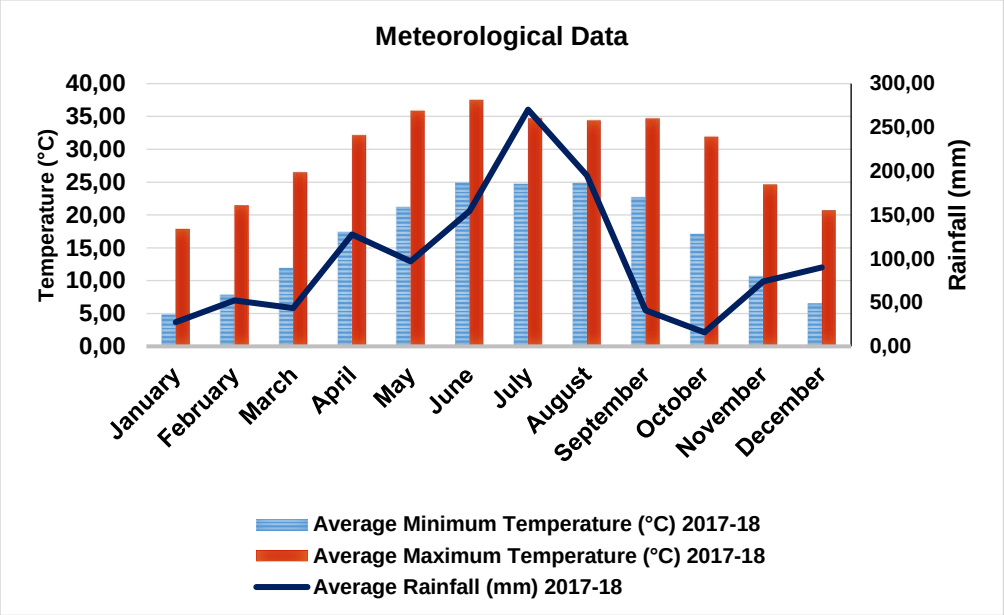

Figure 1. Average minimum, maximum temperature (°C) and rainfall (mm) data of the experimental site during the growing seasons

Plant material and Soil analysis

Twelve olive plants of Frantoio, Coratina, Leccino, and Ottobratica with similar age, height and canopy were selected in three replicates. Similar cultural practices (irrigation, fertilization, hoeing and weeding) were also applied to the used genotypes to reduce the agricultural practices associated variability. Trees were irrigated through high efficiency drip irrigation system (HEIS). The fertilizer was applied at early spring consisting of nitrogen (1500 g), phosphorus (1000 g) and potassium (1000 g). Soil samples were collected under the canopy of selected 48 olive plants at two different depth levels (0–30 cm and 30–60 cm). Then composite sample of both depths of each plant was performed and collected samples were delivered to the laboratory for analysis of pH, particle size according to Robinson pipette method [20] phosphorus by the method of Olsen [21] potassium by the method of Chapman [13] and organic matter by Walkley and Black method [22]. Soil samples were collected at four different mentioned stages to observe the changes in the soil fertility status.

Determination of NPK nutrients in leaves fraction

Nitrogen (N), phosphorus (P) and Potassium (K) contents were determined in olive leaves according to Chapman and Parker [23] method. Twelve olive plants of each variety (Frantoio, Coratina, Leccino, and Ottobratica) having the same age, height and canopy were selected in three replications. One hundred leaves per samples per plant were collected at four different stages viz-a-viz before flowering, after fruit set, at fruit development and after fruit harvesting stages in both growing years. Olive leaves were washed, dried at 56 °C for 50 hours and stainless-steel grinder was used for making powder and kept in plastic bags until analysis. A sample of 1 g olive leaf powder was poured into the Kjeldahl digestion flask, along with sulfuric acid and digestion mixture (K2SO4, CuSO4, and FeSO4). The solution was heated until a translucent green liquid material appeared. The resultant green liquid material was left to cool and collected for distillation in a micro- Kjeldahl apparatus with 40% NaOH. Then titrated against N/10 sulfuric acid until the methyl red colour was recovered. N (%) =[(A-B) x100 x 100 x 0.0014/Vol. of the sample used. Whereas, A = Quantity of N/10 H2SO4 used, B = Blank reading, 100= volume made after digestion, 100 = For percentage, 0.004 = Factor (which is equal to g of N present in 1 ml of N/10 H2SO4). The digestion for calculating P and K content was carried out by taking 0.5 g of oven dried leaf powder and 10 ml of tri-acid mixture (HNO3, HCLO4, H2SO4) in 100 ml flask. Hotplate was used for complete digestion. The digested material was filtered, and then diluted with distilled water to make the volume up to 100 ml. Phosphorus (P) content was measured according to Chapman and Parker technique, and ammonium molybdate and ammonium vanadate was used for this purpose. The absorbance was measured consequently at 420 nm by placing the colored samples in a

spectrophotometer. Moreover, K (%) content was measured by using a flame photometer as per Chapman and Parker's methodology [23].

Statistical analysis

The research was conducted according to two factor factorial arrangements under randomized complete block design (RCBD) with three replications. Genotypic differences were investigated, and least significant difference (LSD) was used to compare the means at p≤0.05) through statistical software XLSTAT, 2014 (v.5.03).

RESULTS AND DISCUSSION

Nitrogen status in the soil and leaves

The of the soil analysis was shown in Table 1. The maximum pH of the soil (8.15) and organic matter content (0.63%), and sandy loam texture was determined at local olive orchard. The nitrogen contents were estimated in the soil and in the leaves at all mentioned four stages under all the treatments. The glance of Figure 2. depicted that maximum nitrogen content in the leaves were recorded in variety V1 (1.62%) followed by variety V2 (1.59%). The variety V3 was ranked at 3rd position (1.43%) while the lowest value was recorded for variety V4 (1.39%). The trend of nitrogen level in leaf against stages was found to decrease with advancing maturity. The highest Nitrogen contents was found at starting maturity stages (S1,1.56%) and the lowest at the final stages of ripening (S4,1.45%). At the flowering stage and fruit set, there was a decreasing nitrogen level from 1.56% to 1.53% followed by a sharp decreasing trend from fruit set to fruit enlargement stage with 1.47%. The average minimum nitrogen depletion was observed between fruit development to fruit harvesting stage (1.45%). The average maximum nitrogen level was found in SN1 (1.52%) as compared to in SN2 (1.48%) which showed that the recommended dose is less for the proper nutritional requirement.

Table 1. Soil analysis in the experiment orchard

Properties	Values
pH	8.15 ± 0.1
Organic matter	0.63 ± 0.02
Soil texture	Sandy loam

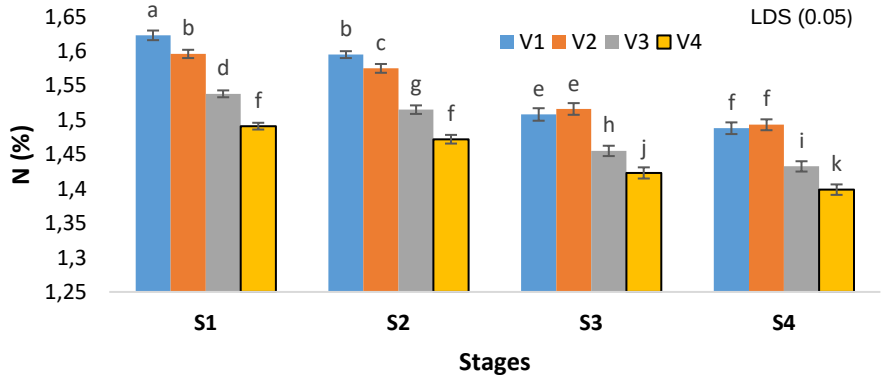

Figure 2. Olive varietal response regarding leaf nitrogen (%) at four different stages of production. Each point represents the mean± SDs of triplicate by LSD test at P < 0.05. Where, V1, Coratina; V2, Frantoio; V3, Ottobratica; V4, Leccino olive varieties; S1, stage 1; before flowering; S2, stage 2; after fruit setting; S3, Stage 3 at fruit development; S4, stage-4; after fruit harvesting.

As for as the nitrogen depletion in the soil was concerned the maximum value for nitrogen level was recorded for variety V3 and V4 (0.043%) with non-significant result in between these two varieties (Table-2). The minimum value was recorded for variety V1 (0.041%). The Table-2 showed that the high yielding cultivar at the end of the growing year led to nitrogen depletion as varieties V2 and V1 showed more yield at harvest and showed less nitrogen contents in the soil. Again, it was found that maximum nitrogen in the soil decreased at the third stage of ripening (from fruit set to fruit development stage) from 0.045% to 0.039%. The interaction between genotype and growing year showed that, except for V4 the nitrogen content in the soil in SN1 might

be due to the fluctuation genotypic differences in fruit load. It was concluded from the nitrogen depletion trend in the soil and leaf that fruit of olive need more nitrogen at fruit setting and fruit development stage. It could also be stated that more soil and leaf nitrogen contents were found in those varieties which gave more yield per plant.

Table 2. Genotypic response regarding soil nitrogen (%) at four different stages of production

Stage (S)	Varieties (V)				Mean (S)
	V1	V2	V3	V4	
	(V×S)				
S1	0.046 ± 0.02 abcd	0.048 ± 0.003 abc	0.050 ± 0.003 a	0.050 ± 0.002 ab	0.048 a
S2	0.043 ± 0.008 d	0.044 ± 0.004 d	0.044 ± 0.004 cd	0.046 ± 0.003 bcd	0.045 b
S3	0.038 ± 0.003 efg	0.039 ± 0.006 ef	0.040 ± 0.002 e	0.040 ± 0.005 e	0.039 c
S4	0.035 ±0.005 g	0.036 ± 0.002 fg	0.038 ± 0.005 efg	0.036 ± 0.004 fg	0.036 d
Mean (V)	0.041 ± 0.003 b	0.042 ± 0.003 ab	0.043 ± 0.004 a	0.043 ± 0.003 a	
SN[c]	(V×SN)				(SN)
SN1	0.041 ± 0.004 bc	0.042 ± 0.002bc	0.041 ± 0.004bc	0.043 ± 0.005ab	0.042 a
SN2	0.040 ± 0.003c	0.042 ± 0.004bc	0.045 ± 0.003 a	0.043 ± 0.005ab	0.042 a

Each data values are represented as means ± SDs of three replications. Different letters are indicated in the table, for each given parameter lower case letter represent significant difference between soil nitrogen and fruit development stages (S1-S4) in four olive varieties (V1-V4) during two seasons (SN1-SN2) and same lower-case letter represent the non-significant difference by LSD test at $P < 0.05$. [a] represent olive cultivars, V1, Coratina; V2, Frantoio; V3, Ottobratica; V4, Leccino. [b] represents stages, S1, stage 1-before flowering; S2, stage 2, after fruit setting; S3, Stage 3, at fruit development; S4, stage-4, after fruit harvesting. [c] represents SN-season; SN1, season-1; SN2, season 2.

The absorption and translocation of micro and macro nutrients in different olive varieties and tree species have been reported by several authors [24-27]. Olive is considered to be a specie with a great capability to survive in poor soils. However, few studies depicted the dynamics of nutrient absorption and genotypic differences among olive varieties [28]. The demand of macro and micronutrients at each step or stage is important indicator to help farmers determine the nutritional deficiency or excess and follow the nutritional demand specifically throughout ripening stages to improve productivity and quality. The data showed that the need were genotype and ripening stage dependent. Our results were in accordance with the findings of various authors who reported variation in leaf nutrient contents among selected olive varieties [29, 30]. Many studies have been conducted under Mediterranean climate conditions and showed that nutrient translocation was variety-dependent under same ecological condition [31, 32]. It has also been well documented that olive yield is dependent upon genotype and proper nutrient management program [33, 34]. Our results were also in agreement with Fahmy, [35] who reported an increase in nitrogen level during “off year” production. Maximum or minimum intake is highly dependent upon species, variety, developmental stage, rootstock, pruning method, climatic conditions along with other minor factors [36-38]. Similar result has been found by Perica, [39] who reported that nitrogen content decreased with advancement of vegetative and reproductive stage and reached the lowest value in summer season. Leaf nutrients were recycled through internal remobilization; therefore, leaf nitrogen accumulation was remobilized from senescing leaves to perennial plant parts [40]. Similar result has been reported that nitrogen concentration was found decreased during the endocarp hardening (fruit development) and increased during the start of rest period in olive leaves [16].

Phosphorous status in the soil and leaves

The phosphorous contents were estimated in the soil and in the leaf at four stages under all the treatments. Maximum phosphorous contents in the leaves were recorded in varieties V3 (0.115%) followed by V1 (0.112%) and V2 (0.112%) without statistical differences among them (Figure 3). The minimum value was recorded for the variety Leccino (0.063%) with significant difference compared to the other genotypes. The comparison between the growing years showed that the highest leaf phosphorous content was found in S1 (0.093%) as compared to S2 (0.073%). The level of leaf phosphorous at different stages showed the highest value (0.108%) at S1 with the regular descending order up to S4 (0.07%). However, relatively sharp decline in phosphorus contents was observed from S1 to S2. Moreover, among interaction of stages and years, maximum phosphorus level was found in SN1 in almost all the stages as compared to SN2.

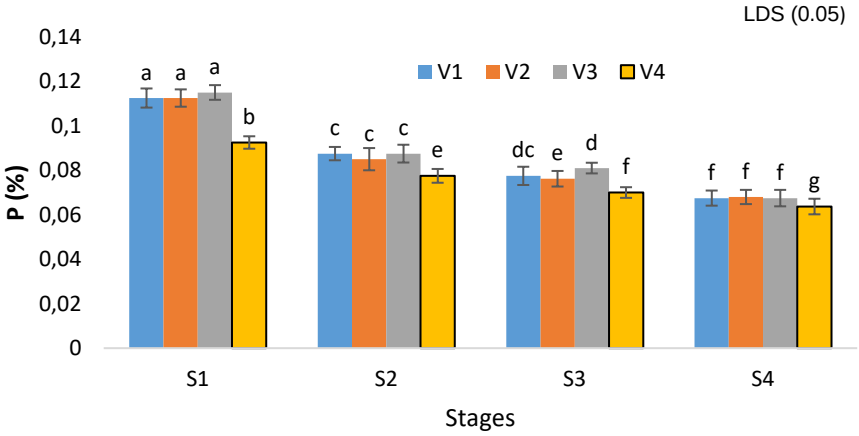

Figure 3. Olive varietal response regarding leaf phosphorus (%) at four different stages of production. Each point represents the mean ± SDs of triplicate by LSD test at $P < 0.05$. Note, V1-Coratina; V2-Frantoio; V3- Ottobratica; V4-Leccino olive varieties; S1-stage 1; before flowering; S2- stage 2; after fruit setting; S3-Stage 3 at fruit development; S4-stage-4; after fruit harvesting.

The results presented depicted that maximum value for phosphorus level was observed in the soil of variety Frantoio (7.66 %) followed by variety V1 (7.61%) and V4 (7.59%) in Table 3. The highest value was recorded in variety V3 (6.42%). the highest soil phosphorus level was found at S1 (7.525%) and regularly found lower value up to S4 (6.709 %). When growing years were compared, the highest phosphorus content was found in season-2 (7.189%) as compared to SN1 (6.938%). Overall, we found that V3 and V1 caused more depletion of phosphorus contents from the soil during the first growing season (SN1). It was observed that phosphorous level in the leaf varied with the stages and varieties. Our results were found similar with the findings of Fahmy and Nasrallah, [41] who found that the leaf phosphorus content in olive vary with the stages and between the growing years but contradiction was found with the statement that leaf phosphorous level increased with the time. However, our results in line with the findings of Fernandez-Escobar et al., [17] who found permanently the lowest value in fruit development stages and the highest value after harvest. Phosphorus is the part of structural components, and also active play important role in physiological and energy processes [42]. Nevertheless, surplus phosphorus concentration normally showed antagonism with other micronutrients such as Zn and Manganese [43. Our result suggested that phosphorus concentration decreased gradually during the S1 and S2 which might contribute to increase the growth and development of fruit.

Table 3. Olive varietal response regarding soil phosphorus (%) at four different stages of production.

Stage (S)	Varieties (V)				
	V1[a]	V2	V3	V4	Mean (S)
	(V×S)				
S1[b]	7.613 ± 0.12 a	7.666 ± 0.13 a	7.225 ± 0.13 bc	7.596 ± 0.20 a	7.525 a
S2	7.172 ± 0.15 bcd	7.354 ± 0.17 b	6.875 ± 0.16 ef	7.211 ± 0.28 bc	7.153 b
S3	6.878 ± 0.30 ef	7.061 ± 0.26 cde	6.563 ± 0.26 gh	6.955 ± 0.11 def	6.864 c
S4	6.728 ± 0.18 fg	6.916 ± 0.21 ef	6.405 ± 0.31 h	6.788 ± 0.10 fg	6.709 d
Mean (V)	7.761 ± 0.19a	7.145 ± 0.19 b	6.902 ± 0.21c	6.444 ± 0.18 d	
SN[c]	(V×SN)				(SN)
SN1	6.954±0.47 d	7.106 ± 0.15 bcd	6.546 ± 0.17e	7.143 ± 0.19 bc	6.937 b
SN2	7.241 ± 0.10ab	7.393 ± 0.36a	6.988 ± 0.20 cd	7.132 ± 0.22 bc	7.188 a

Each data values are represented as means ± SDs of three replications. Different letters are indicated in the table, for each given parameter lower case letter represent significant difference between soil phosphorus and fruit development stages (S1-S4) in four olive varieties (V1-V4) during two seasons (SN1-SN2) and same lower-case letter represent the non-significant difference by LSD test at P < 0.05. a represent olive cultivars, V1, Coratina; V2, Frantoio; V3, Ottobratica; V4, Leccino. b represents stages, S1, stage 1-before flowering; S2, stage 2, after fruit setting; S3, Stage 3, at fruit development; S4, stage-4, after fruit harvesting. c represents SN, season; SN1, season-1; SN2, season 2.

Potassium status in the soil and leaves

Regarding potassium contents, we noticed that the highest level was observed in the leaves of variety Frantoio (0.73%) followed by V3 (0.70%) and V1 (0.69%) in Figure 4. the lowest value was recorded for V4 (0.48%). Among developmental stages, the highest potassium content (0.692%) was found at S1 and regularly found to decrease attaining the lowest value at S4 (0.538%). It is worth mentioning that the lowest values were found at S3 (0.604%) and S4 (0.538%) suggesting that those stages are crucial for optimum provision of potassium.

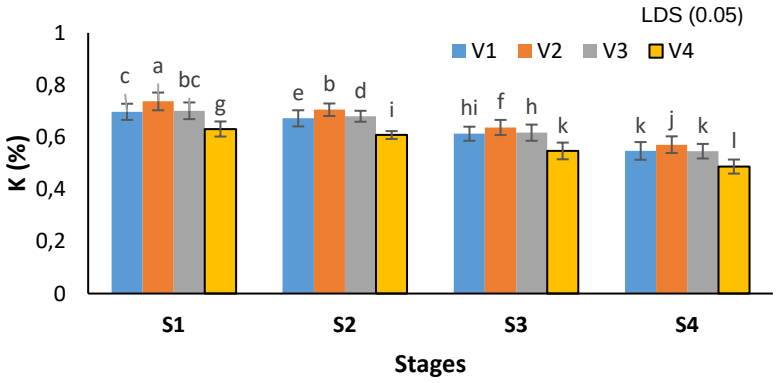

Figure 4. Olive varietal response regarding leaf potassium (%) at four different stages of production. Each data values are represented as means ± SDs of three replications. Different letters are indicated in the graph, for each given parameter lower case letter represent significant difference between soil potassium and fruit development stages and same lower-case letter represent the non-significant difference by LSD test at P < 0.05. Each point represents the mean of triplicate and bars show standard r of the mean, where V1, Coratina; V2, Frantoio; V3, Ottobratica; V4, Leccino olive varieties; S1, stage 1; before flowering; S2, stage 2; after fruit setting; S3, stage 3 at fruit development; S4, stage-4; after fruit harvesting.

As for as the potassium level in the soil was concerned the maximum value for potassium was found in variety V4 (139.16 ppm) as shown in Table 4. All other genotypes were found non-significant among each other with the highest value for variety V1 (134.78 ppm) and the lowest (131.83 ppm) in variety V3. In comparison of years maximum potassium contents in the soil were found in SN2 (136.79 ppm) as compared to SN1 (132.50 ppm). Maximum value of potassium level was found at S1 (155.1 ppm) with progressive decline up to S4 (110.33 ppm).

It was observed that potassium depletion occurred with maximum range at fruit development stage to fruit maturity. These trends were in accordance with the findings of Boulal et al., [44] who suggested, potassium application in the later stages of crop growth and fruit ripening because at this stage need for potassium is generally high in olive plants. It may vary from year to year in relation with crop load with similar suggestions by Fernandez-Escobar [17]. Our results are in agreement with the finding of other researchers who reported that leaf potassium level decreased with the increase of development of fruit and season, which suggested the remobilization of K content from leaf to fruit parts [44, 45]. In general, fruits are considered as great sinker for the accumulation of major nutrients from leaves to fruits in most of the horticultural perennial crops [40, 46, 47]. Higher level of potassium plays important role in the activation of more than 60 enzymes, and involved in different processes such as photosynthesis, ATP-synthesis and CO2 assimilation [48]. It also increases the leaf area, pigments, translocation of carbohydrate contents which increase uptake of nutritional components and increased fruit yield and quality [42].

Table 4. Olive varietal response regarding soil potassium (mg/kg) at four different stages of production.

	Varieties (V)				
Stage (S)	**V1[a]**	**V2**	**V3**	**V4**	**Mean (S)**
	(V×S)				
S1[b]	152.13 ± 3.71 ab	150.92 ± 2.16 ab	147.42 ± 3.29 bc	155.58 ± 3.75 a	151.51 a
S2	148.83 ± 2.25 bc	147.00 ± 3.26 bc	143.92 ± 2.74 c	151.29 ± 4.03 ab	147.76 b
S3	129.00 ± 1.96 de	126.25 ± 1.06 e	126.92 ± 3.17 e	133.71 ± 2.96 d	128.97 c
S4	109.17 ± 2.75 g	107.04 ± 2.36 g	109.08 ± 2.28 g	116.04 ± 3.08 f	110.33 d
Mean (V)	134.78 ± 3.16 b	132.80 ± 3.01 b	131.83 ± 4.15 b	139.16 ± 3.62 a	
SN[C]	**(V×SN)**				**(SN)**
SN1	131.40 cd	130.69 ± cd	129.69 ± d	138.23 ± 3.47 ab	132.50 b
SN2	138.17 ab	134.92 ± bc	133.98 ± bc	140.08 ± 3.16 a	136.79 a

Each data values are represented as means ± SDs of three replications. Different letters are indicated in the table, for each given parameter lower case letter represent significant difference between soil potassium and fruit development stages and same lower-case letter represent the non-significant difference by LSD test at $P < 0.05$. a represent olive cultivars V1, Coratina; V2, Frantoio; V3, Ottobratica; V4, Leccino. b represents stages, S1, stage1, before flowering; S2, stage 2, after fruit setting; S3, stage 3, at fruit development; S4, stage 4, after fruit harvesting. c represents SN, season; SN1, season-1; SN2, season 2.

Relationship between varieties, development stages for seasonal variation of N, P and K and in leaves and soil in experimental olive orchard

Pearson correlation coefficient was calculated to examine the relationship among varieties and stages for N, P and K content in leaves and soil at experimental site (Table 5). No significant correlation was found between varieties and stages, which depicted low degree correlation with respect to all variables, while varieties were negatively correlated with leaf N ($r = -0.675$, $P < 0.001$) and positively correlated with soil P content ($r = 0.081$). Stages were highly negatively correlated with Leaf K ($r = -0.849$, $P < 0.001$), and Soil N ($r = -0.972$, $P < 0.001$), P ($r = -0.851$, $P < 0.001$) and K content ($r = -0.953$, $P < 0.001$) as shown in Table-5.

Leaf N content showed negative correlation with cultivars and stages while significant positive correlation with leaf K (r= 0.881, P < 0.001) and soil P (0.712 P < 0.001) content was found. Moreover, there was no significant correlation between leaf P content and all other traits. However, leaf K content showed highly negative correlation with ripening stages (r= -0.849, P < 0.001) and positive correlation with leaf N content. Soil N, P and K elements were negatively correlated with stages and leaf N, while positively correlated with the corresponding elements in the soil (Table 5). Soil N content was found significantly and positively correlated with soil K content (r = 0.928, P < 0.001) among other elements. Our results are in line with the previous studies highlighting that nutrient translocation under same ecological condition is genotype-dependent [24, 46]. Our results are also in accordance with the previous studies were nutrients contents decreased with advancement of vegetative and reproductive stages in olive cultivars [28, 32].

Table 5. Pearson correlation coefficient between varieties and stages for leaf and soil nitrogen, phosphorus and potassium content.

	V	S	L-N	L-P	L-K	S-N	S-P	S-K
V	1	0.000	-0.675	0.300	-0.354	0.175	-0.113	0.081
S	0.000	1	-0.691	0.460	-0.849	-0.972	-0.851	-0.953
L-N	-0.675	-0.691	1	-0.487	0.881	0.555	0.712	0.609
L-P	0.300	0.460	-0.487	1	-0.533	-0.342	-0.454	-0.466
L-K	-0.354	-0.849	0.881	-0.533	1	0.762	0.697	0.772
S-N	0.175	-0.972	0.555	-0.342	0.762	1	0.791	0.928
S-P	-0.113	-0.851	0.712	-0.454	0.697	0.791	1	0.813
S-K	0.081	-0.953	0.609	-0.466	0.772	0.928	0.813	1

Correlation suggested values in four olive varieties during developmental stages for leaf and soil nutrient status. Where V; varieties, S; stages, L-N; leaf nitrogen content, L-P; leaf phosphorus content, L-K; leaf potassium content, S-N; soil nitrogen, S-P; soil phosphorus content, S-K; soil potassium content.

Regression analysis

The results of linear regression analysis showed significant variation among soil and leaf N, P and K content during developmental stages among four olive cultivars during different growing seasons (Table 6 and 7). Our results revealed that leaf N, P and K content were substantially depleted during stages (S1-S4) in SN2 than SN1 (Table 6). However, soil N, P and K contents were more depleted during stages (S1-S4) in SN1 than SN2 (Table 7). Similar studies have been reported that seasonal conditions may effect on nutrient translocation in varieties [27, 49].

Table 6. Multiple linear regression analysis showing relationships between N, P and K contents in leaf during development stages in different seasons among four verities of olive.

Season			Nutrient content	B	t	p- value	R^2
SN1[c]	**Stages**	**S1[a]**	Nitrogen in leaf	-0.039	-17.473	0.00*	0.930
		S2	Phosphorus in leaf	-0.013	-15.642	0.00*	0.851
		S3	Potassium in leaf	-0.052	-14.454	0.00*	0.845
		S4					
	Varieties	**V1[b]**	Nitrogen in leaf	-0.038	-17.064	0.00*	0.930
		V2	Phosphorus in leaf	-0.003	-3.483	0.001*	0.851
		V3	Potassium in leaf	-0.022	-6.027	0.00*	0.845
		V4					
SN2	**Stages**	**S1**	Nitrogen in leaf	0.008	0.536	0.595^{NS}	0.391
		S2	Phosphorus in leaf	-0.051	-4.472	0.000*	0.388
		S3	Potassium in leaf	-0.057	-8.914	0.012**	0.657
		S4					
	Varieties	**V1**	Nitrogen in leaf	-0.075	-5.348	0.000*	0.391
		V2	Phosphorus in leaf	-0.033	-2.928	0.005^{NS}	0.388
		V3	Potassium in leaf	-0.017	-2.617	0.000*	0.657
		V4					

[a] represents stages, S1-stage1, before flowering; S2, stage 2, after fruit setting; S3, stage 3, at fruit development; S4, stage 4, after fruit harvesting. [b] represents olive cultivars, V1, Coratina; V2, Frantoio; V3, Ottobratica; V4, Leccino. [c] represents SN, season; SN1, season-1; SN2, season 2. Where ^{NS} Non-significant at p >0.05, *Significant at p < 0.001, ** Significant at p< 0.005

Table 7. Multiple linear regression analysis showing relationships between N, P and K contents in soil during development stages in different seasons among four verities of olive

			Nutrient content	B	t	p- value	R^2
SN1[c]	Stages	S1[a]	Nitrogen in soil	-0.004	-21.070	0.00*	0.911
		S2	Phosphorus in soil	-0.274	-11.113	0.148[NS]	0.736
		S3	Potassium in soil	-14.228	-21.871	0.00*	0.915
		S4					
	Varieties	V1[b]	Nitrogen in soil	0.001	3.794	0.00*	0.911
		V2	Phosphorus in soil	-0.036	-1.473	0.00*	0.736
		V3	Potassium in soil	1.213	1.865	0.069NS	0.915
		V4					
SN2	Stages	S1	Nitrogen in soil	-0.002	-2.952	0.005[NS]	0.224
		S2	Phosphorus in soil	-0.249	-5.351	0.000*	0.463
		S3	Potassium in soil	-13.928	-21.219	0.000*	0.909
		S4					
	Varieties	V1	Nitrogen in soil	0.002	2.060	0.045[NS]	0.224
		V2	Phosphorus in soil	-0.149	-3.199	0.003**	0.463
		V3	Potassium in soil	0.880	1.341	0.187[NS]	0.909
		V4					

[a] represents stages, S1, stage1, before flowering; S2, stage 2, after fruit setting; S3, stage 3, at fruit development; S4, stage 4, after fruit harvesting. [b] represents olive cultivars, V1, Coratina; V2, Frantoio; V3, Ottobratica; V4, Leccino. [c] represents SN, season; SN1, season-1; SN2, season 2. Where [NS] Non-significant at $p > 0.05$, *Significant at $p < 0.001$, ** Significant at $p < 0.005$.

CONCLUSION

The present study revealed that olive orchard in Potohar regions were characterized by high soil pH. The investigated experimental site revealed low soil organic matter. Leaf N, P and K content decreased during fruit development stages, suggesting that olive fruit need more N at fruit setting and fruit maturity stages. While phosphorous application is more important during flowering-fruit setting stage following the decline in this element during early stages (S1 & S2). Moreover, potassium depletion occurred with maximum range from fruit ripening stage to fruit maturity which indicated that potassium should be applied at fruit development-maturity stage. Therefore, overall, depletions of these nutrients (N, P and K) are essential for the sustainable production of olive orchard in Potohar regions. Nevertheless, further studies are needed to investigate fertilizer response to build proof-based recommendation regarding fertilizers management fertilizers use efficiency in olive orchard.

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