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The Effect of over Doses in Gibberellic acid in Grape Vineyards (*Vitis Vinifera* L) Leaves using Biophysical Techniques in Agriculture

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ABSTRACT

Grape vineyards have been facing problem of disease like pink berry. The causes of which are still unknown. Earliear researchers had studied possible causes of such disease by studying effects of overdoses of pesticides, antibiotics and growth regulators. This study is based on biophysical techniques such as absorption, fluorescence and thermo luminescence. Though the cause of this disease is unknown it has been observed that the occurrence of the disease is more where application of fertilizers, pesticides, antibiotics and growth regulator is un scientific and heavy. Under such circumstances the study of biophysical applications in using PMT cryostatic method of grape vine yards becomes necessary. Steady state Florescence (FL) spectra and Thermo luminescence (TL) glow curves we are recorded from grape leaves treated with Gibberellic acid individually as well as in combination with endosulfan and Benomyl. The TL peaks were analyzed using a computer assisted model based on the general order kinetics theory, various thermodynamical parameters, such as activation energy (E), entropy (ΔS), frequency factor (So) and free energy (FE), associated with TL peaks were calculated. The TL study shows that GA at a lower concentration improves photosynthetic efficiency of plant by shifting redox potential of Q_b towards Q_a, the thermodynamical parameters also showed increase due to pesticide.

Keywords: Vineyards, Pesticides, Biophysical technique, Florescence spectra and Thermoluminescence, etc

INTRODUCTION

The term biophysical thereby emphasizes that photons are neither particles nor the waves; they are different in that they have both particle and wave nature. It covers all technical applications of light over the whole spectrum from ultraviolet over the visible to the near, mid and far-infrared. Most applications, however, are in the range of the visible and near infrared light. The term photonics developed as an outgrowth of the first practical semiconductor light emitters invented in the early Photonics also relates to the emerging science of quantum information in those cases where it employs photonic methods. Applications of photonics are ubiquitous. Included are all areas from everyday life to the most advanced science, e.g. light detection, telecommunication, information processing spectroscopy, medicine (surgery, vision correction, endoscopy, health monitoring), military technology, laser material processing, visual art, biophotonics, agriculture, and robotics. Thompson seedless, a *vitis vinifera* and its successor varieties are being cultivated widely in India. However, it has been observed that these varieties are more prone to different type of diseases. Hence in order to save the crop and to increase the yield, indiscriminate application of pesticides had become a routine practice, since the biochemical processes of cellular and sub cellular levels of the target organism and the host plant are often quite similar. The possibility of direct effects of the protective chemicals on the host plant cannot be overload.

In the present work the effects of Gibberellic acid when applied individually and also in combination with an insecticide endosulfan and fungicide benomyl on grape leaves have been investigated. The photonic technique such as fluorescence (FL) Thermolumine-scence (TL) has been adopted. Also, the modified TL set- up was developed using PMT.

MATERIALS AND METHODS:

The Commonly cultivated grape varieties in south west India Thompson seedless (*Vitis vinifera* L) were taken. The samples were collected from Narayangaon area in Pune district. TL was recorded from mature leaves at around the sixth nodal position of the shoot for different concentrations of pesticides, viz growth regulator (GA), Insecticide (Endosulfan) and fungicide (Benomyl) were selected. Different concentrations of these pesticides were prepared ranging from normal to heavy dose. Steady state fluorescence was measured from control and treated leaf discs of diameter 1.5 cm. Using Perkin Elmer LS 50 spectroflurometer, fluorescence emission spectra were measured between 650-770 nm by keeping the excitation wavelength fixed at 440nm. The biophysical technique such as thermo luminescence (TL) has been adopted the experimental set-up can be divided in to three parts (Figure) cryostat arrangement to cool the system, the heating system and PMT sliding recording system. Cryostat consists of stainless steel cylindrical container having capacity 5 liters. A copper rod along with copper plate arrangement is dipped in to this container and it acts as a cold finger. When liquid nitrogen is filled in the container the copper rod and copper plate get cooled and sample holder attains low temperature. The sample for the study of TL method needs an adjustable variable temperature in the range between 77K to room temperature (-80°C to +50°C) and this is done by heater. The heating block "K" made up of copper used in the system is circular in shape having 4 cm diameter and 30 cm thickness. In the centre of this block to hold the sample holder cavity 'S' with 2 cm diameter and 25 mm depth has been made sample holder is soldered over the heater. A hole from the surface of the sample holder cavity up to the outer side of the 'K' block has been drilled to hold the thermocouple (TC) so that its one junction is kept in such a position that it permitted a good contact

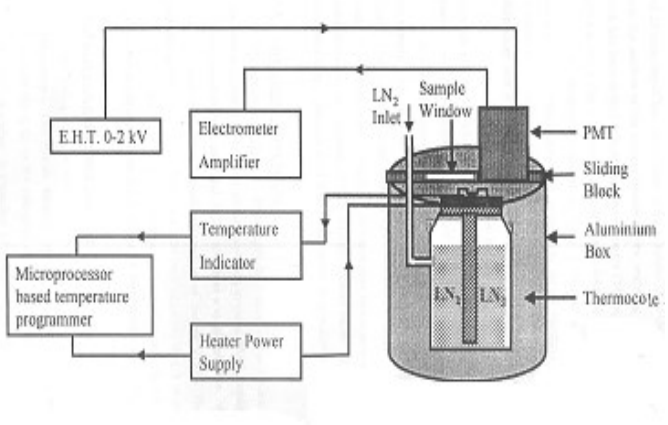

Fig. 1 Block diagram of new TL setup

with the sample holder. At the bottom of this the heater element is of nicrome wire with 26 ohms resistance and of wattage is 200W at 230V A.C. The wire is wound around a Mica sheet and its terminals are taken out for connection. The heater is fitted on the top of copper rod. It is dipped in to LN_2 for cooling purpose. Photomultiplier tube (PMT) is used here to measure the luminescence and it is the heart of recording system, PMT should have large area for light detection, high gain and ability to detect a single photon. The 9798B has a S20 type response which is sensitive from 200 nm to 900 nm. This TL set up requires less LN_2 and less time to cool the system for next observation.

RESULT AND DISCUSSION:

(A) Thermoluminescence: In Thermoluminescence, the fitted TL glow curve from the control untreated grape (*Vitis vinifera* L) leaf disc. It shows the presence of only one peak at about -6^{0}C. As per standard TL nomencla-ture this peak can be assigned as peak II attributed to S_2 /S_3 Q_a recombination 3. The fitted TL spectra recoded from grape leaf disc treated with GA. It shows the presence of the entire peaks viz. peak I, II, III, IV, and V at around -21^{0}C, -4^{0}C, 11^{0}C, 29 ^{0}C and 51 ^{0}C, respectively the intensities of the peak shows increase. Except for peak II all the peaks are broad Table shows the calcula-ted values of all the Thremodynamical parameters.

The present study was planned to check the effect of growth regulator when applied individually as well as in combination with other pesticides on the photosynthetic electron transport chain of grape (*Vitis vinifera* L) leaves. This result confirm that lower concentration of GA improves photosynthetic efficiency of plant by shifting redox potential of Q_b towards Q_a, but when GA was applied with other pesticides damage to the photo-synthetic apparatus started right from lower concentration. Though GA tries to improve photosynthetic efficiency the presence of other pesticides creates stress on the plant. The increase in thermo dynamical parameters also indicates damage to the photosynthetic apparatus due to the pesticides. The studies have indicated that the TL techniques can be used to monitor the effect of pesticides on the photo-synthetic electron transport chain and can detect minute alternation in the photo-synthetic apparatus of plants. Such an alternation is otherwise undetectable and hence may go unobserved till the fruiting period.

Table 1: Thremodynamical parameters of thermo luminescence glow curves recorded from *Vitis vinifera* L. leaves using the general order kinetics theory.

Control (untreated)

Peak	Activation Energy E(eV)	Entropy ($\Delta S/k_B$)	Free Energy F.E.(eV)	Frequency Factor S^{-1}
I	0.677	-1.58	0.712	4.28 X10^{14}
II	1.55	35.34	0.739	4.66 X10^{25}
Effect of GA				
I	0.572	-4.07	0.654	3.52 x 10^8
II	0.774	-1.45	0.715	4.85 x 10^9
III	0.964	0.546	0.61	3.59 x10^{10}
IV	0.993	6.59	0.802	1.52 x 10^{13}
V	1.192	4.93	0.863	2.89 x 10^{12}
Effect of Benomyl with GA				
I	0.861	6.918	0.71	2.1 x10^{13}
II	1.541	35.909	0.736	3.6 x10^{23}
III	1.49	30.803	0.761	5.22 x10^{22}
IV	1.784	40.159	0.793	5.75 x 10^{27}
V	1.257	4.65	0.868	2.97 x 10^{30}

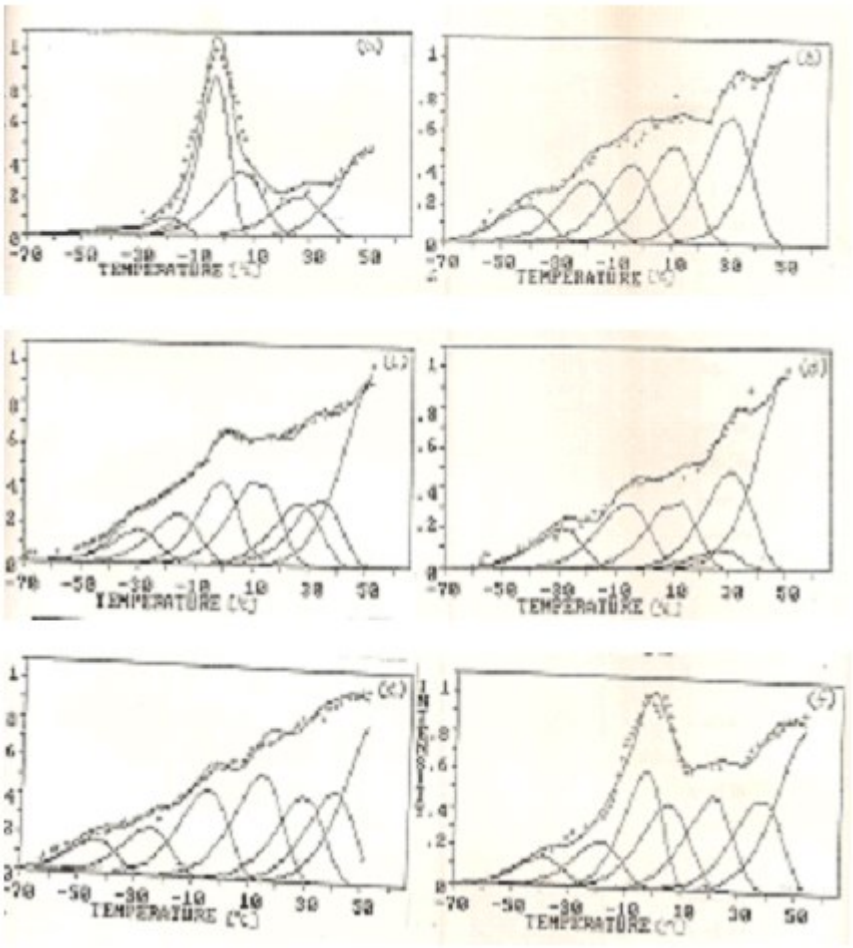

Fig 2 Thermoluminescence glow curve

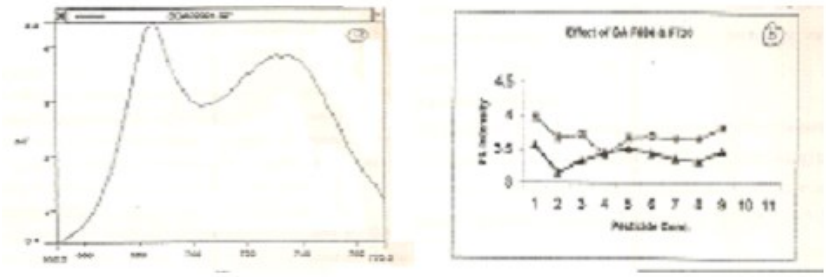

Fig. 3: Fluorescence

(B) Fluorescence: As reported earlier Benomyl treatment results in increasing fluorescence intensity. Hence Benomyl and Gibberellic acid when applied together no variation in fluorescence intensity was observed. The more or less intensity level of FL, at 684 nm as observed for lower concentration of pesticides is indicative of the compensating effects of Gibberellic acid and Benomyl. The heaviest concentration though results in degenerative effects due to application of both the pesticides and the subsequent increase in fluorescence intensity.

Figure 3 (a) shows steady states fluorescence spectra recorded from control (unsaturated) grape leaf disc. It shows one major peak at around 684 nm attributed to PS-II and a shoulder at 730 nm, from PS-I. Figure 3(b) shows variation in florescence intensity for 684 nm as well as 730 nm. Peak recorded from grape leaves

treated with different concentration of GA. For the lower concentration of GA decrease in fluorescence intensity is observed, while for higher concentration the fluorescence intensity is almost constant. This indicates that lower concentration of GA favors forward flow of electron and improves photosynthetic efficiency of grape leaf. Earlier studies have reported that individual application of endosulfan increases fluorescence Intensity, while as mentioned above Gibberellic acid treatment decreases fluorescence intensity. Hence the observed oscillatory pattern indicates that endosulfan and Gibberellic acid when applied in combination compete to demonstrate their individual effects, though Gibberellic acid seems to have an upper hand.

CONCLUSION

The present study was planned to check the effect of growth regulator when applied individually as well as in combination with other pesticides on the photosynthetic electron transport chain of grape (*Vitis vinifera* L) leaves. The FL results have indicated that growth regulator Gibberellic acid for lower concentration improves the photosynthetic efficiency of plants. But when its concentration is increased or when it is applied with other pesticides it affects PS-II adversely. In fact, PS-II is more damaged as compared to PS-I.due to the application of Gibberellic acid and also with pesticides. The TL results confirm that lower concentration of GA improves photosynthetic efficiency of plant by shifting redox potential of Q_b towards Q_a, The increase in thermo dynamical parameters also indicates damage to the photosynthetic apparatus due to the pesticides. This is not the only cause of pesticides but other factors also take part in that like soil nutrients, temperature, and humidity. There for further study will be necessary.

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