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ORIGINAL PAPER

Laser-Induced Chlorophyll Fluorescence: A Technique for Detection of Dimethoate Effect on Chlorophyll Content and Photosynthetic Activity of Wheat Plant

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Abstract Laser-induced chlorophyll fluorescence (LICF) spectra and fluorescence induction kinetics (FIK) curves of wheat plant leaves treated with different concentrations (50, 100 and 200 ppm) of dimethoate are recorded. LICF spectra are recorded in the region of 650–780 nm using violet diode laser (405 nm) and FIK curve at 685 and 730 nm with red diode laser (635 nm) for excitation. The fluorescence intensity ratios (FIR) are determined from LICF spectra and vitality index (R_{fd}) from FIK curves. These parameters along with photosynthetic pigment contents and growth parameters are used to analyze the effect of dimethoate on wheat plants. The result indicates that lower concentration of 50 ppm shows stimulatory response while higher concentrations of dimethoate are hazardous for growth, photosynthetic pigments and activity of wheat plants.

Keywords Laser-induced chlorophyll fluorescence · Fluorescence intensity ratio · Vitality index · Photosynthetic pigment contents (chlorophyll a, chlorophyll b, carotenoids) · Wheat plants · Dimethoate stress

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Introduction

Photosynthesis is the feature, which makes the plants unique in the living world. As the photosynthesis depends upon the chlorophyll (Chl) molecule, thus Chl is the most important molecule and its concentration is one of the basic parameter to be monitored in order to understand the plant's activity and health. Since Chl emits fluorescence in response to irradiation; a technique based on laser-induced fluorescence spectroscopy (LIFS) offers a way of nondestructive/in vivo and non-contact/remote sensing of plants [1, 2]. It has application in the field and remote assessment to detect environmental stress and physiological changes in the plants leaves [3].

The expanding use of pesticides for protecting crop against pests and disease attack is an important part of the modern agriculture. Dimethoate (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate) is foliar applied systemic thio-organophosphorus insecticide. This is used in various countries to control the insect population on wide variety of crops. Dimethoate produce their insecticidal effect through inhibition of acetylcholinesterase, an enzyme that terminate the action of acetylcholine by catalyzing its hydrolysis, thereby disrupting the normal functioning of nerve transmission, producing hyperexcitability, convulsion, muscular paralysis, and respiratory failure [4].

Studies of the phytotoxic effect of orgnophosphorus insecticides on phytoplanktons suggested that this type of toxicant reduces growth rates and inhibits chlorophyll, protein and carbohydrate biosynthesis when algae are exposed for several days [5, 6]. Dimethoate 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 [7, 8]. Dimethoate negatively affects the electron transport between PSII and PSI; significantly inhibit the photosynthesis and dark respiration increased at higher concentration [9]. 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 [10].

Light energy absorbed by the Chl molecule can undergo one of the three processes; (i) to drive photosynthesis (photochemistry), (ii) excess energy can be dissipated as heat and (iii) re-emitted as – Chl fluorescence. These three processes occur in competition. In the green photosynthetically active tissue light absorbed by the photosynthetic pigments (Chl and carotenoids) is primarily used in the photosynthetic light reaction for the photosynthetic quantum conversion. Under optimum condition for photosynthesis only a small proportion of absorbed light is lost as heat and as red Chl fluorescence [11–14].

Various stress conditions may reduce the rate of the photosynthesis (CO_2-assimalation), and disturb or block the light–driven photosynthetic electron transport as well as the photosynthetic pigment apparatus, without affecting the process of light absorption. This then leads to an increased de-excitation of absorbed light energy via Chl fluorescence and heat emission. The study of the laser-induced in vivo Chl fluorescence of leaves of green plant provides basic information on the functioning of the photosynthetic apparatus and on the capacity and performance of photosynthesis.

The in vivo Chl fluorescence spectra of green leaves show at room temperature two maxima, one in the spectral region near 685 nm and other in the region of about 735 nm. The shape of the Chl fluorescence spectra and value of the fluorescence intensity ratio (FIR) at the two maxima (F685/F735) depends on the Chl content of the leaf. Chl fluorescence depends to a great extent on Chl content and the absorbance of the leaves [15–20]. In the green leaves about 90% of the emitted Chl fluorescence at 685 nm, is reabsorbed by the Chl molecules of the leaf [21]. It is found that the effect of reabsorption of the red Chl fluorescence at 685 nm is particularly large when leaves possess a moderate to high Chl content. Thus, as the Chl content decreases, the peak intensity at the 685 nm region increase much more in comparison to 735 nm and the fluorescence spectra show only one maximum peak in the 685 nm region with a shoulder near 735 nm. When the Chl content is very low, the absorbed light energy, not being used for the photochemical work and transferred to heat, is emitted as Chl fluorescence, which can leave the leaf tissue with no or little hindrance through re-absorption process.

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 (F_m) which is completed in 100 to 500 msec and (2) the slow fluorescence decrease ($F_d = F_m - F_s$) to the steady state (F_s), where photosynthesis and fluorescence are in steady states, which is completed in 3 to 5 min. Higher the variable fluorescence (rise above F_o to F_m) and larger the slow fluorescence decrease (from F_m to F_s), higher the photosynthetic capacity of leaf. The fluorescence decrease from F_m to F_s is paralleled by increasing rate of oxygen evolution and the photosynthetic CO_2-fixation [2, 22, 23]. The relative extent of the fluorescence decrease is therefore an approximate measure of the degree of photosynthetic quantum conversion of leaf. Several other factors may participate in this fluorescence decrease, e.g. thermal quenching, phosphorylation of the light-harvesting chlorophyll-proteins, etc. [24, 25]. The ratio of the fluorescence decrease to the study state fluorescence ($R_{fd} = F_d/F_s$) is a very suitable indicator of vitality and stress of the plant, and has been termed as vitality index [22, 26]. The level of the R_{fd}-values is, in fact a measure of the potential photosynthetic activity and capacity of the leaf [11, 22, 26]. It signals the intactness and functionality of the internal photosynthetic apparatus, even with closed stomata.

As the dimethoate is highly hazardous for the plant health, the present paper deals with the study of dimethoate effect on the pigment contents and photosynthetic activity of the leaves of wheat plants by using the technique laser-induced chlorophyll fluorescence.

Material and Method

Plant Growth and Treatment with Insecticide

Healthy and uniform sized seed of *Triticum estivum* L. (Var. PBW 343) were surface sterile in 4% sodium hypochlorite solution (*v/v*, in double distilled water) for 20 min and presoaked for 20 h in distilled water and wrapped in wet cloth overnight. Selected uniform germinated seed and transferred into cup contain acid washed sterilized sand (≈260–270 g). Plants were grown under illumination of 4500 Lux and 23±2 °C temp in 14 h photoperiod. Plants were irrigated with 0.2% modified Rorrison medium after 3 days of germination. The basic components of Rorrison medium are as follows: 0.4 mM $Ca(NO_3)_2$, 0.2 mM $MgSO_4.7H_2O$, 0.2 mM KH_2PO_4, 0.1 μm $CuSO_4$. $5H_2O$, 0.2 μm $ZnSO_4.7H_2O$, 9.2 μm H_3BO_3, 1.8 μm $MnCl_2.4H_2O$, 0.2 μm $NaMoO_4.2H_2O$ and 10 μm FeEDTA. The 50, 100 and 200 ppm of dimethoate treatment were given to the plant along with nutrient medium at alternate day and the first treatment was given after the 6 days of germination. Plant leaves were used

Table 1 Plant Growth Parameters (Shoot length, Root length, Fresh weight and Dry weight) of control as well as dimethoate treated wheat plants

± Values indicate standard deviation (mean $n=3$). The value in parenthesis shows percent decrease/increase over control plant

Treatment of dimethoate	Control	50 ppm	100 ppm	200 ppm
Shoot length (cm)	20.74±0.21	21.28±0.24 (2.6)	20.08±0.20 (−3.18)	17.34±0.19 (−16.39)
Root length (cm)	7.90±0.29	8.72±0.22 (10.38)	7.8±0.24 (1.27)	7.32±0.21 (−7.39)
Fresh weight (gm)	1.55±0.31	1.59±0.26 (2.58)	1.46±0.28 (−5.81)	1.13±0.22 (−27.1)
Dry weight (gm)	0.171±0.25	0.179±0.29 (4.68)	0.170±0.31 (−0.58)	0.169±0.24 (−1.17)

after 10 days of first treatment to analyze the effect of dimethoate.

Determination of Pigment

Twenty milligrams of plant leaves from control as well as dimethoate treated *Triticum astivum* L. extracted in 3 ml 80% acetone (*v/v*, in double distilled water) and the extract were used for the measurement of pigment contents. The pigment contents were determined from the transparent, centrifuged acetone extract solution by measuring the absorbance in the region 380–700 nm by using the UV/VIS spectrometer (Perkin Elmer lambda 35). The pigment concentrations were determined according to the method of Lichtenthaler and Welburn [27].

Laser-Induced Chlorophyll Fluorescence Spectra by Violet Diode Laser (405 nm)

LICF spectra were recorded using computer control Acton 0.5 M triple grating monochromator, Hamamastu R928 PMT as a detector, exited with a 405 nm violet diode laser (Oxxus CE, made in france, modal PS-001, Power 50 mW) light. The intensity of 405 nm violet diode laser is 140 μmol photons m^{-2} s^{-1}. The beam expander was aligned to obtain 4.0 cm^2 expanded laser light on leaves. The fluorescence radiation was collected on the entrance slit of monochromator. Leaf fluorescence was exited and sensed in a angle 45° to the leaf plain.

LICF spectra were recorded in the region of 650–780 nm with 1800 grooves/mm grating blazed at 500 nm wavelength using survey mode of spectra sense software. Time necessary for detection of one spectrum was about 78 s. These spectra were analyzed using GRAMS 32 software with curve fit Array basic program. Spectral correction was made from the response curve of PMT and grating of monochromator.

Fluorescence Induction Kinetics by 635 nm Red Diode Laser

Same experimental setup was used for the fluorescence induction kinetics with 635 nm red diode laser (Power 10 mW). The intensity of 635 nm red diode laser is 28 μmol photons m^{-2} s^{-1}. The fluorescence induction kinetics was measured by exposing the 20 min pre darkened plants leaves by red diode laser. The fluorescence intensity was recorded as the function of time at two different wavelength 685 and 730 nm for 5 min of nearly 4.0 cm^2 area of plant leaves using Intensity vs. Time mode of spectra sense software. Time resolution during detection

Table 2 Photosynthetic pigment contents and pigment ratios of control as well as dimethoate treated wheat plants

Treatment of dimethoate	Control	50 ppm	100 ppm	200 ppm
Chl a (μg/ml)	6.39±0.14	7.63±0.16 (19.4)	5.86±0.11 (−8.29)	4.18±0.12 (−34.59)
Chl b (μg/ml)	2.01±0.16	2.30±0.08 (14.43)	1.65±0.10 (−17.91)	1.18±0.06 (−41.29)
Total Chl (μg/ml)	8.39±0.15	9.93±0.09 (18.36)	7.51±0.13 (−10.49)	5.36±0.09 (−36.11)
Chl a/b	3.18±0.12	3.32±0.21 (4.07)	3.55±0.16 (11.29)	3.54±0.17 (10.66)
Car (μg/ml)	1.26±0.20	1.45±0.19 (15.07)	1.19±0.09 (−5.56)	0.89±0.14 (−29.37)
Chl/Car	6.65±0.18	6.86±0.19 (3.15)	6.30±0.20 (−5.26)	6.03±0.16 (−9.32)

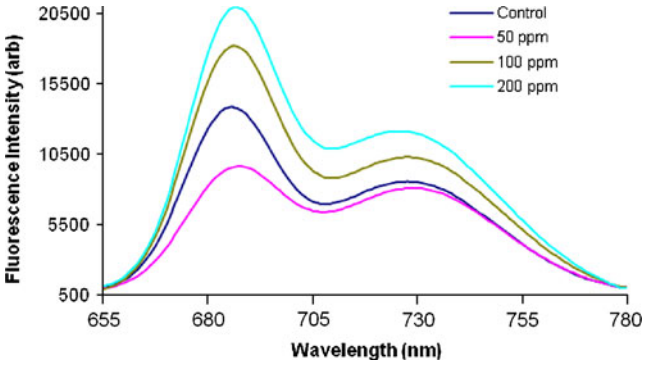

Fig. 1 Gaussian curve-fitted LICF spectra of the control and dimethoate treated wheat plant leaves excited by 405 nm violet diode laser

of the FIK curve is 500 msec. Various parameter such as fluorescence maximum (F_m), steady state level (F_s) and fluorescence decrease (F_d) were calculated from these curves. Vitality index ($R_{fd} = F_d/F_s$) was calculated from these parameters for all the samples.

Curve-Fitting

Interactive non-linear curve fitting was made using Levenberg-Marquardt algorithm method. Two Gaussian peaks have been used for fitting one at 685 and other at 730 nm. Three Gaussian parameters (Centre, Height and Width) were used for the fitting of each peak. The fitted parameters of the Gaussian curves to spectra based on the wave length on x-axis. After choosing the Gaussian spectral function, the individual component peaks were 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 bandwidth (full width at half intensity maximum).

Results

Growth and Photosynthetic Pigment Contents

Wheat plants treated with 50 ppm of dimethoate shows an increase in the shoot and root length, fresh and dry weight where as treatment of 100 and 200 ppm of dimethoate shows significant decrease in these plant growth parameters (Table 1). Similar to growth, 50 ppm of dimethoate shows increase in Chl *a*, Chl *b* and carotenoid contents. Wheat plants treated with 100 and 200 ppm of dimethoate shows significant decrease in the pigment contents, where as pigments ratio shows variable response. The relative decrease in the Chl content is significantly more than that of carotenoid contents. Change in Chl, carotenoid contents and pigments ratios are given in the Table 2. The decrease in the Chl *a* are 8.29, and 34.59% and decrease in the Chl *b* 17.91 and 41.29% over the control plant for 100 and 200 ppm respectively. The decrease in the Chl *b* are comparatively higher than the Chl *a*, that is why the ratio of Chl *a*/Chl *b* increase significantly with increase in concentration of dimethoate treatment. Carotenoid contents also decrease but the ratio of total Chl/Car increases for 50 ppm of dimethoate and decreases for 100 and 200 ppm concentration of dimethoate.

LICF Spectra

The LICF spectra of wheat plants are given in the Fig. 1, and F685/F730 ratios for peak height, band width and band area of the curve fitted spectra of control as well as dimethoate treated wheat plant leaves are given in the Table 3. These ratios show considerable variation over control plant. The ratio of peak height, FIR decrease by 15.86% and increases by 11.03, and 15.86% over the control plant for 50, 100 and 200 ppm of dimethoate treatment respectively. Band width (full width at half intensity maxima) is increase for 50 ppm and decrease for 100 and 200 ppm of dimethoate where as band area are decrease for 50 ppm and increase for 100 and 200 ppm of dimethoate.

Fluorescence Induction Kinetics

FIK curves are shown in the Fig. 2. Fluorescence decrease ratio or plant vitality index are given in the Table 4. The R_{fd} value shows an increase for 50 ppm of dimethoate. It increases by 12.93% for R_{fd} 685 and 14.93% for R_{fd} 730 over the control plants. Decrease in the R_{fd} 685 is 12.07 and

Table 3 The F685/F730 ratios for the peak height, band width and band area of the curve fitted spectra of control as well as dimethoate treated wheat plant leaves exited by 405 nm violet diode laser

Treatment of dimethoate	Control	50 ppm	100 ppm	200 ppm
Peak height	1.01±0.01	0.85±0.02 (−15.86)	1.13±0.01 (11.03)	1.18±0.02 (15.86)
Band width[a]	0.48±0.01	0.54±0.02 (12.5)	0.46±0.02 (−4.17)	0.47±0.01 (−2.08)
Band area	0.70±0.01	0.66±0.01 (−5.71)	0.75±0.01 (7.14)	0.80±0.01 (14.29)

[a] full width at half intensity maxima (FWHM)

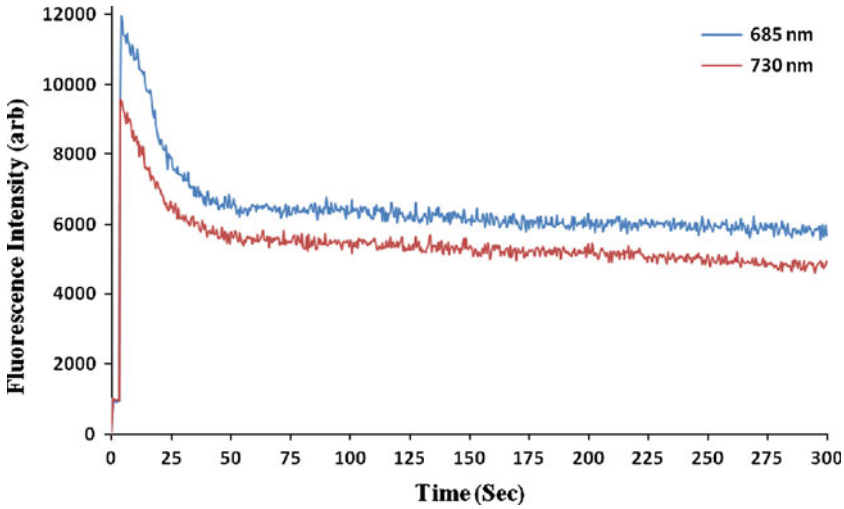

Fig. 2 Fluorescence induction kinetics curve of wheat plant leaves exited by 635 nm red diode laser

16.37% and decrease in the R_{fd} 730 is 8.33 and 13.10% over the control plant for 100 and 200 ppm of dimethoate respectively.

Discussion

The decrease in the FIR is related with the increase in the Chl content. The Chl fluorescence of leaves consists of two maxima in the red (near 685–690 nm), and far-red region (near 730–740 nm). The intensity and the shape of the Chl fluorescence spectra depends on the concentration of the fluorophore Chl *a* and to a lower degree also on the leaf structure, the photosynthetic activity and the leaf's optical properties. The latter determine the penetration of excitation light into the leaf as well as the emission of Chl fluorescence from different depths of the leaf. The increase of Chl fluorescence with increasing Chl concentration is mainly detected in the long wavelength range (far-red fluorescence), whereas short-wavelength red fluorescence levels off and then decreases due to the re-absorption of the emitted red Chl fluorescence by the Chl absorption bands, which reduce the short-wavelength fluorescence with rising Chl content [28]. 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, 15, 21]. Since the red Chl fluorescence maxima near 690 is more strongly affected by the re-absorption 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 Chl content and photosynthetic activity of the leaf [29, 30]. FIR value is decrease for 50 ppm of dimethoate. This suggests that application of low concentration of insecticide increases the pigment contents of the leaf. However pigment contents decrease at higher concentration of insecticide i.e. 100 and 200 ppm of dimethoate.

The increase in the R_{fd} value is related with the increase in the potential photosynthetic activity and capacity of plant leaves. In the dark-adapted leaf, the primary quinone electron acceptor (Q_A) and secondary quinone electron acceptor (Q_B) are thought to be fully oxidized and the reaction centre of PSII are "open". Upon illumination Q_A become reduced and transfer the electron to Q_B and during the rise to the maximum fluorescence (F_m) the plastoquinone pool is successively reduced by Q_B. At the point of the F_m, Q_A, Q_B and PQ pool are fully reduced by PSII. The reaction centre of PSII is now closed. With the onset of PSI

Table 4 Fluorescence-induction kinetics parameters for the control and dimethoate treated wheat plant leaves at 685 and 730 nm exited by 635 nm red diode laser

Treatment of dimethoate	Control	50 ppm	100 ppm	200 ppm
Rfd 685	1.16±0.01	1.31±0.02	1.02±0.01	0.97±0.01
		(12.93)	(−12.07)	(−16.37)
Rfd 730	0.84±0.01	0.96±0.01	0.77±0.01	0.73±0.02
		(14.29)	(−8.33)	(−13.10)

activity, which reoxidize the PQ pool as well as Q_A and Q_B, the F_m slowly decrease to terminal steady state and the fluorescence decrease from F_m to F_s is paralleled by increasing rate of oxygen evolution and the photosynthetic CO_2-fixation [1, 13, 14, 22, 31]. The R_{fd} value increases at 50 ppm of dimethoate. This suggests that application of low concentration of insecticide increases the photosynthetic activity and capacity of leaves. However at higher concentration of the insecticide i.e. 100 and 200 ppm of dimethoate, the photosynthetic activity decreases subsequently.

The FIR and R_{fd} values are further supported by plant growth parameters and photosynthetic pigment contents. Wheat plants treated with 50 ppm of dimethoate shows an increase in plant growth parameters. The increase in the plant growth parameter may be due to the possible cellular degradation of organophosphrus insecticide increased phosphate content that accelerated the plant growth. In addition, low doses of dimethoate probably increase the cell membrane permeability, which enhanced nutrient influx to the root cells, and their subsequent transportation to leaf and shoot may accelerate plant growth performance. In contrast to 50 ppm at a higher concentration (100 and 200 ppm) the decrease in root shoot length is due to the arrest of key physiological and biochemical steps. Dimethoate accumulates in plant leaves [32], which are the major sites of metabolic activities and thus inhibit the plant growth. Such inhibition in plant morphology may be associated with changes in cell division and cell elongation [33] or due to conversion of indole-3 acetic acid (IAA) into various photo oxidative products [34] and excess of reactive oxygen species (ROS) production.

Similar to the growth, 50 ppm of dimethoate shows increase in the photosynthetic pigment contents, which could be due to release of phosphorus following partial degradation of dimethoate and thereby, stimulating the growth and Chl biosynthesis. Oranophosphurus insecticide inhibits chlorophyll, protein and carbohydrate biosynthesis when algae are exposed for several days [5, 6]. It significantly inhibit the synthesis of Chl *a* under various dimethoate level [9]. It could be due to inhibition of Chl biosynthesis by inhibiting δ- aminolevulinic acid dehydratase and proto-chloropyllide reductase activities and breakdown of pigment or their precursors as reported for other stresses [35, 36]. Mishra et al. [37] also have observed similar inhibition in the Chl content in cowpea seedlings by dimethoate and UV-B exposure. Luier [32] has studied the metabolism of 32P and 14C-carbonyl labeled dimethoate in bean plants and conversion of dimethoate to the oxygen analog is correlated with translocation of dimethoate to the leaf tissue. Mishra et al. [10] has reported the production and accumulation of $O_2^{\cdot-}$ and H_2O_2 in leaves exposed to dimethoate and UV-B irradiation. H_2O_2 and $O_2^{\cdot-}$ are relatively less damaging themselves, but they can form other species such as hydroxyl radical ($OH^{\cdot}$) that can initiate lipid peroxidation, and thus causes membrane leakage. Chl present in the chloroplast acts as aphotosensitizer and may enhance lipid peroxidation by the formation of singlet oxygen [38]. Therefore, the decrease in the photosynthetic activity in of dimethoate treated wheat plants could be due to impairment of the electron transport chain modulated by ROS.

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References

1. Lichtenthaler HK (1990) In: Steven M, Clark JA (eds) Application of remote sensing in agriculture. Butterworths Scientific Ltd, London, pp 287–305
2. Saito Y, Kurihara K, Takahashi H, Kobayashi F, Kawahara T, Nomura A, Takeda S (2002) Remote estimation of the chlorophyll concentration of living trees using laser-induced fluorescence imaging lidar. Opt Rev 9(2):37–39
3. Saito Y (2007) Laser-induced fluorescence spectroscopy/technique as tool for field monitoring of physiological status of living plants, Proc SPIE-Int Soc Opt Eng 6604, 66041 W 1–12
4. O'Brien RD (1967) Biochemistry of insects. Academic, New York
5. Macro E, Martinez F, Orus MI (1990) Physiological alteration induced by the organophorus insecticide trichlorfon in *Anabaena* PCC 7119 grown with nitrates. Environ Exp Bot 30:119–126
6. Mohapatra PK, Mohanty RC (1992) Growth pattern changes of *Chlorella vulraris* and *Anabaena doliolum* due to toxicity of dimethoate and endosulfan. Bull Environ Contam Toxicol 49:576–581
7. Mohapatra PK, Schubert H, Schiewer U (1997) Effect of dimethoate on photosynthesis and pigment fluorescence of *Synechocystis* sp. PCC 6803. Ecotoxicol Environ Saf 36:231–237
8. Mohapatra PK, Schiewer U (1998) Effect of dimethoate and chlofenvinphos on plasma membrane integrity of *Synechocystis* sp. PCC 6803. Ecotoxicol Environ Saf 41:269–274
9. Chen Z, Juneau P, Qiu B (2007) Effect of three peticides on the growth, photosynthesis and photoinhibition of edible cyanobacterium Ge-Xian-Mi (*Nostos*). Aquat Toxicol 81:256–265
10. Mishra V, Srivastava G, Prasad SM (2009) Antioxidant response of bitter gourd (*Momordica charantia* L.) seedlings to interactive effect of dimethoate and UV-B irradiation. Sci Hortic 120:373–378
11. Lichtenthaler HK (1987) Chlorophyll fluorescence signatures of leaves during the autumnal chlorophyll breakdown. J Plant Physiol 131:101–110
12. Lichtenthaler HK, Buschman C, Rinderle U, Schmuck G (1986) Application of chlorophyll fluorescence in ecophysiology. Radiat Environ Biophys 25:297–308
13. Lazár D (1999) Chlorophyll *a* fluorescence induction. Biochim Biophys Acta 1412:1–28
14. Lazár D, Schansker G (2009) In: Laisk A, Nebdal L, Govindjee (eds) Photosynthesis in silico: understanding complexity from molecules to ecosystems. Springer Science+Business Media B.V, A A Dordrecht, The Netherland, pp 85–123

15. Gopal R, Mishra KB, Zeeshan M, Prasad SM, Joshi MM (2002) Laser induced chlorophyll fluorescence spectra of mung plants growing under nickel stress. Curr Sci 83(7):880–884
16. Mishra KB, Gopal R (2005) Study of laser induced fluorescence signatures from the leaves of wheat seedlings growing under cadmium stress. Gen Appl Plant Physiol 31(3–4):181–196
17. Mishra KB, Gopal R (2008) Detection of nickel induced stress using laser induced fluorescence signature's from the leaves of wheat seedlings. Int J Remote Sens 29(1):157–173
18. Strasser RJ (1986) Laser–induced fluorescence of plants and its application in environmental research, *Proceeding International Geoscience and remote sensing Symposium*, *IGARSS-86*, Zurich, III, ESA Publication Division, Noordwijk
19. Buschmann C, Lichtenthaler HK (1988) In: Lichtenthaler HK (ed) Application of chlorophyll fluorescence in photosynthesis research, stress physiology, hydrobiology and remote sensing. Kluwer Academic, Dordrecht, pp 325–332
20. Dahn GH, Gunther KP, Lodecker W (1992) Characterization of drought stress of maize and wheat by means of spectral resolved laser-induced fluorescence. EARSeL Adv Remote Sens 1:12–19
21. Gitelson AA, Buschmann C, Lichtenthaler HK (1998) Leaf Chlorophyll fluorescence corrected for reabsorption by means of absorption and reflectance measurements. J Plant Physiol 152:283–296
22. Lichtenthaler HK, Rinderle U (1988) The role of Chl fluorescence in the detection of stress condition in plant. CRC Crit Rev Anal Chem 19(1):S29–S85
23. Walker DA (1988) In: Lichtenthaler HK (ed) Application of chlorophyll fluorescence in photosynthesis research, stress physiology, hydrobiology and remote sensing. Kluwer Academic, Dordrecht, pp 13–20
24. Papageorgiou G (1975) In: Govindjee (ed) Bioenegetics of photosynthesis. Academic, New York, pp 319–372
25. Krause GH, Weis E (1983) Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynthetic Res 5:129–157
26. Haitz M, Lichtenthaler HK (1988) In: Lichtenthaler HK (ed) Application of chlorophyll fluorescence in photosynthesis research, stress physiology, hydrobiology and remote sensing. Kluwer Academic, Dordrecht, pp 249–254
27. Lichtenthaler HK, Welburn AR (1983) Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc Trans 11:591–592
28. Buschmann C (2007) Variability and application of the chlorophyll fluorescence emission ratio red/far-red of leaves. Photosynth Res 92:261–271
29. Maurya R, Gopal R (2008) LIF ratios of *Cajanus cajan* L. Under the stress of cadmium and its correlation with pigment content and pigment ratios. Appl Spectrosc 62(4):433–438
30. Maurya R, Prasad SM, Gopal R (2008) LIF technique offers the potential for the detection of cadmium-induced alteration in photosynthetic activity of *Zea mays* L. J Photochem Photobiol, C 9:29–35
31. Lichtenthaler HK, Buschmann C, Knapp M (2005) How to correctly determine the different chlorophyll fluorescence parameters and chlorophyll fluorescence decrease ration Rfd of leaves with the PAM fluorometer. Photosynthtica 43(3):379–393
32. Lucier GW (1967) Metabolism of dimethoate in bean plants in relation to its mode of application, *Entomology Ph.D. Thesis*, University of Maryland College Park, MD
33. Tevini M, Teramura AH (1989) UV-B effects on terrestrial plants. Photochem Photobiol 50:463–473
34. Ros J (1990) On the effect of UV-radiation on elongation growth of sunflower seedling (*helianthus annuus* L.) (Thesis), in: Karls, Beitr, Entw. (Eds. Tevini M) Bot Inst II Karlsruhe. Okophysiol 8:1–157
35. Teramura AH, Sullivan JH (1994) Effect of UV-B radiation on photosynthesis and growth of terrestrial plants. Photosynth Res 39:463–473
36. Ouzounidou G (1995) Cu ions mediated change in growth, chlorophyll and other ion content in a Cu-tolerate *Koeleria splendens*. Biol Plant 37:71–79
37. Mishra V, Srivastava G, Prasad SM, Abraham G (2008) Growth, photosynthetic pigments and photosynthetic activity during seedling stage of cowpea (*Vigna unguiculata*) in response to UV-B and dimethoate. Pesticide Biochem Physiol 92:30–37
38. Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and Stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198