Ain Shams University
Faculty of science
Physics Department

Spectroscopic Studies on the effect of Nitrogen Fertilization levels on the soil on the concentrations of Chlorophyll, Nitrogen and Minerals of plant leaves

THESIS

Submitted to the faculty of Science – Ain Shams University In partial fulfillment for Degree of M.Sc in Biophysics

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APPROVAL SHEET

Title of the M.Sc Thesis

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Abstract

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, Nitrogen and Minerals in grape leaves. 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). To establish a range of leaf chlorophyll levels, each treatment was applied to definite line; thus there were 5 different lines, three vines in each line were selected to make the application.

The spectral analysis of the soil sample representing the area of the study revealed that the soils under analysis are sandy soils consist of major part of SiO_2, traces of aliphatic hydrocarbons and carbonates compounds.

Careful examination of the spectra of leaves revealed that the treatment of the soil with N-fertilizer causes no observable changes in their spectral features of the spectra apart from slight changes in the intensities of the absorption bands.

To evaluate the changes in the spectral features on quantitative bases the absorbance of the peaks at 2921 cm^{-1} (C-H), 1738 cm^{-1}(C=O), 1625(Amide), 1049 cm^{-1} (starch) were determined and the absorbances ratios A1738cm^{-1} / A2921 cm^{-1}, A1625cm^{-1} / A2921cm^{-1} and A1049cm^{-1} / A2921cm^{-1} were calculated.

Examination of the spectra of sample which was taken shortly after the beginning of vegetation growth revealed that the increasing in the N-fertilizer levels results in a significant increasing of the values of the absorbance ratios. This result was attributed to the fact that the photosynthetic process is very active in order to continue the life cycle of the grape vine. The study of the spectra of sample which was taken after two weeks of the beginning of vegetation growth showed that the rates of changes of the above mentioned absorbance ratios increase with the increasing the N-fertilizer level. It was found that the absorbance ratio A1738 cm^{-1}/A2921cm^{-1} suggests the lowest rate of change while the ratio A1625cm^{-1} /A2921 cm^{-1} suggests the highest values. This stage was considered as the flowering stage. As a result the vegetative growth of the vine is completed subsequently the photosynthesis process reached the maximum. So the rate of change of the ratio A1738cm^{-1}/ A2921cm^{-1} for any given level is less than the corresponding values for previous sample while the reverse is true for the other two ratios A1625 cm^{-1} /A2921 cm^{-1} and A1049 cm^{-1} /A2921 cm^{-1}. Analysis of the spectra of sample which was taken after four weeks of the beginning of vegetation growth gave strong evidence that the rate of changes of the absorbances ratios still increases with increasing N-fertilizer level. It was found that for sample which was taken after six weeks of the beginning of vegetation growth the rates of change of the ratios A1625cm^{-1}/A 2921 cm^{-1} and A1738 cm^{-1}/A 2921 cm^{-1} increases with increasing the N-fertilizer level. Whereas, the ratio A1049 cm^{-1}/ A 2921 cm^{-1} decreases with increasing N–fertilizer level. Careful study of the given results of sample which was taken after eight weeks of the beginning of vegetation growth reveals that for any N-level the rates of changes of ratios A1625cm^{-1}/ A 2921 cm^{-1} and A1738 cm^{-1}/ A2921 cm^{-1} increase with increasing N-fertilizer level up to 100% level then the rates of changes decrease with increasing N-level for 133% and 166%. While the absorbance ratio A1049 cm^{-1}/A2921 cm^{-1} decreases with increasing N-fertilizer level for all levels. Careful examination of the given data for sample which was taken after ten weeks of the beginning of vegetation growth indicated that the rate of change of ratio A1625 cm^{-1}

/ A2921 cm^{-1} increases with increasing N-fertilizer level up to 100% level and with increasing N-level to 133%, 166% the rate of change decreases as the corresponding ratios of previous sample.

In addition to the above the concentrations of 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.

Contents

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1-1 INTRODUCTION

Chlorophyll is the primary photoreceptor pigment for photosynthesis, the process used in plants for converting light energy into chemical energy. It is also responsible for the green appearance of leaves, stems, and fruits before they ripen. Two forms of chlorophyll, a and b, are used for photosynthesis. Chlorophyll a is the predominant catalyst of light harvesting and is found in all plants; Chlorophyll b is that absorbs the energy from wavelengths of light accessory pigment that differ from chlorophyll a. Chlorophylls are probably the most well known plant chemicals. Chlorophylls capture light energy and convert it into ATP, nature's universal currency of energy. During this process, absorption of light by chlorophyll causes electron transfer reactions that form oxidized and reduced products. These oxidized and reduced chemicals set up a pH gradient that eventually leads to ATP energy formation.

It is Known that chlorophyll was a mixture of two compounds, chlorophyll-a and chlorophyll-b:

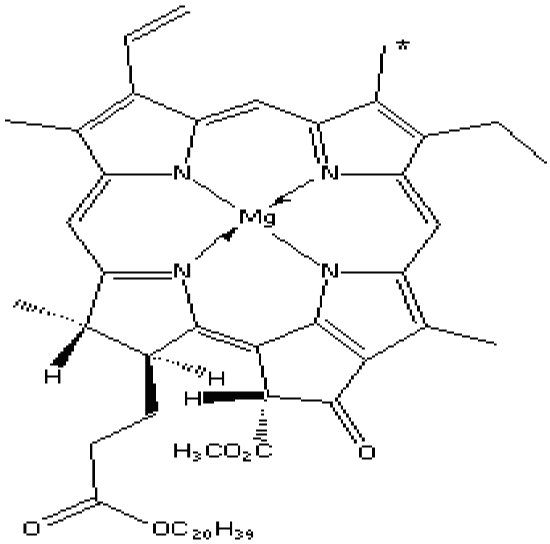

Fig (1-1)(Chlorophyll-*a* ($C_{55}H_{72}MgN_4O_5$, mol. Wt.: 893.49). The methyl group marked with an asterisk is replaced by an aldehyde in chlorophyll-*b* ($C_{55}H_{70}MgN_4O_6$, mol. Wt.: 906.51).

The two components were separated by shaking a light petroleum solution of chlorophyll with aqueous methanol: chlorophyll-a remains in the light petroleum but chlorophyll-b is transferred into the aqueous methanol. Chlorophyll-a is a bluish-black solid and chlorophyll-b is a dark green solid, both giving a green solution in organic solutions. In natural chlorophyll there is a ratio of 3 to 1 (of a to b) of the two components. The intense green color of chlorophyll is due to its strong absorbance in the red and blue regions of the spectrum; shown in fig (1-2) Because of this absorbance the -light it reflects and transmits appears green.

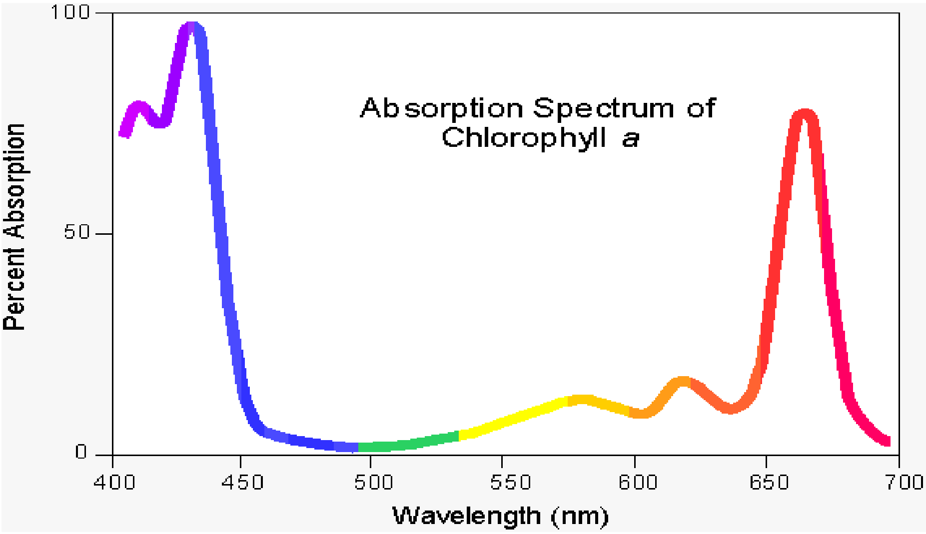

Fig (1-2) Shows the UV/Visible absorption spectrum for chlorophyll. (**Richard 1989)**

Due to the green color of chlorophyll, it has many uses as dyes and pigments. It is used in coloring soaps, oils, waxes and confectionary. Chlorophyll's most important use, however, is in nature, in photosynthesis. It is capable of channeling the energy of sunlight into chemical energy through the process of photosynthesis. In this process the energy absorbed by chlorophyll transforms carbon dioxide and water into carbohydrates and oxygen:

$$CO_2 + H_2O \longrightarrow (CH_2O) + O_2$$

Note: CH_2O is the empirical formula of carbohydrates.

The chemical energy stored by photosynthesis in carbohydrates drives biochemical reactions in nearly all living organisms.

In the photosynthetic reaction electrons are transferred from water to carbon dioxide that is carbon dioxide is reduced by water. Chlorophyll assists this transfer as when chlorophyll absorbs light energy, an electron in chlorophyll is excited from a lower energy state to a higher energy state. In this higher energy state, this electron is more readily transferred to another molecule. This starts a chain of electron-transfer steps, which ends with an electron being transferred to carbon dioxide. Meanwhile, the chlorophyll which gave up an electron can accept an electron from another molecule. This is the end of a process which starts with the removal of an electron from water. Thus, chlorophyll is at the centre of the photosynthetic oxidation-reduction reaction between carbon dioxide and water as shown in figure(1-3).