Study on the effect of N-level fertilization on constituents of grape leaves and fruit quality using FT-IR spectroscopy

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Abstract: This study was carried out during 2005 and 2006 in a private orchard locates at 62 km. of the Cairo – Alexandria desert road.Fourier Transform Infrared (FT-IR) spectroscopic technique was used in the present work to study the effect of N-fertilization levels on the concentrations of chlorophyll, A , B and caretenoids in grape leaves and fruit quality of grapes . Ammonium nitrate (N-fertilizer) was applied after the beginning of vegetation growth to supply 33.3%, 66.6%, 100%, 133.3%, 166.6% of the recommended N-fertilizer level. (The recommended N-fertilizer level according to the ministry of Agriculture is 50 units N and this consider as the level of 100% for comparing during N application). Chlorophyll A, Chlorophyll B and total Carotenoieds were determined by using visible spectroscopic techniques at wave lengths 470nm, 645nm, and 662nm respectively. It was found that for the leaves of the first three samples the concentrations of Chlorophyll A, Chlorophyll B and Total Carotenoieds increase with increasing of N-level. For the forth sample the rate of increasing of the concentration of pigment is characterized by a first sharp increase up to 100% followed by slow increase up to 166%. As the fifth and sixth sample the increases in the pigment concentrations with increasing N-level assumes the maximum values at 100% N-level then decrease with increasing N-level. This results obtained by using visible spectroscopic techniques is in good agreement with the results obtained by FT-IR spectroscopy. The determined values of ash content of leaves indicated that the fraction of ash content increases with the increase of the N-level from 33% to 100% and then slightly decreases with increasing the N-level to 133% and 166%. This means that the ash content of the leaves is influenced by the N-level.The fruiting data give strong evidence that the length of the clusters increases with increasing N-level until reaching the maximum in 100% treatment (the recommended level), also the T.S.S (Total Soluble Solid) and T.S.S/ Acidity ratio recorded the highest values at 100% treatment which reflect the degree of maturity and the quality of the grape production. [Journal of American Science. 2011;7(1):126-134]. (ISSN: 1545-1003).

Keywords: Species richness; beta-diversity; taxonomic diversity; forest

1. Introduction

The pattern and relationships between species diversity and ecosystem functioning are the current areas of great ecological interest throughout the world. Species diversity incorporates two components (Stirling and Wilsey, 2001); evenness (how evenly abundance or biomass is distributed among species) and richness (number of species per unit area). High evenness can increase invasion resistance, below-ground productivity and reduce total extinction rates (Smith et al., 2004). The spatial variations in biodiversity generally include species diversity in relation to size of the area, relationship between local and regional species diversity and diversity along gradients across space, and environmental factors such as latitude, altitude, depth, isolation, moisture and productivity (Gaston, 2000). In addition, species richness of a taxon is not only sufficient to express diversity but the equitability is also a important factor because communities however vary in properties of the total importance of the species and share their functional contribution (Tilman, 2000).

A fundamental characteristic of mountain ecosystems is to the drastic change in vegetation as well as in climatic conditions from the base to the summit of the mountain. Elevation gradients create varied climates, along with resultant soil differentiation; promote the diversification of plant species (Brown, 2001). Many studies have investigated on species richness along elevation gradient across habit and taxa (Sanders et al., 2003), as part efforts to understand ecosystem effects on biodiversity and maintenance of biodiversity (Gytnes and Vetaas, 2002). Furthermore, the observation relations between species distribution and elevation bands may also help to understand the possible effects of climate change, e.g. by providing baseline information to measure the effect of climate change and anthropogenic changes on vegetation.

The forest herbs, which play important role for rural communities for example, the livestock totally dependent on them for fodder and as traditional medicines, have been hardly studied from diversity standpoint (Singh and Singh 1987). Quantitative information on the forest floor species of the Central Himalaya region is generally lacking except for studies done by Rawat and Singh (1989), and Singh and Singh (1992). Interestingly, most of the recent major field experiments addressed questions relating to species diversity which has been carried out in grasslands. But forest herbs of the Himalayan region remain poorly studied.

In the present study we investigate herb species richness (spermatophyte) in terms of taxonomical diversity and species composition in relation to oak and pine forests in Central Himalayan forests.

2. Material and Methods

A study site was located at Cairo- Alex. Desert road, and selected under the same environmental condition, age, and plant growth condition.

Ammonium nitrate was applied after planting to supply 33.3%, 66.6%, 100%, 133.3%, 166.6% of the recommended rate (The 100% N application equals 50 units N and it is considered the recommended dose according to the ministry of Agriculture)

Leaves samples:

Shortly after a thesis fully expanded leaves which sites at number 5, 6 from the base of the original branch of the remarked vines were excised and placed in a plastic bag in an ice chest and transported into the laboratory for spectral measurement.

Ash content of the leaves:

The fresh leaves were weighted then the leaves were dried at $60C^0$ for time enough to maintaining a constant weight. The dry leaves were ignited in muffle furnace at 650 C^0 and weighted until their weights being contestant and then the samples were stored for FT-IR examination.

Chlorophylls *a* and *b* and total carotenoids determination in plant leaves:

Before FT-IR measurements were carried out, chlorophyll concentration of the same leaves were determined. A circular disks which have 6 mm in diameter, were punched from the same general area of the leaf for which FTIR were measured. The disks were placed immediately into 8 ml of 100% acetone, and pigments were allowed to extract in the dark at $37C^0$ for 24 h. Absorbances of the clear extract at 470nm, 645nm, and 662nm were recorded by using (spectrophotometer 640) and concentrations of chlorophyll a, b, and carotenoids were calculated by using the equation of lichtenthaler's equations (1983). The concentrations were measured in mg/cm^2.

Fruit quality determination

Length of the cluster (cm.), length of berry (cm.), T.S.S. (%), acidity and T.S.S. / Acidity were determined at the collection time of the crop after reaching maturity stage.

3. Results and Discussion

The effect of N-Level on pigment analysis:

Tables (1.2.3.4.5.and 6) shows the concentrations of Chlorophyll A, chlorophyll B and Total Carotenoieds for sample No.1, 2, 3, 4, 5, 6 respectively, The relation between the concentrations of Chl.A, Chl.b, Total Carotenoieds and N-fertilizer levels is shown in and figures (1.2.3.4.5.and 6) respectively.

It is clear from the data that Chlorophyll A, Chlorophyll B and Total Carotenoieds concentrations increases with increase N-Level till the third sample.

As the forth sample the rate of increases after 100% N-level was slightly lowered. As the fifth and sixth sample there were increases in the pigment concentrations with increasing N-level to get maximum values at 100% N-level then decrease with increasing N-level.

This result could be explained that in the samples from first to third, the leaves growth is occurred rapidly that means that in this period the vines mainly concerned with leaves growth. Also the results could be explained that from the forth till the sixth samples the vines are concerned with fruiting.

Table (1): Concentrations of Chlorophyll A, Chlorophyll B and Total Carotenoieds in grape leaves versus N-levels

N-Level	Chlorophyll A ($\mu g/cm^2$)	Chlorophyll B ($\mu g/cm^2$)	Total Carotenoied ($\mu g/cm^2$)
33.3%	2.77635	1.13295	0.885879
66.6%	3.70461	1.49047	0.915758
100%	4.06539	1.62643	1.143425
133.3%	4.37258	1.65166	1.145062
166.6%	4.65632	1.76244	1.169516

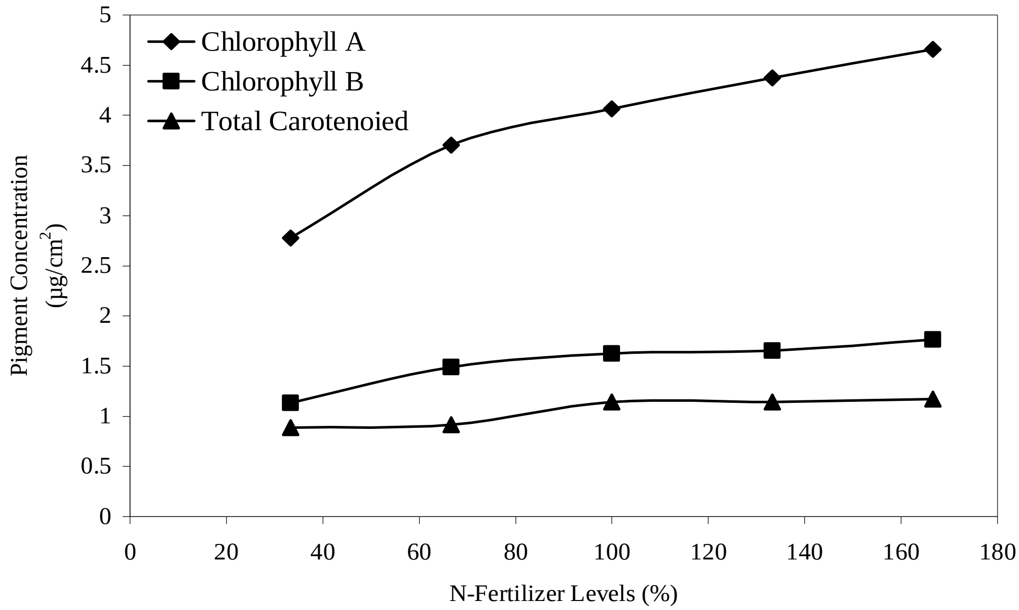

Figure (1): The relationship between Pigment concentrations in grape leaves versus N-fertilizer Level.

Table (2): Concentrations of Chlorophyll A, Chlorophyll B and Total Carotenoieds in grape leaves versus N-levels.

N- level	Chlorophyll A ($\mu g/cm^2$)	Chlorophyll B ($\mu g/cm^2$)	Total Carotenoieds ($\mu g/cm^2$)
33.3%	2.43253	1.12281	0.551865
66.6%	3.29131	1.56587	0.732982
100%	4.00337	1.57609	1.008791
133.3%	4.6602	2.0644	0.995875
166.6%	5.3366	2.1652	1.014839

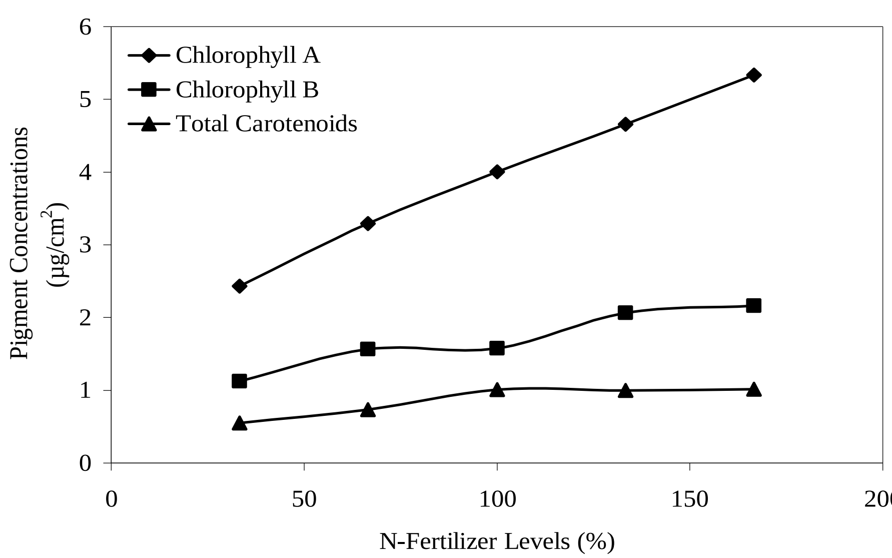

Figure (2): The relationship between Pigment concentrations in grape leaves versus N-fertilizer level**.**

Table (3): Concentrations of Chlorophyll A, Chlorophyll B and Total Carotenoieds in grape leaves versus N-levels.

N-level	Chlorophyll A (µg/cm^2)	Chlorophyll B (µg/cm^2)	Total Carotenoied (µg/cm^2)
33.3%	2.23988	0.93656	0.468165
66.6%	2.97007	1.64129	0.465171
100%	3.92071	1.59117	0.751972
133.3%	5.33379	2.18533	1.058521
166.6%	5.88722	2.50754	1.021871

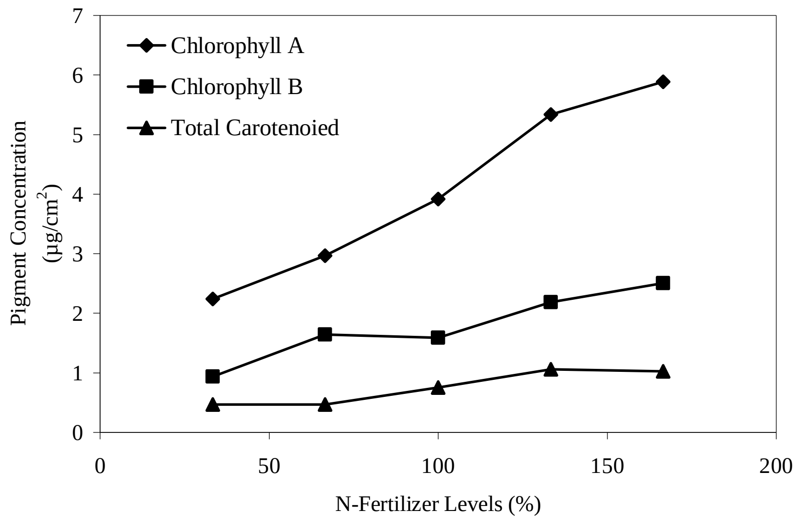

Figure (3): The relationship between Pigment concentrations in grape leaves versus N-fertilizer level.

Table (4): Concentrations of Chlorophyll A, Chlorophyll B and total Carotenoieds in grape leaves versus N-levels.

N-level	Chlorophyll A (µg/cm^2)	Chlorophyll B (µg/cm^2)	Total Carotenoieds (µg/cm^2)
33.3%	1.87154	0.68988	0.590892
66.6%	2.78197	1.09269	0.77649
100%	4.28139	1.48053	1.123457
133.3%	4.4909	1.546	1.086039
166.6%	4.49846	1.65672	1.06604

Table (5): Concentrations of Chlorophyll A, Chlorophyll B and total Carotenoieds in grape leaves versus N-levels.

N-level	Chlorophyll A (µg/cm^2)	Chlorophyll B (µg/cm^2)	Total Carotenoieds (µg/cm^2)
33.3%	1.974405	0.694935	0.556258
66.6%	2.17592	0.73524	0.574536
100%	5.10364	2.18528	1.119128
133.3%	5.01245	2.01415	1.12836
166.6%	4.84713	2.04431	0.936307

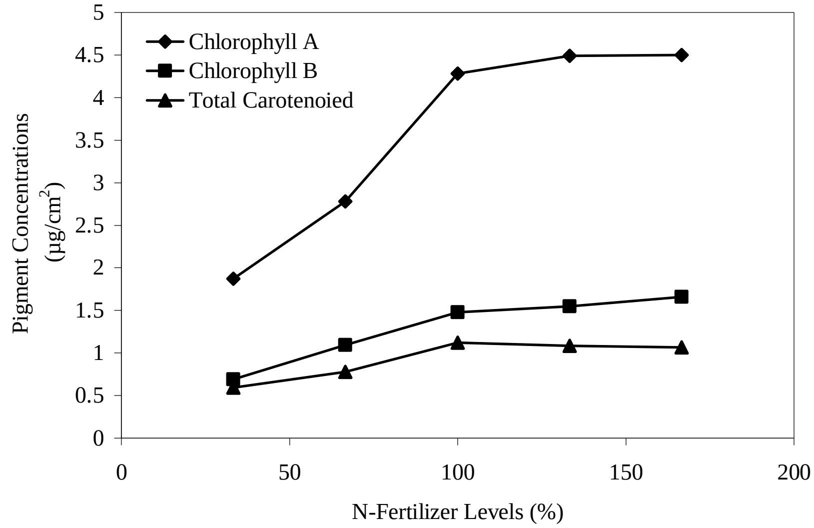

Figure (4) : The relationship between Pigment concentrations in grape leaves versus N-fertilizer level.

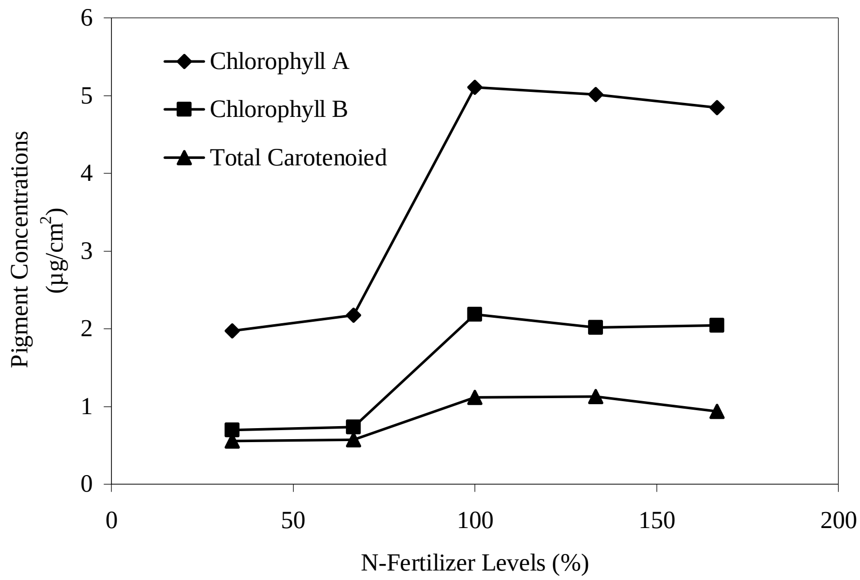

Figure (5): The relationship between Pigment concentrations in grape leaves versus N-fertilizer level.

Table (6): Concentrations of Chlorophyll A, Chlorophyll B and total Carotenoieds in grape leaves versus N-levels.

N-level	**Chlorophyll A ($\mu g/cm^2$)**	**Chlorophyll B ($\mu g/cm^2$)**	**Total Carotenoieds ($\mu g/cm^2$)**
33.3%	2.0763	0.6245	0.428469
66.6%	2.20509	0.85603	0.58045
100%	5.15335	1.99405	1.016596
133.3%	4.80575	1.92855	0.94148
166.6%	4.5474	1.8832	0.90871

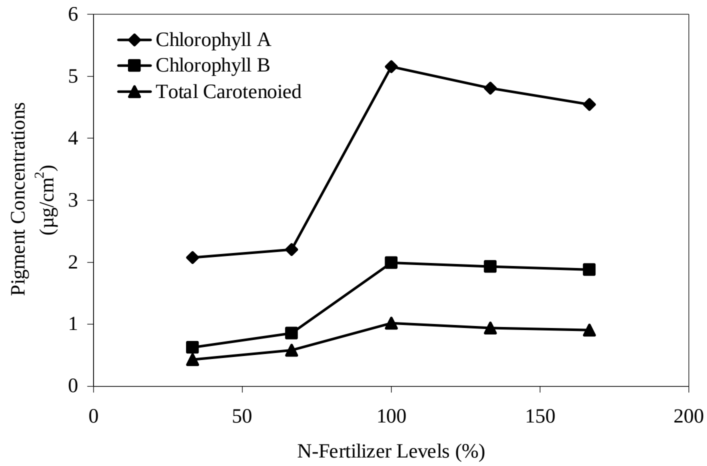

Figure (6): The relationship between Pigment concentrations in grape leaves versus N-fertilizer level

Ash content of the plant leaves

The plant leaves were ignited in muffle oven at 650C^0 until the samples gives a constant weight. Figure (7) shows the FT-IR spectrum of the ash of plant leaves.

The spectrum shows peaks at 3641 cm^{-1}, 1645 cm^{-1} and 567 cm^{-1} which are assigned as stretching and bending vibrations of the (PO_4) ions. The peaks at 1791 cm^{-1}, 1410 cm^{-1} and 882 cm^{-1} which are represent the stretching vibration and bending vibration of the (CO_3) ions. The spectrum also contains peaks at 1113 cm^{-1}, 1046 cm^{-1} and 672 cm^{-1}which are attributed to stretching and bending vibrations of the (SO_4) ions. Therefore, from the above assignment can concluded that the ash of the plant leaf contains PO_4, CO_3 and SO_4 ions.

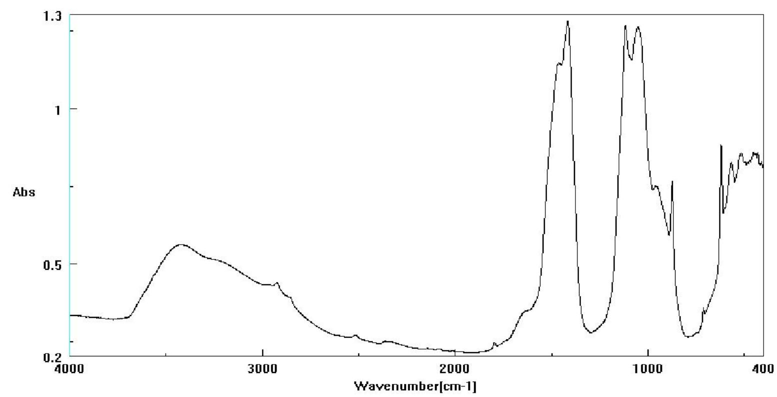

Figure (7): FT-IR spectrum of the ash powder of grape leaves.

Table (7): The wet, dry and ash weights of the grape leaves and their percentage weights.

N-level	Wet Weight(g)	Dry Weight(g)	Ash Weight(g)	Dry/Wet	Ash/Wet	Ash/Dry
33%	3751	910.5	57	24.27 %	1.519 %	6.260 %
66%	4046	1013	63.7	25.03 %	1.574 %	6.288 %
100%	3838.3	1073	70.7	27.95 %	1.841 %	6.589 %
133%	3924.8	1080.3	69.2	27.52 %	1.763 %	6.405 %
166%	3722.6	1036	66.4	27.83 %	1.78 %	6.409 %

Analysis of the data given in table (7) reveals that the fraction of the ash contents assumes a maximum values in the leaves treated with 100% N-level and a minimum values in the leaves treated with 33% N-level.It appears also that this fraction increases with the increase of the N-level from 33% to 100% and then slightly decreases with increases the N-level to 133% and 166%.

This means that the ash content of the leaves is influenced by the N-level.

The effect of N-Fertilizer levels on grape quality.

Length of the clusters is considered as one of the important feature of grape production.

It is clear from data given in table (8) that the length of the clusters increase with increasing N-level reaching the maximum in 100% treatment (the recommended dose). The length of the cluster in 33% treatment recorded 20.6 cm in comparing with 23 cm in 100% treatment. The percent of increment reached 11.7%. The same findings are noticed in the effect of N-level on length and diameter of berries per cluster. The percent of increment in 100% treatment comparing with 33% treatment recorded 14.9% and 10.8% respectively. It's clear from the figures (8, 9, and 10) that the T.S.S (Total soluble solid) and T.S.S/ Acidity ratio recorded the highest values at 100% treatment which reflect the degree of maturity and the quality of the grape production.

Development of sugar, which is measured in terms of TSS content, is a better measure of ripening. At maturity TSS in grapes vary from 16 to 18 percent and to developed quality control for the grape production determination of the shape, size and T.S.S of barriers are highly recommended. K.N. Tiwari (2005).

Table (8): Yield and Physical characteristics of grape Fruits versus N-fertilizer levels.

N-Level / characteristics	33%	66%	100%	133%	166%
Length of the cluster(cm)	20.6	19.3	23	20.7	22.8
Length of Berry (cm)	1.61	1.85	1.85	1.87	1.84
Diameter of Berry (cm)	1.57	1.73	1.74	1.78	1.73
T.S.S (%)	17.50	17.17	18.00	17.60	17.92
Acidity (%)	7.14	6.96	6.25	7.08	7.17
T.S.S/Acidity	2.45098	2.467	2.88	2.486	2.499

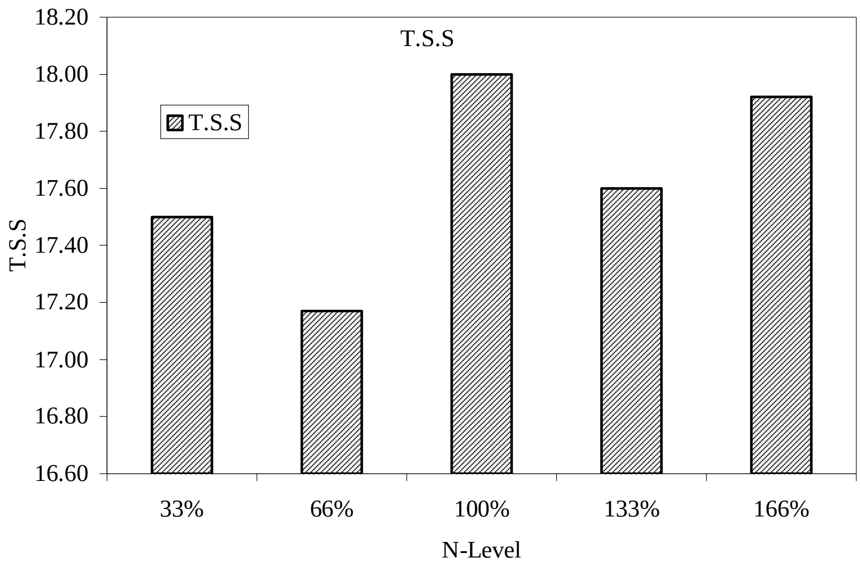

Figure (8): TSS versus N-fertilizer levels of grape fruits

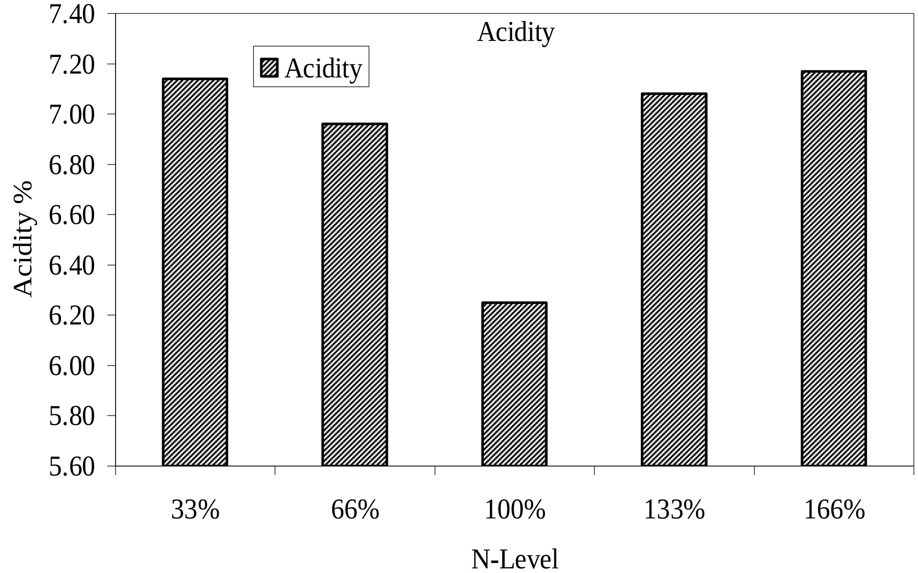

Figure (9): Acidity percent versus N-fertilizer levels of grape fruits

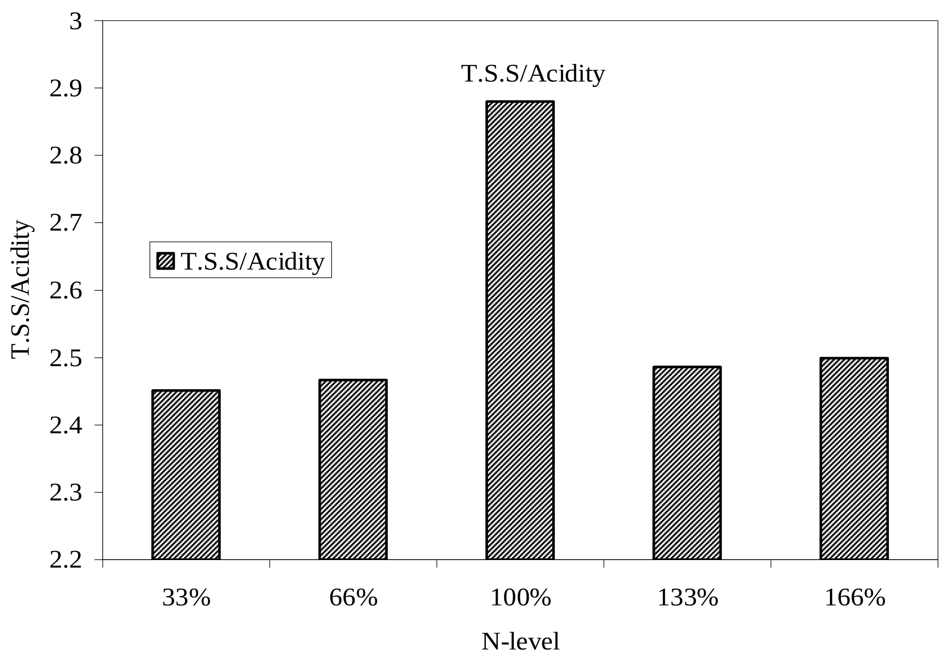

Figure (10) : TSS/Acidity of grape fruits versus N-fertilizer levels

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