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Study the effect of insecticide dimethoate on photosynthetic pigments and photosynthetic activity of pigeon pea: Laser-induced chlorophyll fluorescence spectroscopy

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ABSTRACT

Pigeon pea is one of the most important legume crops in India and dimethoate is a widely used insecticide in various crop plants. We studied the effect of dimethoate on growth and photosynthetic activity of pigeon pea plants over a short and long term exposure. Plant growth parameters, photosynthetic pigment content and chlorophyll fluorescence response of pigeon pea (*Cajanus cajan* L.) plants treated with various concentrations of the insecticide dimethoate (10, 20, 40 and 80 ppm) have been compared for 30 days at regular intervals of 10 days each. Laser induced chlorophyll fluorescence spectra and fluorescence-induction kinetics (FIK) curve of dimethoate treated pigeon pea plants were recorded after 10, 20 and 30 days of treatment. Fluorescence intensity ratio at the two fluorescence maxima (F685/F730) was calculated by evaluating curve-fitted parameters. The variable chlorophyll fluorescence decrease ratio (R_{fd}) was determined from the FIK curves. Our study revealed that after 10 days of treatment, 10 ppm of dimethoate showed stimulatory response whereas 20, 40 and 80 ppm of dimethoate showed inhibitory response for growth and photosynthetic activity of pigeon pea plants, but after 20 and 30 days of treatment all the tested concentrations of dimethoate became inhibitory. This study clearly shows that dimethoate is highly toxic to the pigeon pea plant, even at very low concentration (10 ppm), if used for a prolonged duration. Our study may thus be helpful in determining the optimal dose of dimethoate in agricultural practices.

1. Introduction

During the recent past, several toxic chemicals are continuously being spilled in agricultural fields as a consequence of modern agricultural practices, heavy industrialization and faster urbanization. In agriculture, pesticides are used mainly to control insects but this practice has resulted in pesticide poisoning in both plants as well as in animals [1]. Indiscriminate use of various types of pesticides in modern age has led to serious environmental hazards [2]. Dimethoate (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate) is a systemic thio-organophosphorus insecticide with foliar application [3]. It is used in various countries to control the insect population in a wide variety of crops. A large number of crop plants including fruits, cereals, legumes, vegetables, field crops, fodder crops and other economically important crops are registered from various countries for the use of dimethoate (see reference [3], and the references cited therein). Dimethoate produces its insecticidal effect through inhibition of acetylcholinesterase, an enzyme that terminates 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 on the phytotoxic effects of organophosphorus insecticides on phytoplankton have suggested that this type of toxicant reduces growth rates and inhibits chlorophyll (Chl), protein and carbohydrate biosynthesis [5,6]. Dimethoate has been shown to induce enhancement of respiratory O_2 consumption in cyanobacteria [6,7]. It is reported that dimethoate affects the fluidity and integrity of the lipid membrane [8]. Organophosphorus (OP) insecticide affected the lipid membranes by disorganization of phospholipid core [9] and lowering of membrane transition temperature [10]. Dimethoate caused significant reduction in photosynthesis, transpiration and stomatal conductance [7,11]. It is also reported to reduction in Photosystem II (PS II) activity and photophosphorylation in *Synechocystis* cells and cause inhibition of the photosynthetic

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electron transport [7,8]. Reports suggest that dimethoate negatively affects electron transport between PS II and PS I at the level of plastoquinone pool PQ [6,12]. Inhibition of photosynthetic electron transport resulted in increased PSII fluorescence and reduced CO_2 fixation [7]. At the elevated concentration dimethoate modifies the photosystem fluorescence of green algae and cyanobacteria [5,12,13]. Dimethoate caused inhibition of thylakoid ATPase [7,8,13] which results in decreased thylakoid pH, which caused increased non-photochemical quenching, increased initial fluorescence, decreased maximum fluorescence and decrease in variable fluorescence [11–13]. It also causes production and accumulation of reactive oxygen species (ROS) and increased activity of antioxidants such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) [14].

Laser-induced fluorescence (LIF) measurements have application in the field and in remote assessment of plant stress and physiological changes in leaves [15]. Chlorophyll fluorescence measurement is a non-invasive, rapid and highly sensitive probe for the analysis of photosynthesis not only for studying primary processes but also for physiological reaction processes [16]. It is used as one of the most powerful tools for probing excitation energy transfer, primary photochemistry, and electron flow on both the electron donor and electron acceptor side of PS II. It is further very useful in the quick assay of PS II mutations, down regulation and other adjustments to stress (such as excess light, heat, heavy metal, nutrients and pesticides) [16].

Chlorophyll fluorescence exhibits two maxima in 685 and 730 nm region [17]. The shape of the Chl fluorescence spectra and the value of fluorescence intensity ratio (FIR) at two fluorescence maxima (F685/F730) depend upon the Chl content of leaf [18]. At low Chl content, the fluorescence emission spectrum shows only one maximum peak at 685 nm with a shoulder near 730 nm. Parallel to the chloroplast and thylakoid multiplication, which proceed during the endogenous development and greening of the leaf, there occurs a massive accumulation of the light-harvesting Chl *a*/*b* pigments protein, which mainly functions in light absorption. This not only increases light absorption of leaves, but also causes an increase in re-absorption of the emitted fluorescence. Therefore with increasing Chl content, the shorter wavelength fluorescence becomes increasingly suppressed due to re-absorption of the emitted fluorescence by Chl and the shoulder at 730 nm develops as a second maximum [19]. Therefore, a decrease in the ratio F685/F730 is observed [20]. Since Chl fluorescence ratio F685/F730 is dependent on the concentration of Chl, it can be used to monitor changes in Chl content during leaf development [21], autumnal Chl breakdown [22], during re-greening of yellowish leaves [23] and also as a result of natural and anthropogenic stress and/or damage events [24–26].

In a dark-adapted plant leaf (placed in dark for 20 min or more), the cooperation of the two photosynthetic photosystems is impaired [19]. In dark-adapted plant leaf, the photosynthetic apparatus is in its nonfunctional state [27]. Upon irradiation of dark adapted leaves it takes a few minutes to induce cooperation in both the photosystems again and to bring about the joint photosynthetic electron transport reaction that leads to proper water splitting, oxygen evolution, as well as $NADP^+$ reduction and ATP formation [28]. This is required for photosynthetic CO_2 assimilation [27,29–31]. Chl fluorescence induction kinetics is characterized by a fast rise from the initial fluorescence level (F_0) to a maximum fluorescence level (F_m) within 500 ms. From the maximum fluorescence level, Chl fluorescence gradually declines to a much lower steady state fluorescence (F_s) within 3–5 min [32]. The greater this Chl fluorescence decrease F_d, from F_m to F_s, higher is the net photosynthetic rate of the leaf [16,19,33,34].

The present paper deals with the study of effect of dimethoate on growth, pigment content and photosynthetic activity of pigeon pea plants using laser-induced chlorophyll fluorescence spectroscopy at regular intervals of 10 days each until a total time span of 30 days. We have done this experiment because India is the largest producer and consumer of pulses in the world and pigeon pea is the second most important pulse crop in India after chick pea (*Cicer arietinum*) [35]. Dimethoate is extensively used in India. Nearly 636 metric tonnes of dimethoate was used in India during 2009–2010 [36]. Dimethoate is registered for 24 crops by Central Insecticides Board and Registration Committee of India. Food Safety and Standards Authority of India did not set Maximum Residue Limits for 10 crops including pigeon pea [36]. On 6th October 2011, Australian Pesticides and Veterinary Medicines Authority suspended the use of dimethoate on many food crops due to potential dietary risks [37]. Pigeon pea is a widely grown crop and dimethoate is an extensively used insecticide. So, there is a need to study the response of pigeon pea to dimethoate treatment. Thus, the objective of the present study was to test the potential application of LICF spectroscopy as a non-invasive and non-destructive tool to investigate the influence of dimethoate treatment on pigeon pea plants. The Chl fluorescence intensity ratio F685/F730 and variable Chl fluorescence decrease ratio (R_{fd}) values derived from Chl fluorescence measurements were utilized for assessing the impact of dimethoate treatment on growth and photosynthetic activity of this crop.

2. Materials and methods

2.1. Plant growth and treatment with the insecticide dimethoate

Healthy and uniform sized seeds of pigeon pea (*Cajanus cajan* L.), Var. SARJU-12, were surface sterilized with 4% sodium hypochlorite solution (v/v, in double distilled water) for 20 min and presoaked for 20 h in distilled water and kept wrapped in wet cloth overnight. Uniformly germinated seeds were selected and transferred into small plastic pots containing acid-washed sterilized sand (≈260–270 g). Plants were grown in a growth chamber under photosynthetically active radiation (PAR) of 90 μmol photons m^{-2} s^{-1} obtained from four fluorescent tubes (*Philips*, India) at 23 ± 2 °C in 14:10 h light: dark regime. Plants were irrigated with 0.2% modified Rorrison medium after 3 days of germination. The Rorrison medium contained: 0.4 mM $Ca(NO_3)_2$, 0.2 mM $MgSO_4{\cdot}7H_2O$, 0.2 mM KH_2PO_4, 0.1 μM $CuSO_4{\cdot}5H_2O$, 0.2 μM $ZnSO_4{\cdot}7H_2O$, 9.2 μM H_3BO_3, 1.8 μM $MnCl_2{\cdot}4H_2O$, 0.2 μM $Na_2MoO_4{\cdot}2H_2O$ and 10 μM FeEDTA. Dimethoate treatment of 10, 20, 40 and 80 ppm was given to the plants along with nutrient medium on alternate days and the first treatment was given after six days of germination. Plant leaves were used for analyzing the effects of dimethoate after 10, 20 and 30 days of first treatment.

2.2. Determination of photosynthetic pigments

Leaves (20 mg) from control as well as dimethoate treated *Cajanus cajan* L. plants were extracted in 3 ml 80% acetone (v/v, in double distilled water) and the extract was used for estimating the photosynthetic pigment content. The photosynthetic pigment content was determined according to the method of Lichtenthaler and Welburn [38] by recording the absorbance of the centrifuged, transparent acetone extract at 380–700 nm (UV/VIS spectrophotometer *Perkin Elmer lambda 35*, USA).

2.3. Laser-induced chlorophyll fluorescence spectra by excitation with 405 nm diode laser light

LICF spectra were recorded using computer controlled Acton 0.5 M triple grating monochromator (*Acton Research Corporation*,