LIF SPECTRA OF DIMETHOATE TREATED CAJANUS CAJAN L.

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Abstract: The effect of dimethoate on the photosynthetic activity and pigments content on Cajanus cajan L. is determined by laser induced chlorophyll fluorescence (LICF) and fluorescence induction kinetics (FIK) of leaves. The LICF spectra is recorded in the region of 650-780 nm by using 405 nm violet diode laser and FIK curves are recorded at 685 and 730 nm by using 635 nm red diode laser as the excitation source and PMT as a detector. The fluorescence intensity ratio (FIR) of control and as well as dimethoate treated C. cajan L. plants are calculated by evaluating curve-fitted parameters using Gaussian spectral function. Vitality index is calculated by FIK curve. All these parameters along with photosynthetic pigments content are used to analyze the effect of dimethoate on C. cajan L. treated with different conc. of dimethoate (20, 40 and 80 ppm). It is found that dimethoate inhibits the plant growth even at very low concentration of 20 ppm.

1. INTRODUCTION

Laser induced fluorescence (LIF) is a nondestructive technique for detection of plant stress responses. The LICF spectrum of green leaves exhibits two fluorescence maxima: one near 685nm and other around 735nm. The shape of the chlorophyll fluorescence spectra and the value of the fluorescence intensity ratio (FIR) at the two maxima (F685/F735) depend largely upon the chlorophyll (Chl) content of leaves and to a lower degree also on the leaf structure, the photosynthetic activity and the leaf’s optical properties [1-3].

Upon reillumination of a 20 min predarkened leaf the Chl fluorescence undergoes induction kinetics, which is known as Kautsky effect. Two part’s of these induction kinetics can be distinguished: (1) the fast fluorescence rise to the maximum fluorescence (fmax) which is completed in 100 to 500 m sec and (2) the slow fluorescence decrease (fd) to the steady, where photosynthesis and fluorescence are in study state condition, which is completed in 3to 5 min. higher the variable fluorescence (rise above Fo to Fm) and larger the slow fluorescence decrease (from Fm to Fs), higher the photosynthetic capacity of leaf. The fluorescence decrease from Fm to Fs is paralleled by increasing rate of oxygen evolution and the photosynthetic CO\(_2\) fixation [4].

The use of organophosphorus insecticide has increased threefold during the past three decades. Dimethoate is a systemic insecticide; it could affect plasma membrane, PSII activity and photophosphorylation of Synechocystis cells and cause the inhibition of photosynthetic electron transport and enhancement of respiratory \(O_2\) consumption, and chlorophyll biosynthesis. Dimethoate negatively affects the electron transport between PSI and PSII; significantly inhibit the photosynthesis and increases dark respiration at higher concentration. It accumulate in the thylakoid membrane and increase the fluidity of the lipid bilayer making the membrane less integrated, causing detachment of phycobilisomes and inhibiting photosynthetic electron transport. It also inhibits thylakoid ATPase by increasing acidity of thylakoid lumen of intact Synechocystis cells [5, 6]. Dimethoate also cause production and accumulation of reactive oxygen species (ROS) and increased activity of antioxidants such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) with increasing concentration of dimethoate [7].

The recommended doses of dimethoate by WHO ranges from 300-600 ppm and earlier results are reported near this conc. It is found that it inhibits the growth of plants. The present paper deals with the study of the effect of the dimethoate on C.cajan L. treated with the comparatively much lower conc. of dimethoate.

2. MATERIAL AND METHODS

2.1 Plant growth and treatment with insecticide

Healthy and uniform sized seed of C.cajan L were surface sterile in 4% sodium hypochlorite solution (v/v, in double distilled water) for 20 min and presoaked for 20 hr in distilled water and wrapped in wet cloth overnight. Selected uniform germinated seed and transferred Plants in the growth chamber and stress of dimethoate (20, 40, 80 ppm) is given with the modified Rorrison medium on alternate day. Plant leaves are used after 10 days of first treatment to analyze the effect of dimethoate.

2.2 Determination of pigment

The pigments content are determined from the transparent, centrifused acetone extract solution of plant leaves by measuring the absorbance in the region 380-700 nm by using the UV/VIS spectrometer (Perkin Elmer lambda 35). The pigment...
conc. determined according to the Lichtenhaler & Welburn [8].

2.3 Laser-induced chlorophyll fluorescence
LIF spectra are recorded in the region of 650–780 nm by using violet diode laser (405 nm), the fluorescence induction kinetics (FIK) are recorded at 685 and 730 nm using red diode laser (635 nm), as the excitation source with computer controlled Acton 0.5 M triple grating monochromator and PMT R928 as a detector. FIR’s of control as well as dimethoate treated plants are calculated form LICF spectra by evaluating curve fitted parameter using a Gaussian spectral function. Various parameter such as fluorescence maximum (Fm), steady state level (Fs) and fluorescence decrease (Fd) are calculated from FIK curve. Vitality index (Rfd = Fd/Fs) is calculated from these parameter for all the samples.

2.5 Curve-fitting
Using Levenberg-Marquardt algorithm method makes interative non-linear curve fitting. After choosing the Gaussian spectral function, the individual component peaks are selected. Peak widths were adjusted so as to much approximately the line shape of the spectrum. It provides a reasonable matching fit of the spectral data with good F-statistics, standard error for peak amplitude, peak center and bandwith (full width at half intensity maximum).

3. RESULTS AND DISCUSSION
3.1 Photosynthetic pigment content
The leaves of the dimethoate treated C. cajan L. show 7.2, 8.0, and 16% decrease in the pigments content over the control plants for 20, 40 and 80 ppm of dimethoate treatment respectively. Oranophosphurus insecticide inhibits chlorophyll, protein and carbohydrate biosynthesis when alga are exposed for several days [9] it significantly inhibit the synthesis of Chl a under various dimethoate level [10]. It could be due to inhibition of Chl biosynthesis by inhibiting δ- aminolevulinic acid dehydrogenase and proto-chloropyllide reductase activity and breakdown of pigment or their precursors as reported for other stresses [11, 12]. Mishra et al [7] has reported the production and accumulation of O₂⁻ and H₂O₂ in leaves exposed to dimethoate and UV-B irradiation. H₂O₂ and O₂⁻ are relatively less damaging themselves , but they can form other species such as hydroxyl radical (OH⁻) that can initiate lipid peroxidation, and thus causes membrane leakage.

3.2. LICF Spectra
The treatment of the dimethoate increases the FIR. The increase in the FIR ration due to the dimethoate treatment is given in the Table: 1, the increase in the FIR ratio is the function of dimethoate treatment, as the conc. of dimethoate treatment increase the FIR ratio is also increase. Fig.1 shows the curve fitted LICF spectra of C. cajan L. leaves.

![Figure 1: Gaussian curve-fitted LICF spectra of the control and dimethoate treated wheat plant leaves excited by 405 nm violet laser.](image)

The effect of the decrease in the Chl content is

<table>
<thead>
<tr>
<th>Treatment of dimethoate</th>
<th>FIR</th>
<th>Rfd 685</th>
<th>Rfd 730</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.92</td>
<td>0.66</td>
<td>0.49</td>
</tr>
<tr>
<td>20 ppm</td>
<td>2.11</td>
<td>0.62</td>
<td>0.46</td>
</tr>
<tr>
<td>40 ppm</td>
<td>2.16</td>
<td>0.60</td>
<td>0.45</td>
</tr>
<tr>
<td>80 ppm</td>
<td>2.31</td>
<td>0.56</td>
<td>0.42</td>
</tr>
</tbody>
</table>

The effect of the decrease in the Chl content is
mainly detected in short-wavelength range (red Chl fluorescence), where short-wavelength red Chl fluorescence increases with decrease in the Chl content due to the reduction of the re-absorption of the emitted red Chl fluorescence by the Chl absorption band. In the green leaves about 90% of the emitted Chl fluorescence at 685 nm, reabsorbed by the Chl molecules of the leaf and the re-absorption is caused by the overlapping of short-wavelength range of the Chl fluorescence emission spectrum with the long-Wavelength of the Chl absorption spectrum [3, 13]. Since the red Chl fluorescence maxima near 690 is more strongly affected by the re-abotion than the long-wavelength maximum near 730-740 nm, the ratio F685/F735 increase with decreasing Chl content and vice-versa. Thus FIR is strongly influenced by variation in chlorophyll content and photosynthetic activity of the leaf.

3.3 Fluorescence Induction kinetics

Dimethoate treatment decreases in Vitality index (Rfd) value (Table 1). The decrease in the Rfd value is also the function of dimethoate treatment, its decrease with increase in the dimethoate concentration. The FIK curves at 685 and 730 nm of C. cajan L. leaves are shown in Fig-2. The ratio of the fluorescence decrease (Rfd = Fd/Fs) is an approximate measure of the potential photosynthetic activity of a leaf and can be used for the fast screening of the presence of photosynthetic function. The decrease in the vitality index shows the effect of dimethoate on photosynthetic activity of plant leaves.

Thus the result clearly shows the extent of damaging effect of dimethoate on the photosynthetic pigments, activity and plant health at a comparatively low concentration.

4. ACKNOWLEDGEMENT

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5. REFERENCING

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