EXECUTIVE SUMMARY ON MAJOR RESEARCH PROJECT

Title: "Study Of Physico-Chemical Stress On Photosynthetic Activity In Soyabean Plant By Laser Induced Fluorescence"

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Introduction:-

All stress factors acting at any of the plant are it roots of leaf will have in the course of time an indirect or a direct effect on photosynthesis and should thus be detectable by measurements of the in vivo chlorophyll fluorescence. For this reason, chlorophyll fluorescence can be a valuable tool to detect the induction of stress and damage as well as the regeneration of the system when the stress factor is removed. The physical and chemical factor implies the stress on the plant which arrests the vegetative growth of the plant and ultimately lowers the crop yield. The changes in these physical factors like light, temperature etc as well as the stress due to changes in soil composition should be control to increase crop yield. The principal physiological process in plant responsible for growth is photosynthesis so the study of these factors on photosynthetic activity has been studied in present work by laser induced fluorescence technology. The followings objectives were keep in mind during the study of present work

- To develop simple and suitable experimental setup for recording chlorophyll florescence
- To evaluate different methods and techniques of analyzing chlorophyll florescence spectra
- To study the effect of different physical factors like water, temperature, light etc on the rate of photosynthesis
- To study the stresses of micronutrients and nonessential elements present in the soil on the rate of photosynthesis with special reference to heavy meal like mercury, aluminum, copper etc.
- To study the fluorescence of plant excited by different wavelength laser light.

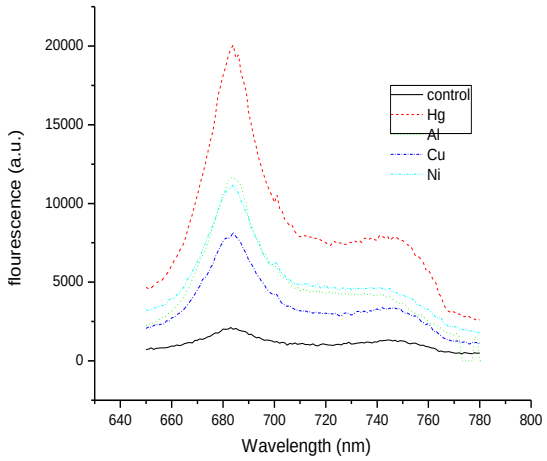
Photosynthetic activities of green plants depend on the chlorophyll content of their leaves. In

photosynthesis, chlorophyll acts as a photosynthesiser. The level of fluorescence emitted by plants upon exposure to light is governed by photosynthetic efficiency. The study of in vivo chlorophyll fluorescence (ChlF) of green plants is highly correlated to knowledge of the mechanism of photosynthesis. It provides basic information on the functioning of the photosynthetic apparatus and on the capacity and performance of photosynthesis. Chlorophyll fluorescence is also used to indicate and evaluate stress levels or physiological damage of plant, such as photo-inhibition, deficiency of water and nutrients, damage caused by pollution, temperature, and diseases. In addition, the ratio of the red to far red fluorescence is a good indicator of stress and chlorophyll content.

Materials and Method:-

Leaves of the Soybean plants used for LICF study were rinsed in distilled water, dried at 80°C for 24 h and wet-ashed in nitric and perchloric acid mixture (3/1 v/v) on an electric thermostatic plate (300°C). Chlorophyll content was determined by atomic absorption spectrophotometry18 (Perkin–Elmer 2380). The laser spectrofluorometer used in the present study is a computer-controlled data acquisition system, which provides fluorescence information. A cw argon ion laser (Spectra Physics, USA model 2016) operating at 488 nm was used for exposing the full intact leaf with the help of the beam expander. The fluorescence light was collected with the help of a convex lens on the slit of computer controlled 0.5 M monochromator (Acton Research Corporation, USA) having a resolution of 0.03 nm and reciprocal linear dispersion of 1.1 nm/mm, with R928 PMT detector. The PMT signals were sent to the computer and the data have been collected and analyzed using Grams-32 (Galactic) software. Laser light intensity was measured with the help of power meter (Spectra Physics, USA model 407A-2). The LICF spectra for the control and plants harvested after 5 days of metal treatment, excited by 488 nm of argon ion laser (power 30.0 mw) have been recorded in the region 600–800 nm with two peaks lying nearly at 680 and 730 nm, which are due to chlorophyll from PS II and PS I respectively. The curve fitting has been done in the region between 650 and 800 nm using the Grams-32 software with the Curve fit program. This curve-fit is based on the original algorithm of nonlinear peak fitting as described by Marquardt and also known as the Levenberg–Marquardt method.

Observations:-



The Gaussian spectra resulting from the curve-fitting analysis of LICF for the control and the mercury, Aluminium, Copper and Nickel-stressed Soybean plants.

Plant Treatment	Red Band			
	Peak position in nm	Peak height a.u. x 10^2	Band width in nm	Band area a.u. x 10^4
Controlled plant	680	1427	31	55959
Copper (Cu)	680	14727	24	435507
Aluminum (Al)	680	9114	24	272976
Nickel (Ni)	680	6028	25.2	190423
Mercury (Hg)	680	7791	23.5	229724

Plant Treatment	Far Red Band			
	Peak position in nm	Peak height a.u. x 10^2	Band width in nm	Band area a.u. x 10^4
Controlled plant	730	844	35	37122
Copper (Cu)	730	5028	42.41	267327
Aluminum (Al)	730	3317	42.10	175037
Nickel (Ni)	730	2042	38.49	98515
Mercury (Hg)	730	2592	41.36	134385

The curve-fitting parameters such as peak centre, peak height, bandwidth (FWHM) and the area under each Gaussian curve for both the control and metal-treated plants

In the present work the ratio F680/F730 for peak height is maximum for nickel treated plant and lowest for aluminium treated plant. It indicates the chlorophyll content is more in aluminium treated plant and is less in nickel treated plant. Nickel affects adversely the growth of Soybean plant compared to aluminium.

The pigments content were determined from the transparent, centrifuged acetone extract solution by measuring the absorbance using UV/Vis spectrophotometer (Unicam 5600 series, model UV/Vis 5625). The pigments concentrations were calculated by the equations allowing a simultaneous determination of Chl *a*, Chl *b* and carotenoid in the same solution as determined by Lichtenthaler [47].

Effect of metal stress on chlorophyll content of leaf is given in following table Chlorophyll a, chlorophyll b and total chlorophyll content is minimum in mercury stressed plant and maximum for copper stressed plant after control plant.

Sr. No.	Metal Stress	Chlorophyll a	Chlorophyll b	Total Chlorophyll
1	Controlled plant	34.488676	18.380584	52.869256
2	Aluminum (Al))	34.180301	16.669652	50.849954
3	Copper (Cu)	27.850763	9.9201193	37.770882
4	Nickel (Ni)	24.954886	7.830877	32.785764
5	Mercury (Hg)	24.265267	8.401796	32.667063

Effect of heavy metal stress on chlorophyll content of Soybean plant

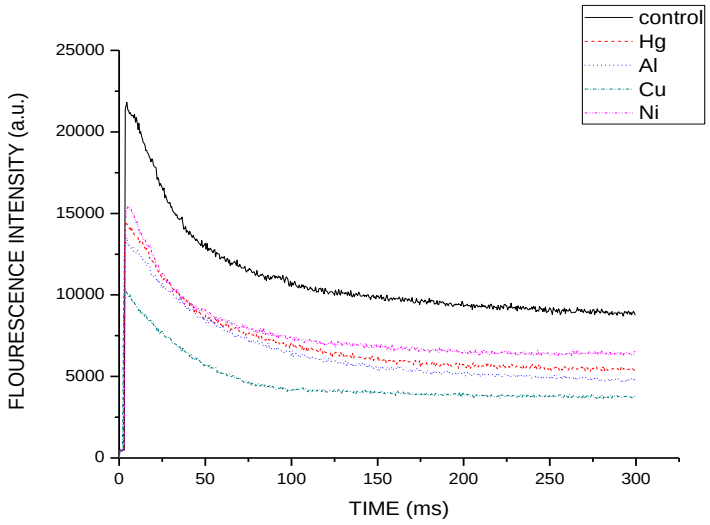
Result and Discussion:-

Soybean plants grown in controlled condition and in different metals treated plans (Al, Cu, Ni and Hg) showed variation in photosynthetic pigment content and their ratios (Table 1). Plants treated with Aluminum exhibited decrease in the value of Chl *a slightly* by 0.90 %, than control while plant treated with copper shows decrease in chlorophyll a content somewhat more by 19.24 % whereas plant treated with Nickel and Mercury shows marked decrease in chlorophyll a content by 27.64% and 29.64 % respectively. About chlorophyll b and other pigment also the result are approximately same. The decrease in chlorophyll content is due to stress obtained by heavy metal treatment which decreases the photosynthetic activity in Soybean plant. The values of Chl *a as* well as chl b were significantly different in plants treated with different metals. The Chls of higher plants consist of Chl *a* as the major pigment and of Chl *b* as an accessory pigment, however, both Chls are genuine components of the photosynthetic membranes. The carotenoid is regular components of the photosynthetically active thylakoids of chloroplasts of higher plants.

Fluorescence Induction Kinetics of Soybean Plant under Various Metal Stress

Soybean leaves of controlled and stressed plant are kept in dark for 20 minutes. Then illuminated by He-Ne laser and fluorescence kinetics recorded at wavelength of 690 nm. Fluorescence

induction kinetic curves are shown in the following graph. From kinetic curves vitality index for control and stressed plant calculated and is given in following table.



Induction Kinetics curves of Soybean under different stress

Material Stress	F_{max} In 6.5ms	$f_d = F_{max} - f_s$	f_s After 299.5 ms	R_{fd} = fd/fs
Controlled	21817	13057	8760	1.4905
Aluminium	13520	8949	4571	1.9578
Copper	10298	6543	3755	1.7424
Nickel	15445	8859	6586	1.3451
Mercury	14513	9108	5405	1.6851

Vitality index of soybean under different stresses

Variations in the intensity of the fluorescence induction kinetics curve were found which might depend on the leaf Chls content (Fig. 1). The fluorescence induction kinetics parameters *F*m, *F*s and *F*d, were measured from fluorescence induction kinetics curve recorded at 690 nm, are given in following table. The *R*fd values calculated from fluorescence induction kinetics parameters at 690 nm were found to be decreased with the Metal treated plant over controlled plant. Activity of PS II and PS I as well as *R*fd 690 values decreased in plants treated with Al, Cu, Ni and Hg as compared to

controlled plant. As metals blocks electron transfer from PS II to PS I when the plants are treated with the heavy metal treated *Soybean* was significantly low in 690 nm.

The variation of the Chl FIR F680/F730 of control as well as Al, Cu, Ni & Hg treated *Soybean* is due to the different Chl and carotenoid contents of the plants. The variation in Chl FIR F680/F730has been determined by reabsorption of the emitted fluorescence by overlapping of the absorption bands of Chl–carotenoid protein complex in the photochemically active thylakoids of chloroplast .This FIR F680/F730 was the hightest in the case of Hg treated plants. **Conclusion:-**

The overall results indicated that the Soybean treated with metal such as Al, Cu, Ni & Hg with high concentration were found to be hazardous and FIRs, F685/F735 and F470/F540were also correlated with photosynthetic pigment contents and their ratios.

Fluorescence induction kinetics parameters for the study of PS II system	Fluorescence induction kinetics Intensity of Soyabean leaves in different stressed conditions				
	Control Plant	Hg stressed	Ni stressed	Cu stressed	Al stressed
F_o	532	494	483	419	524
$F_v = F_m - F_o$	21164	20906	14799	9752	12698
F_m	21696	21400	15282	10171	13222
$F_d = F_m - F_s$	12876	12451	8885	6390	8495
F_s	8820	8949	6397	3781	4727
$R_{fd} = F_d/Fs$	1.45986	1.39132	1.38893	1.69002	1.79712
$\Phi = F_v/F_m$	0.97547	0.97691	0.96839	0.95880	0.96036

Fluorescence induction kinetics parameter of controlled and stressed plant.

PAPERS PUBLISHED/ COMMUNICATED

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2 Talwankar D. S., Hargunani S. P.& Talekar A. B., Study of Different Metal Stress on Soybean Plant. *Int. J. pure App. Biosci.* **2015,** Vol. 3 (5), 56-61pp

3 Talwankar D. S. and Hargunani & Sonekar R. P. 2012;Chlorophyll Fluorescence in the leaves of JS-335; JS-305 Soybean Affected by High Temperature stress; Proc. Nat. Conf. Recent Trends in Bio-Sciences at G. S. College, Khamgaon ISBN:978-81-922866-1-7, 210-212pp