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RESEARCH PAPER

Nutrient imbalance diagnosis in walnut orchards by using DRIS and PCA Approaches in the northern part of Erbil governorate-Iraq.

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ABSTRACT:

The use of Diagnosis and Recommendation Integrated System (DRIS) in studying the concept of a plant's nutritional balance has become an efficient method for assessing plant nutritional status; this method limits nutrient location depending on plant demand, enabling the nutritional balance between the nutrients in the leaf sample. Aim of this study is to establish walnut DRIS norms and diagnosis nutrient imbalance by using DRIS and PCA (Principal component analysis) methods. One hundred four leaf samples were collected during two consecutive years (2016 and 2017) from three walnut orchards in Erbil governorate/ Iraq. The results showed that the smallest average NBI (Nutrient Balance Index) values are 125.6 and 27.2 respectively for both studied years which was coincided by high walnut productivity. The most limiting nutrient descending order as Fe>Na>Ca>K>P>N>Mg respectively for first-year samples, and N>Na>P>Fe>Mg>Ca>K respectively for the second year. From the result of PCA reveal that the nutrient concentration was explained in the low and high yield subgroups and the DRIS index by 61.95%, 87.75% and 58.67% respectively for first-year and 79.22%, 60% and 65.95% respectively for second-year sampling. Several nutrient interventions in a single PC indicate that no nutrient imbalance in isolation could be diagnosed.

KEY WORDS: Walnut; Foliar nutrient concentration; Nutrient imbalance; Diagnosis and Recommendation Integrated System (DRIS); Principal Component Analysis (PCA).
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1. INTRODUCTION

Common walnut species (*Juglans regia* L.) belongs to the Juglandaceae family, one of the most important horticultural crops in northern Iraq (Townsend and Guest, 1980). It is a valuable tree attained great concern from the farmers and villagers in the region from their economically and ecologically importance (Gauthier and Jacobs, 2011, Sytykiewicz *et al.*, 2019).

Leaf analysis plays the main role in the physiological processes of the plants. Therefore, it becomes a successful tool for diagnosing the nutritional requirement of forest tree and crops (Carneiro *et al.*, 2015). Nutritional diagnosis is an appropriate way of detecting deficiencies and allowing more efficient application of fertilizers (Carneiro *et al.*, 2015, Silveira *et al.*, 2005).

Diagnosis and Recommendation Integrated System (DRIS) is regarded as an accurate system that uses pairwise relationship among nutrients and compare it with the high population productivity (references norm) which represented by the mean, variance and coefficient of variance (Silveira *et al.*, 2005, Matos *et al.*, 2018, Dias *et*

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al., 2011). Many studies urge to established regional DRIS norm, that adapted for varieties of crops (Carneiro *et al.*, 2015), that driven from the same local soil and climate condition (Talpur, 2015). The mean of the deviations from the DRIS norm compose nutrient index (Bailey *et al.*, 1997, Yao *et al.*, 2013). The sum of absolute values of nutrient indices forms nutrient balance index (NBI) (Yao *et al.*, 2013). Their value ranges from negative to positive with always the sum value of zero. The excessive nutrient contents of crops are expressed by (positive indices), deficient (negative indices) and adequate (zero indices) (Geiklooi *et al.*, 2018, Darwesh, 2011).

Principal component analysis (PCA) is a multivariate mathematical reduction procedure, which reduced a large data set of variables (Principal component- PC) that contains most of the information in the original data set. PCA has been performed on DRIS indices by many investigators (Wairegi and Van Asten, 2011). However, several authors in Iraq have successfully used the DRIS method to interpret the results of leaf analysis for a wide range of plants such as lentils (Darwesh, 2011), wheat (Esmail, 2012, Esmail *et al.*, 2019), soybean (Darwesh and Mustafa, 2012), barley (Dizayee and Hussein, 2017), Broccoli (Dizayee and Saleh, 2017), . Whereas, there is not the availability of such information about nutrient balance for a walnut tree for local agriculture manager and orchard farmers who complain about the lack of production of these trees. The aims of this study is to establish the norm of walnut and assess nutrient balance index.

2. MATERIALS AND METHODS

2.1 Samples collection

For this study, three areas in the northern part of Erbil province / Iraq were selected. In the following counties with their geographical coordinates, plant samples were collected using (GPS) a Global Positioning System (UTM): Shaqlawa District (38s E0439944 latitude N4028711 , elevation 963m above sea level); Malakan village (38s E0463373 N4035073, elevation 1366 above sea level); Choman (Warda village) (38s E0489275 N4057261, elevation 1514m above sea level) (Figure 1). The climate in the area is semi-arid and, characterised by wet, cold winter (December- March) and hot summer (Al-Kubaisi and Gardi, 2012).

In each studied area a specific walnut orchard selected after previous obtained permission from the orchard owners. Two consecutive years of plant sampling were conducted in June 2016 and June 2017. Eighteen samples per study orchards were taken.

2.2 Laboratory analysis

The leaves samples have been dried at 65C° for 48 hrs., ground and wet digested using H_2SO_4: H_2O_2 method (Cotteine, 1980). From the digestive samples N were determined using the Micro - Kjeldahl method ; P was determined using the Molybdenum Blue method and measured by the Spectrophotometer; K using the Flame Photometer, while Ca and Mg was assayed by EDTA titrimetric method (Allen *et al.*, 1974).

2.3 DRIS calculation

According to Beaufils (1971), the mean, variance and coefficient of variation (CV) for each possible nutrient pair ratio were calculated for both high and low yield populations. For each pair of nutrients, the mean and CV ratios that maximized the variance ratio between the low and high population were selected as norms for that pair of nutrients as described by Walworth and Sumner (1987). For current study the reference population was defined as 12.6 $Kg.tree^{-1}$ or 3.54 $t.ha^{-1}$, a population exceeded this limit considered as high yield and lower of this point regarded as low yielding population.

DRIS provides a means of ordering nutrient ratios or products into a meaningful expression called the DRIS index. A nutrient index is the mean of deviations of the ratios containing a given nutrient from their respective optimum or norms value. All indices were balanced to nearly zero. Therefore, nutrient indices to sum zero. The more negative an index is the more insufficient nutrient compared to other nutrients being used in the diagnosis. Alternatively, a positive index showed that the corresponding nutrients were found in relatively excessive quantities. DRIS indices could be calculated for nutrients from following generalized equation as an example:

$$N\ index = [f\left(\frac{N}{P}\right) + f\left(\frac{N}{Ca}\right) + \ldots\ldots - f\left(\frac{Na}{N}\right) - f\left(\frac{Fe}{N}\right)]$$

$$When\frac{N}{P} > \frac{n}{p}\, then\ f(N/P) = \left(\frac{\frac{N}{P}}{\frac{n}{p}} - 1\right)\left(\frac{1000}{CV}\right)$$

$$When\frac{N}{P} < \frac{n}{p}\ then\ f(N/P) = \left(1 - \frac{\frac{n}{p}}{\frac{P}{N}}\right)\left(\frac{1000}{CV}\right)$$

$$When\ \frac{N}{P} = \frac{n}{p}\ \ then\ f\left(\frac{N}{P}\right) = 0$$

Where N/P is the ratio of nutrient from analyzed leaves tissue n/p is the ratio of nutrient from DRIS norm of paired nutrients from the population CV is the coefficient of variance(n/p) of the references (high yield population).

Nutrient imbalance index (NBI) calculated from the sum of absolute values of the nutrient indices. The smaller the absolute sum (NBI), the lesser imbalance among nutrients. Whereas the larger the sum index, the more imbalance within nutrients (Agbangba *et al.*, 2011, Wairegi and Van Asten, 2011, Snyder and Kretschmer Jr, 1988).

2.4 Principal component analysis (PCA)

The PCA was performed separately on the nutrient concentration data for low and high yield populations and DRIS indices (Sharma *et al.*, 2005). PCs with eigenvalues greater >1 regarded as significant. Only PC loadings in the eigenvectors having values greater than the selection criterion (SC) were considered as the significant loads. The selection criterion was as follows (Raghupathi *et al.*, 2005, Geiklooi *et al.*, 2018):

$$SC = 0.5/(PC\ eigen\ values)\text{^}0.5$$

2.5 Statistical analysis

Descriptive, multivariate (Principal component analysis), normal distribution and DRIS calculation for parameters were performed by using (SPSS version 25) program and Excell spreadsheets.

3. RESULTS AND DISCUSSION

3.1 Diagnosis and Recommendation Integrated System (DRIS)

The results show the descriptive statistics for leaf tissues of the walnut tree (Table 1). The data for the high and low yielding population was relatively less skewed, with most nutrients having a skewed value of less than 1. It is an indication that most data is normally distributed, therefore, suitable for the deriving the DRIS norm (Chanan *et al.*, 2019). Because the symmetrical data provided a realistic approximation of the possibility of interactive effect variations in the productivity of walnut of different nutrients. The only marginal difference was noticed throughout the study period in the concentration of various nutrients between high and low yielding plants. More than 66% of walnut trees in first year sampling considered as high yielding population coincided with higher nutrients concentration compared with only 55% of high yield population for second year samples with decreases in nutrients concentration for most cases.

All ratios of nutrients are presented in Tables (2 and 3) for both studying years with their mean, variance and coefficient of variance. Twenty - one ratios of nutrients were chosen as those with the highest ratio variants between the high and low yielding population and became the DRIS norm. These norms were used to calculate nutrient indices and nutrient imbalance indices. The choice of nutrient ratio with maximum variants of high and low yielding populations was used to maximize the potential of differentiated walnut with high yielding compared to inadequate yielding populations. The variant of the ratio indicated the importance of certain nutrient ratios against the results parameter with a very high ratio, which shows that the nutrient is essential for the plant (Raghupathi *et al.*, 2005). The similar selected nutrient ratios for both studying years are: N/P, N/K, Fe/N, Mg/Na, Fe/Mg; and K/Na had greater physiological effects in leaf tissue. Photosynthesis rate influences K content through involvement in osmoregulation, protein synthesis and enzymes activation (Gardner *et al.*, 2017). Among the essential functions of Fe in the plant, the body is the constituent of the enzyme as a metal activator. The Fe deficiency causes chlorophyll formation to fail because Fe is essential for chlorophyll molecule biosynthesis through decreasing chloroplast size (Barker and Pilbeam, 2015). On the other hand, the intensive review of the literature showed that no study had been done to establish the DRIS walnut norms. However, the use of universal DRIS norms is suitable for several factors, such as soil type, climate and crop genotypes (Talpur, 2015).

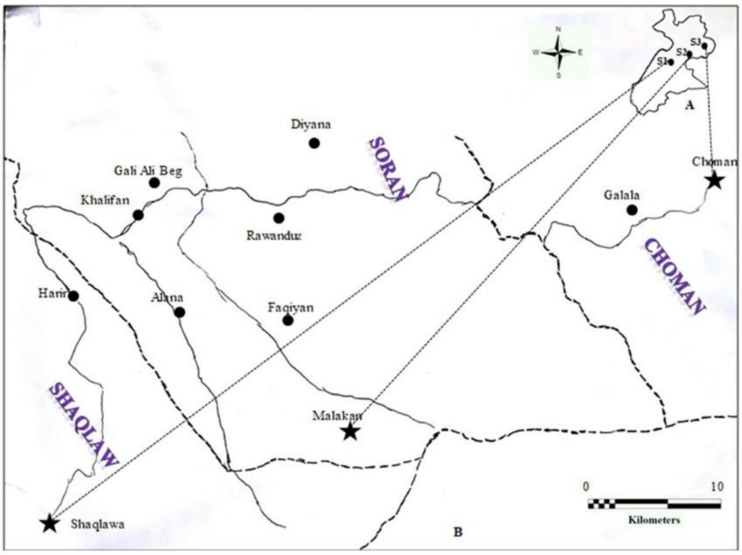
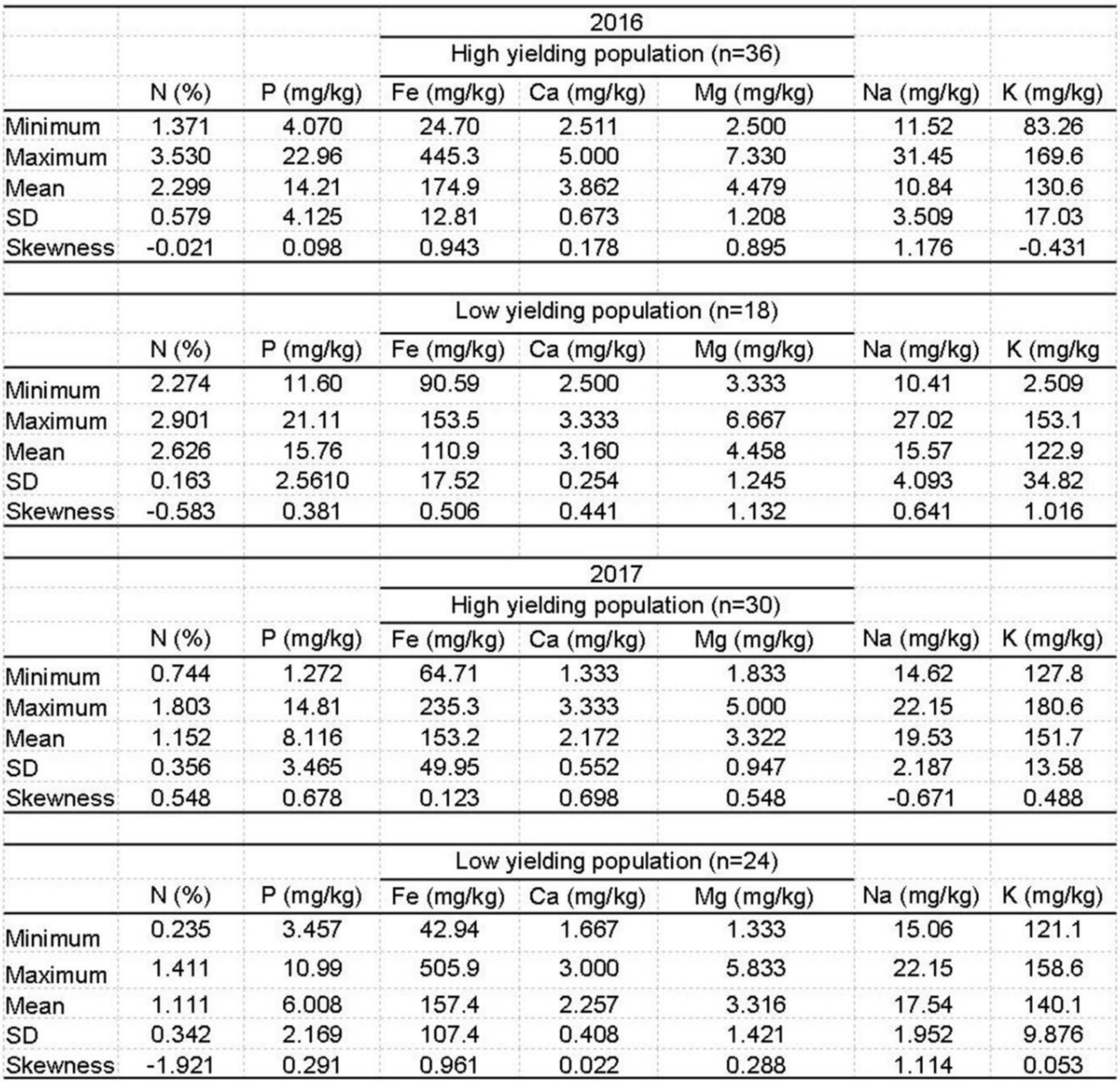


Figure 1: A. Locations of the studied area in northern part of Erbil province/Iraq. B. Study sites.

Table 1: Descriptive statistics of nutrients in the walnut leaves from high and low yielding population for two consequence years 2016 and 2017.



2016

High yielding population (n=36)

	N (%)	P (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)
Minimum	1.371	4.070	24.70	2.511	2.500	11.52	83.26
Maximum	3.530	22.96	445.3	5.000	7.330	31.45	169.6
Mean	2.299	14.21	174.9	3.862	4.479	10.84	130.6
SD	0.579	4.125	12.81	0.673	1.208	3.509	17.03
Skewness	-0.021	0.098	0.943	0.178	0.895	1.176	-0.431

Low yielding population (n=18)

	N (%)	P (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg
Minimum	2.274	11.60	90.59	2.500	3.333	10.41	2.509
Maximum	2.901	21.11	153.5	3.333	6.667	27.02	153.1
Mean	2.626	15.76	110.9	3.160	4.458	15.57	122.9
SD	0.163	2.5610	17.52	0.254	1.245	4.093	34.82
Skewness	-0.583	0.381	0.506	0.441	1.132	0.641	1.016

2017

High yielding population (n=30)

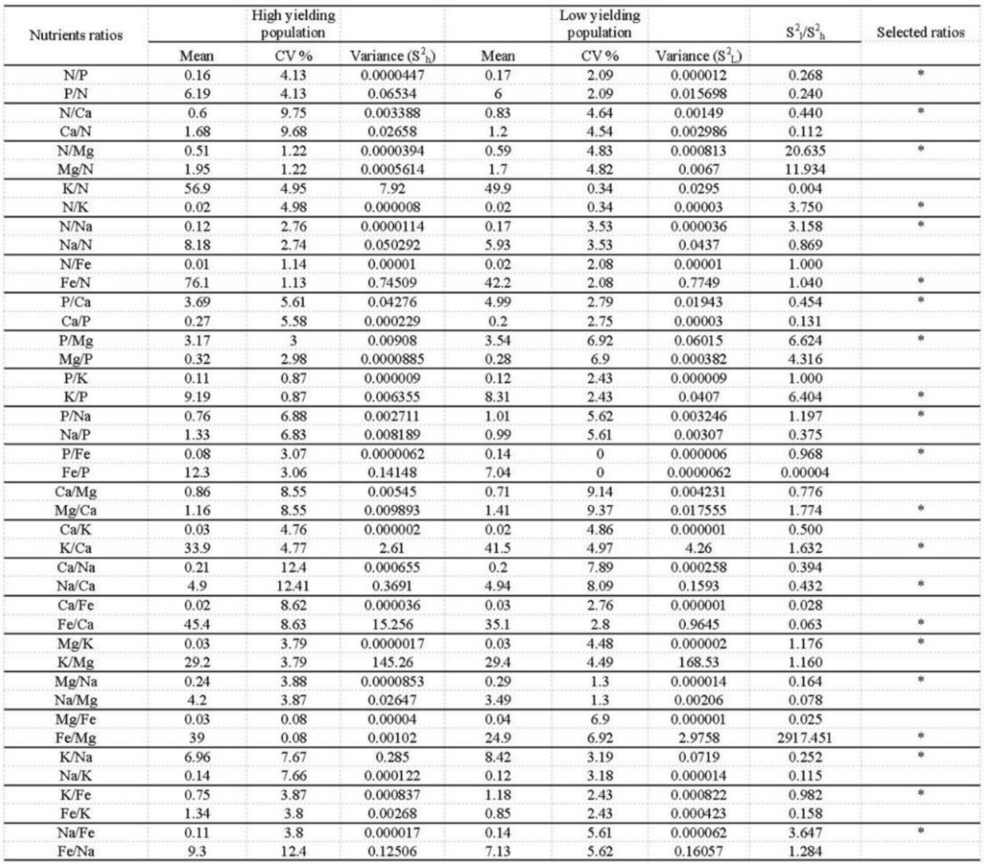
	N (%)	P (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)
Minimum	0.744	1.272	64.71	1.333	1.833	14.62	127.8
Maximum	1.803	14.81	235.3	3.333	5.000	22.15	180.6
Mean	1.152	8.116	153.2	2.172	3.322	19.53	151.7
SD	0.356	3.465	49.95	0.552	0.947	2.187	13.58
Skewness	0.548	0.678	0.123	0.698	0.548	-0.671	0.488

Low yielding population (n=24)

	N (%)	P (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)
Minimum	0.235	3.457	42.94	1.667	1.333	15.06	121.1
Maximum	1.411	10.99	505.9	3.000	5.833	22.15	158.6
Mean	1.111	6.008	157.4	2.257	3.316	17.54	140.1
SD	0.342	2.169	107.4	0.408	1.421	1.952	9.876
Skewness	-1.921	0.291	0.961	0.022	0.288	1.114	0.053

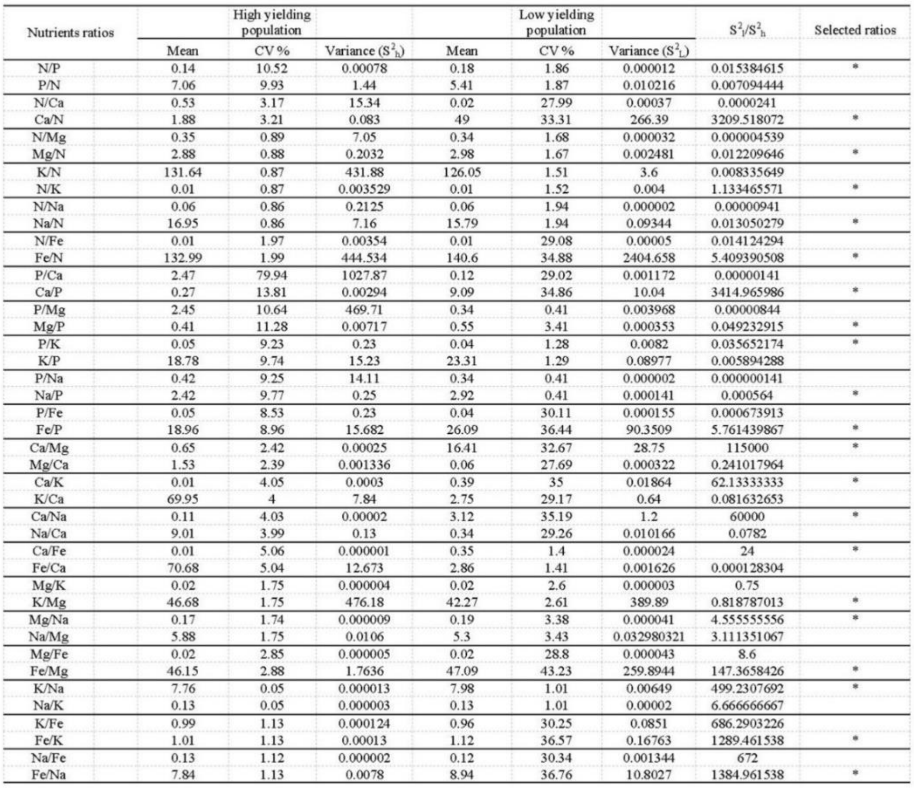
Table **2:**

Comparison of leaf nutrients, nutrient ratio means and coefficient of variance (CV), variance between high and low yielding population in walnut during June 2016.



Nutrients ratios	High yielding population			Low yielding population			S^2_l/S^2_h	Selected ratios
	Mean	CV %	Variance (S^2_h)	Mean	CV %	Variance (S^2_L)		
N/P	0.16	4.13	0.0000447	0.17	2.09	0.000012	0.268	*
P/N	6.19	4.13	0.06534	6	2.09	0.015698	0.240	
N/Ca	0.6	9.75	0.003388	0.83	4.64	0.00149	0.440	*
Ca/N	1.68	9.68	0.02658	1.2	4.54	0.002986	0.112	
N/Mg	0.51	1.22	0.0000394	0.59	4.83	0.000813	20.635	*
Mg/N	1.95	1.22	0.0005614	1.7	4.82	0.0067	11.934	
K/N	56.9	4.95	7.92	49.9	0.34	0.0295	0.004	
N/K	0.02	4.98	0.000008	0.02	0.34	0.00003	3.750	*
N/Na	0.12	2.76	0.0000114	0.17	3.53	0.000036	3.158	*
Na/N	8.18	2.74	0.050292	5.93	3.53	0.0437	0.869	
N/Fe	0.01	1.14	0.00001	0.02	2.08	0.00001	1.000	
Fe/N	76.1	1.13	0.74509	42.2	2.08	0.7749	1.040	*
P/Ca	3.69	5.61	0.04276	4.99	2.79	0.01943	0.454	*
Ca/P	0.27	5.58	0.000229	0.2	2.75	0.00003	0.131	
P/Mg	3.17	3	0.00908	3.54	6.92	0.06015	6.624	*
Mg/P	0.32	2.98	0.0000885	0.28	6.9	0.000382	4.316	
P/K	0.11	0.87	0.000009	0.12	2.43	0.000009	1.000	
K/P	9.19	0.87	0.006355	8.31	2.43	0.0407	6.404	*
P/Na	0.76	6.88	0.002711	1.01	5.62	0.003246	1.197	*
Na/P	1.33	6.83	0.008189	0.99	5.61	0.00307	0.375	
P/Fe	0.08	3.07	0.0000062	0.14	0	0.000006	0.968	*
Fe/P	12.3	3.06	0.14148	7.04	0	0.0000062	0.00004	
Ca/Mg	0.86	8.55	0.00545	0.71	9.14	0.004231	0.776	
Mg/Ca	1.16	8.55	0.009893	1.41	9.37	0.017555	1.774	*
Ca/K	0.03	4.76	0.000002	0.02	4.86	0.000001	0.500	
K/Ca	33.9	4.77	2.61	41.5	4.97	4.26	1.632	*
Ca/Na	0.21	12.4	0.000655	0.2	7.89	0.000258	0.394	
Na/Ca	4.9	12.41	0.3691	4.94	8.09	0.1593	0.432	*
Ca/Fe	0.02	8.62	0.000036	0.03	2.76	0.000001	0.028	
Fe/Ca	45.4	8.63	15.256	35.1	2.8	0.9645	0.063	*
Mg/K	0.03	3.79	0.0000017	0.03	4.48	0.000002	1.176	*
K/Mg	29.2	3.79	145.26	29.4	4.49	168.53	1.160	
Mg/Na	0.24	3.88	0.0000853	0.29	1.3	0.000014	0.164	*
Na/Mg	4.2	3.87	0.02647	3.49	1.3	0.00206	0.078	
Mg/Fe	0.03	0.08	0.00004	0.04	6.9	0.000001	0.025	
Fe/Mg	39	0.08	0.00102	24.9	6.92	2.9758	2917.451	*
K/Na	6.96	7.67	0.285	8.42	3.19	0.0719	0.252	*
Na/K	0.14	7.66	0.000122	0.12	3.18	0.000014	0.115	
K/Fe	0.75	3.87	0.000837	1.18	2.43	0.000822	0.982	*
Fe/K	1.34	3.8	0.00268	0.85	2.43	0.000423	0.158	
Na/Fe	0.11	3.8	0.000017	0.14	5.61	0.000062	3.647	*
Fe/Na	9.3	12.4	0.12506	7.13	5.62	0.16057	1.284	

Table 3: Comparison of leaf nutrients, nutrient ratio means and coefficient of variance (CV), variance between high and low yielding population in walnut during June 2017.



Nutrients ratios	High yielding population			Low yielding population			S^2_l/S^2_h	Selected ratios
	Mean	CV %	Variance (S^2_h)	Mean	CV %	Variance (S^2_L)		
N/P	0.14	10.52	0.00078	0.18	1.86	0.000012	0.015384615	*
P/N	7.06	9.93	1.44	5.41	1.87	0.010216	0.007094444	
N/Ca	0.53	3.17	15.34	0.02	27.99	0.00037	0.0000241	
Ca/N	1.88	3.21	0.083	49	33.31	266.39	3209.518072	*
N/Mg	0.35	0.89	7.05	0.34	1.68	0.000032	0.000004539	
Mg/N	2.88	0.88	0.2032	2.98	1.67	0.002481	0.012209646	*
K/N	131.64	0.87	431.88	126.05	1.51	3.6	0.008335649	
N/K	0.01	0.87	0.003529	0.01	1.52	0.004	1.133465571	*
N/Na	0.06	0.86	0.2125	0.06	1.94	0.000002	0.00000941	
Na/N	16.95	0.86	7.16	15.79	1.94	0.09344	0.013050279	*
N/Fe	0.01	1.97	0.00354	0.01	29.08	0.00005	0.014124294	
Fe/N	132.99	1.99	444.534	140.6	34.88	2404.658	5.409390508	*
P/Ca	2.47	79.94	1027.87	0.12	29.02	0.001172	0.00000141	
Ca/P	0.27	13.81	0.00294	9.09	34.86	10.04	3414.965986	*
P/Mg	2.45	10.64	469.71	0.34	0.41	0.003968	0.00000844	
Mg/P	0.41	11.28	0.00717	0.55	3.41	0.000353	0.049232915	*
P/K	0.05	9.23	0.23	0.04	1.28	0.0082	0.035652174	*
K/P	18.78	9.74	15.23	23.31	1.29	0.08977	0.005894288	
P/Na	0.42	9.25	14.11	0.34	0.41	0.000002	0.000000141	
Na/P	2.42	9.77	0.25	2.92	0.41	0.000141	0.000564	*
P/Fe	0.05	8.53	0.23	0.04	30.11	0.000155	0.000673913	
Fe/P	18.96	8.96	15.682	26.09	36.44	90.3509	5.761439867	*
Ca/Mg	0.65	2.42	0.00025	16.41	32.67	28.75	115000	*
Mg/Ca	1.53	2.39	0.001336	0.06	27.69	0.000322	0.241017964	
Ca/K	0.01	4.05	0.0003	0.39	35	0.01864	62.13333333	*
K/Ca	69.95	4	7.84	2.75	29.17	0.64	0.081632653	
Ca/Na	0.11	4.03	0.00002	3.12	35.19	1.2	60000	*
Na/Ca	9.01	3.99	0.13	0.34	29.26	0.010166	0.0782	
Ca/Fe	0.01	5.06	0.000001	0.35	1.4	0.000024	24	*
Fe/Ca	70.68	5.04	12.673	2.86	1.41	0.001626	0.000128304	
Mg/K	0.02	1.75	0.000004	0.02	2.6	0.000003	0.75	
K/Mg	46.68	1.75	476.18	42.27	2.61	389.89	0.818787013	*
Mg/Na	0.17	1.74	0.000009	0.19	3.38	0.000041	4.555555556	*
Na/Mg	5.88	1.75	0.0106	5.3	3.43	0.032980321	3.111351067	
Mg/Fe	0.02	2.85	0.000005	0.02	28.8	0.000043	8.6	
Fe/Mg	46.15	2.88	1.7636	47.09	43.23	259.8944	147.3658426	*
K/Na	7.76	0.05	0.000013	7.98	1.01	0.00649	499.2307692	*
Na/K	0.13	0.05	0.000003	0.13	1.01	0.00002	6.666666667	
K/Fe	0.99	1.13	0.000124	0.96	30.25	0.0851	686.2903226	
Fe/K	1.01	1.13	0.00013	1.12	36.57	0.16763	1289.461538	*
Na/Fe	0.13	1.12	0.000002	0.12	30.34	0.001344	672	
Fe/Na	7.84	1.13	0.0078	8.94	36.76	10.8027	1384.961538	*

Depending on DRIS chart Figures (2 and 3), the origin point in the center of the chart is the mean of each expression N/P, N/K and K/P for the population of high yielding plants, this is the composition required to increase the possibility of gaining a high yield. In another word, the ratio within the origin (in the center of the small circle) represents the optimum (balance) values of the studied nutrient concentrations for high yield of the plant. The nutrient ratio values situated between the two circles represented the critical value (imbalance). The outer values of both circles are high sufficiency or high deficiency depending on the direction of the arrow. Arrow points up as an index to sufficiency, while the downward arrow represents deficiency and the right the optimum or adequacy. The results in Figure 2 explain that, if the ratio between N/P is greater than 0.1776, the concentration of nitrogen increases while the concentration of phosphorus decreases, in this case, the phosphorus requirement of the plant increases. Moreover, if the N/P ratio is lower than 0.1424 the plant requirement for nitrogen increases, the same explanation for other nutrient ratios is true (Beaufils, 1971, Beaufils, 1973).

The results of DRIS indices are shown in Tables (4 and 5). The most limiting nutrient descending order are as Fe>Na>Ca>K>P>N>Mg respectively for first-year samples, and are arranged as N>Na>P>Fe>Mg>Ca>K respectively for the second-year samples. Nitrogen deficiency is claimed to be the lack of the main nutrient that curbs plant growth and can also regulate forest ecosystem succession patterns (Kirchmann *et al.*, 2007). Compost or manure is commonly used biofertilizer as a source of N to minimize the input on the system. Adding these nutrients can give a positive effect on the soil's availability of P (Ohno *et al.*, 2005). Residues of plant deciduous leaves and animal waste decomposed as elements fulfil the need for essential nutrients for walnut productivity.

The smallest NBI value was 879 with an average NBI 125.6 for sample 8 during 2016, which produced from DRIS indices 219.9, 118.9, -8.06, 80.79, 19.99, -48.03 and -383.5 for N, P, Ca, Mg, K, Na and Fe respectively and gave a yield of 15.54 Kg.tree^{-1}. While, sample 4 for second-year samples 2017 was more close to zero with NBI value 192.4 and an average NBI 27.2, which attained from DRIS indices 22, 2.51, -10.2, -75.94, 32.6, 39.12 and -10.1 for N, P, Ca, Mg, K, Na and Fe respectively and produce a yield of 13.27 Kg.tree^{-1}. The highest NBI values for both years were 19063.4 and 4137.3 respectively for samples 2 and 18 respectively with deficiencies Fe for the first year and each of N and Fe for the second-year sample. Generally, increases in Fe deficiency during second-year sampling were observed compared to first-year sampling.

3.2 Principal component analysis (PCA):

PCA was performed for high and low yield population and DRIS indices as shown in Table (6). Retained PCs with eigenvalue more than one represented the greatest variability in the data set was consist of the first three PCs for high yield, and two PCs for low yield and DRIS indices with explained 61.95%, 87.75% and 58.67% respectively for first-year sampling 2016. Under each PC, the nutrient with eigenvector larger than the selection score (SC) was selected. The first PC of the high yield population had a positive correlation with N, P, and K and negative loading for Ca and Na, and it designated as (N+P+Ca-Na-K+). The fundamental role of calcium in membrane stability and cell integrity by bridging phosphate and carboxylate groups of phospholipids and proteins, preferably on membrane surfaces, may involve an exchange of calcium at these binding sites with other cations (K+ and Na+) (Marschner, 2011). A very high correlation in PC1 represented by N, K and P respectively. there is an inverse relationship between foliage nutrients (N, P, K) in one direction and Ca and Na on the opposite direction. For the second PC, was expressed as loading for (Fe+Mg+Na+). All variables were changed in the same direction. While PCA conducted on the low yield population produced three PCs with eigenvalues up to 3.22 and total variance of 87.75%. The first three PCs variances were explained by 46.11%, 24.47% and 17.16% respectively. PC1 had positive loading with N, P and inverse negative loading with Fe and Mg, as it designed (N+P+Fe-Mg-Na+). The contrasting relationship between N+P+Na and Mg+Fe was obtained. Fe deficiency may occur in plants grown on alkaline soils rich in Ca and Mg (Darwesh, 2011). While (Havlin *et al.*, 1999) stated that excessive phosphate anions could prevent Fe uptake and used by plants.

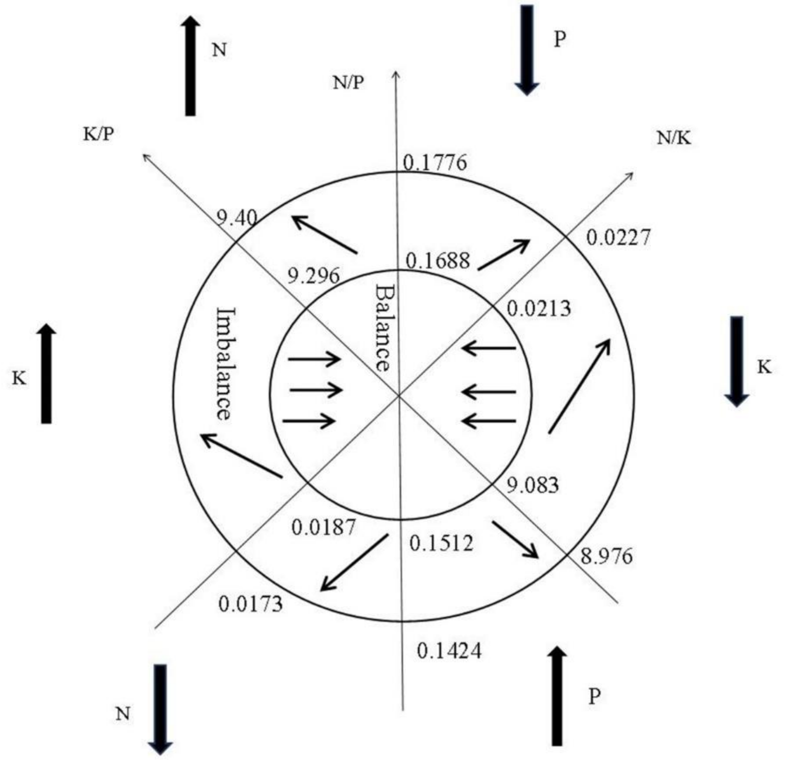


Figure 2: DRIS indices chart explain the optimum and critical values of some nutrients ratio for first-year samples 2016.

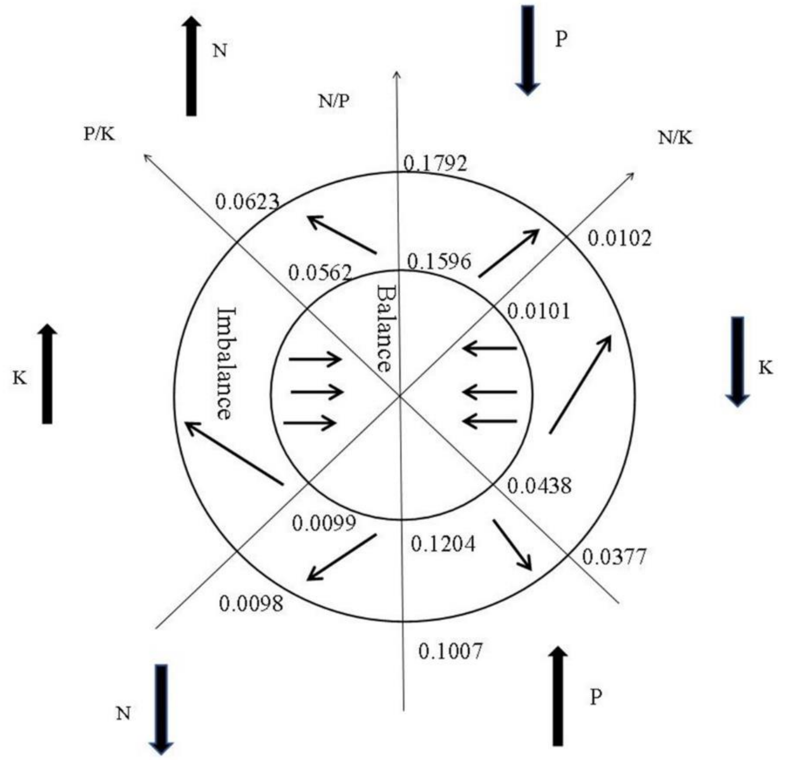


Figure 3: DRIS indices chart explain the optimum and critical values of some nutrients ratio for second-year samples 2017.

Table 4: Diagnosis of nutrient imbalance by DRIS indices in walnut during June 2016.

	NI	PI	CaI	MgI	KI	NaI	FeI	NBI	Average NBI
1.00	-70.87	-17.33	28.70	1413.84	43.33	27.22	-1424.88	3026.16	432.31
2.00	140.31	99.70	139.86	8686.13	215.87	249.83	-9531.70	19063.40	2723.34
3.00	70.77	-160.41	109.74	1973.71	87.86	152.98	-2234.64	4790.10	684.30
4.00	-193.87	-157.67	21.13	-2843.15	-93.40	-74.85	3341.82	6725.90	960.84
5.00	-368.11	-339.57	20.72	-1144.20	16.40	15.44	1799.32	3703.76	529.11
6.00	-145.74	-48.58	-62.60	-289.28	-82.18	-37.35	665.74	1331.47	190.21
7.00	61.07	-65.52	-9.59	1938.09	31.59	-171.71	-1783.93	4061.50	580.21
8.00	219.93	118.89	-8.06	80.79	19.99	-48.03	-383.51	879.20	125.60
9.00	98.33	-17.90	-18.19	-600.49	2.67	-40.99	576.58	1355.15	193.59
10.00	381.14	23.80	-35.97	1105.27	119.41	-31.73	-1561.92	3259.24	465.61
11.00	214.85	71.50	-11.37	1451.88	10.37	-30.65	-1706.59	3497.21	499.60
12.00	225.44	73.96	-9.59	1309.74	19.31	-28.09	-1590.77	3256.90	465.27
13.00	144.25	194.21	17.97	759.27	-22.67	0.33	-1093.37	2232.07	318.87
14.00	163.50	237.69	-32.97	1277.08	-22.57	49.93	-1672.66	3456.39	493.77
15.00	47.83	102.94	18.30	480.67	-10.52	20.75	-659.96	1340.95	191.56
16.00	243.30	107.21	-24.97	339.21	63.13	49.90	-777.79	1605.52	229.36
17.00	291.82	159.08	-19.15	1501.62	13.98	-3.35	-1944.00	3933.00	561.86
18.00	198.74	265.28	-4.67	636.73	-112.23	-25.06	-958.79	2201.50	314.50

Table 5: Diagnosis of nutrient imbalance by DRIS indices in walnut during June 2017.

	NI	PI	CaI	MgI	KI	NaI	FeI	NBI	Average NBI
1.00	-232.64	-25.36	240.15	-214.93	-695.89	966.49	-37.83	2413.27	344.75
2.00	50.92	-13.97	-27.69	80.50	684.82	-666.46	-108.13	1632.49	233.21
3.00	198.71	-17.43	-37.71	-197.28	154.62	-322.31	221.39	1149.44	164.21
4.00	22.03	2.51	-10.21	-75.94	32.60	39.12	-10.10	192.49	27.50
5.00	-131.20	-116.20	-72.84	359.84	-760.26	589.51	131.16	2161.02	308.72
6.00	-474.66	-21.00	-32.47	418.92	872.28	-927.40	164.33	2911.07	415.87
7.00	-72.99	-121.11	85.18	-16.56	533.93	-482.10	73.66	1385.53	197.93
8.00	-187.05	-0.53	13.35	234.53	48.99	-1.55	-107.74	593.74	84.82
9.00	-238.61	-77.58	153.63	-166.70	-317.59	385.15	261.70	1600.96	228.71
10.00	-526.19	-39.96	10.48	335.10	-75.23	263.49	32.31	1282.77	183.25
11.00	83.64	-71.64	71.33	140.96	-74.36	-187.96	38.04	667.94	95.42
12.00	-168.56	-57.34	31.54	239.99	-275.52	194.65	35.23	1002.84	143.26
13.00	286.60	-14.59	-3.63	-118.95	255.50	-264.19	-140.73	1084.19	154.88
14.00	-214.74	122.23	-33.29	133.47	-279.85	926.01	-653.83	2363.42	337.63
15.00	474.78	84.59	-54.36	-343.11	1042.99	-956.25	-248.64	3204.72	457.82
16.00	244.05	-27.20	82.45	-336.25	629.93	-716.64	123.67	2160.18	308.60
17.00	366.54	52.78	152.40	-745.74	-268.51	183.50	259.04	2028.50	289.79
18.00	-1827.70	63.80	211.76	153.11	1371.37	268.66	-240.99	4137.39	591.06

Table 6: Principal component analysis of nutrient concentration for high and low yield population, as well as DRIS indices in walnut during June 2016.

	High yield		Low yield			DRIS index	
Nutrient	PC1	PC2	PC1	PC2	PC3	PC1	PC2
N	*0.825**	0.140	*0.280**	*0.849**	-0.252	-0.182	*0.744**
P	*0.767**	-0.130	*0.905**	-0.180	-0.016	0.087	*0.760**
Fe	0.115	*0.840**	*-0.754**	-0.009	*0.482**	*0.896**	0.261
Ca	*-0.754**	0.019	0.025	0.002	*0.963**	*0.625**	-0.294
Mg	-0.030	*0.845**	*-0.965**	-0.144	-0.014	*0.838**	0.036
Na	*-0.458**	*0.435**	*0.791**	*0.431**	0.145	*0.635**	-0.063
K	*0.793**	0.027	-0.136	*0.927**	0.194	*0.502**	*0.475**
Eigen value	2.702	1.635	3.228	1.713	1.202	2.631	1.477
% Variance	38.59	23.36	46.11	24.47	17.16	37.58	21.09
Total variance %	38.59	61.95	46.11	70.58	87.75	37.58	58.67
Selection Criteria	0.304	0.391	0.278	0.382	0.456	0.308	0.411
	PC1=N+P+Ca-Na-K+		PC1=P+Fe-Mg-Na+			PC1= Fe+Ca+Mg+Na+K+	
	PC2=Fe+Mg+Na+		PC2= N+Na+K+			PC2=N+P+K+	
			PC3= Fe+Ca+				

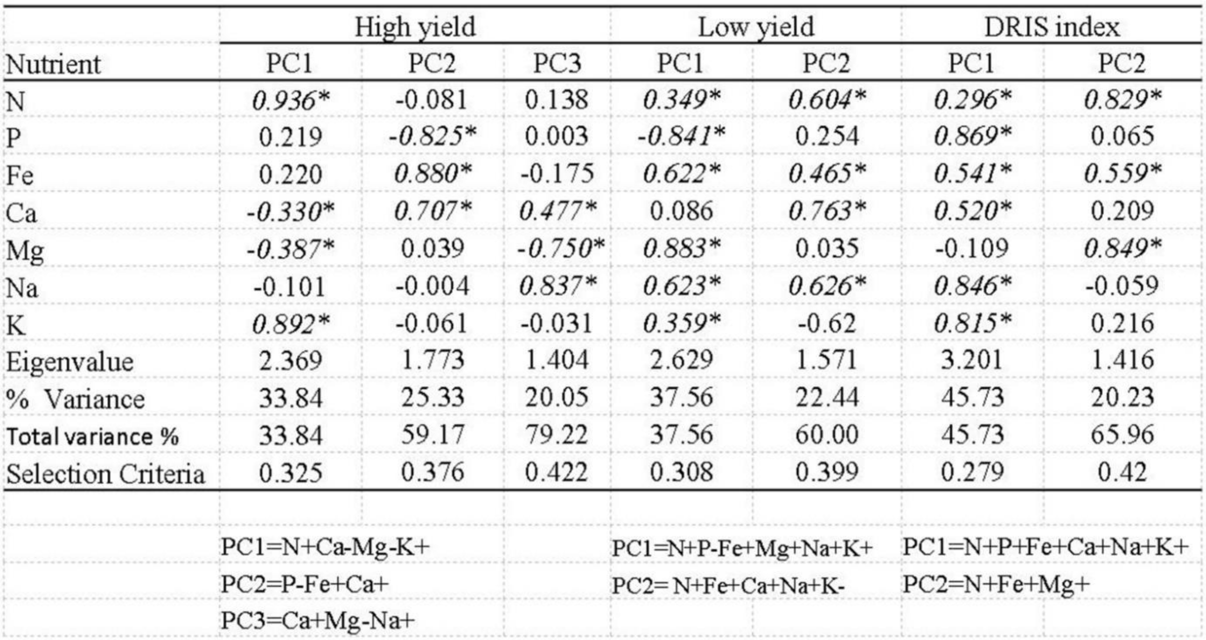
The PC2 and PC3 (N+Na+K) and (Fe+Ca) was performed with a positive relationship and concentration changes in one direction. PCA on DRIS indices indicated of nutrients in two PCs that was designated as (N+P+Fe+Ca+Na+K) and (N+Fe+Mg+) respectively with total variance explained by 37.58% and 21.09% respectively. The positive relationship between nutrients obtained with highest loads for Fe and Mg in PC1and P, N in PC2. The positive interaction between P and N become commonly held view (Raghupathi *et al.*, 2005).

For second-year sampling 2017, PCA conducted on high yield population produced three PCs that represented by 33.84%, 25.33% and 20.05% of the variances respectively Table (7). Several nutrients involvement in PC1 indicated an interaction between foliar nutrients, with positive loading for both N(0.936) and K(0.892) and negative loading for each one Ca (-0.330) and Mg(-0.387) as designated (N+Ca-Mg-K+). Deficiency of Ca and Mg in leaf tissues leads to a decrease in plant productivity. Ca play an essential role in membrane permeability, protein synthesis and carbohydrate transfer; while Mg is the main component in chlorophyll structure(Hirons and Thomas, 2018, Taiz and Zeiger, 2006). On the other hand, PC2 had shown positive loading for Fe and Ca with opposite change direction for P with high negative loading (-0.825). The third PC related to a positive correlation with Ca and Na and negative correlation to Mg(-0.75).

For the low yielding population, only two PCs were retained with explaining 60% of total variance. The PC1 was effective in separating chemical nutrients into two groups based on their mobility. Phosphorus behalves in one direction with negative correlation (-0.841), while N, Fe, Mg, Na and K designated as (N+Fe+Ca+Na+) in the other direction with 22.44% of total variance. All these nutrients are essential in plant metabolic processes, such as synthesis protein, enzymes activity, chlorophyll, osmoregulation, phosphorylation and enzyme activation(Duca, 2015). The calcareous soil is widely distributed worldwide including soils in the Kurdistan region. The availability of phosphorus in this soil is low due to the high content of calcium carbonate ($CaCO_3$), which has led to phosphorus being chemically fixed (Esmail, 2012).

Finally, PCA was performed on DRIS indices which produced two PCs with 45.73% and 20.23% of explained total variance. Both PCs was designated as (N+P+Fe+Ca+Na+K+) and (N+Fe+Mg) respectively, in positive correlation and one direction change. Mg (a component of the chlorophyll molecule) and P and Fe play a direct role in photosynthesis. If these elements are not present in sufficient levels, photosynthetic activity will be significantly reduced (Jones Jr, 2012, Barker and Pilbeam, 2015).

Table 7: Principal component analysis of nutrient concentration for high and low yield population, as well as DRIS indices in walnut during June 2017.



	High yield			Low yield		DRIS index	
Nutrient	PC1	PC2	PC3	PC1	PC2	PC1	PC2
N	0.936*	-0.081	0.138	0.349*	0.604*	0.296*	0.829*
P	0.219	-0.825*	0.003	-0.841*	0.254	0.869*	0.065
Fe	0.220	0.880*	-0.175	0.622*	0.465*	0.541*	0.559*
Ca	-0.330*	0.707*	0.477*	0.086	0.763*	0.520*	0.209
Mg	-0.387*	0.039	-0.750*	0.883*	0.035	-0.109	0.849*
Na	-0.101	-0.004	0.837*	0.623*	0.626*	0.846*	-0.059
K	0.892*	-0.061	-0.031	0.359*	-0.62	0.815*	0.216
Eigenvalue	2.369	1.773	1.404	2.629	1.571	3.201	1.416
% Variance	33.84	25.33	20.05	37.56	22.44	45.73	20.23
Total variance %	33.84	59.17	79.22	37.56	60.00	45.73	65.96
Selection Criteria	0.325	0.376	0.422	0.308	0.399	0.279	0.42
	PC1=N+Ca-Mg-K+			PC1=N+P-Fe+Mg+Na+K+		PC1=N+P+Fe+Ca+Na+K+	
	PC2=P-Fe+Ca+			PC2= N+Fe+Ca+Na+K-		PC2=N+Fe+Mg+	
	PC3=Ca+Mg-Na+						

4. CONCLUSIONS

By developing the first DRIS walnut standards for northern Iraq, the results indicated that the local or regional DRIS norm is more convenient for local areas derived from special environmental conditions. It enables DRIS to demonstrate more reliable nutrient diagnosis deficiencies in the walnut tree. The obtained results from PCA reveal that it gives an opportunity for more accurate nutrient diagnosis deficiencies which confirm the DRIS approach. Depending on the average NBI values of the second-year samples were closer to adequate condition than the first-year samples. Generally, Fe was the most deficient nutrient. The amount of nutrient inputs back to the soil comes only from leaves falling plants it is not sufficient to a fulfilled deficit of nutrients. Application of bio-fertilizer compost and manure may be the best choice to compensate for nutrient deficiencies.

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Conflict of Interest (1)

There is no conflict of interest among the authors.

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