Bull Environ Contam Toxicol (2010) 85:67–71
DOI 10.1007/s00128-010-0036-7

Development of Tolerance of Egg Plant (*Solanum melangena* L.) to Field Application of Dimethoate

R. Khillar · S. Acharya · P. K. Mohapatra

Received: 21 November 2009 / Accepted: 12 May 2010 / Published online: 26 May 2010

Abstract Dimethoate, at field concentration (1.419 mg g^{-1} fr wt), caused inhibition of photosynthesis, transpiration and stomatal conductance of *Solanum melangena* L. on first treatment but subsequent treatments caused adaptation and recovery of these parameters. The variable fluorescence (F_v), dissipation (DI_0/RC), 2 ms relative variable fluorescence (V_j), net rate of PS II closure (M_0), and maximum trapping rate of active PS II (TR_0/RC) increased initially but reduced to the control value with repetition of treatment. However, fluorescence yield (TR_0/Abs), electron transport probability (ET_0/TR_0) and activity of RC (ET_0/RC) increased with each treatment. With each subsequent treatment there was enhancement of activities of esterases and decrease of insecticide content of leaves.

Keywords Dimethoate · Tolerance · *Solanum melangena* · OJIP

Insecticides are used worldwide in agriculture in vast amount every year. The most commonly used organophosphorus (OP) insecticides are ethion, dimethoate, monocrotophos and chlorpyriphos in India. The widespread use of these insecticides over decades has led to their residual toxicity in the environment affecting many non-target organisms (Megharaj et al. 1994; Schäfer et al. 1994; Mohapatra and Schiewer 2000). Dimethoate (CAS 60-51-5) is a dimethyl substituted OP insecticide having a comparatively long half life in plant tissue than in soil. Because of its systemic nature the insecticide is applied on various vegetable crops, often for multiple times in one cropping season, to control the sulking and chewing pests like leaf hoppers, plant hoppers, stem borers and thrips (Wauchope et al. 1992). The insecticide is known to inhibit photosynthesis and modify photosystem fluorescence (Schäfer et al. 1994; Mohapatra and Schiewer 2000; Mohapatra et al. 1996, 2010). However, there is no information on the fate of the insecticide in the plant tissue even though its microbial degradation has been documented (Kuo and Raushel 1994; Liu et al. 2001). Repeated insecticide application induces the resistance of pests but response of the crop plant has not yet been properly documented. This paper deals with the study of the tolerance of the vegetable crop *Solanum melangena* L. (Family: *Solanaceae*) to repeated application of dimethoate through the analysis of the photosynthetic and OJIP fluorescence parameters.

R. Khillar · S. Acharya · P. K. Mohapatra (✉)
PG Department of Botany, Utkal University,
Bhubaneswar 751 004, India
e-mail: pradiptamoha@yahoo.com

Materials and Methods

Three weeks old seedlings of *Solanum melangena* L. (*Solanaceae*), raised in seed beds devoid of detectable level of pesticides, were transplanted in 5 L polyethylene bags containing 4 kg homogenized soil-manure mixture. Prior to transplantation, the soil was irrigated everyday for 10 days to allow weeds to come out. Proper deweeding was made before and after transplantation on daily basis. The experimental plants were maintained in a poly house having ambient temperature regime (35–38°C), relative humidity (59–71%) and irradiance (1,000–1,300 µmol photon m^{-2} s^{-1}) measured at 12.00 noon.

The commercial grade of dimethoate (CAS: 60-51-5) [O,O- dimethyl-S-(methylcarbamoylmethyl) phosphorothioate] was purchased from All India Medical Corp.,

Mumbai. The purity and content of the a.i. present in the commercial preparation were 98.7% and 294 ± 7.85 mg a.i. mL^{-1}, respectively. The insecticide stock solution (900 mg L^{-1}) was prepared fresh in double distilled water and was sprayed on the plants, 6 weeks after transplantation, to achieve a concentration of 1.419 ± 0.086 mg g^{-1} fr wt of leaf (at 95% CI). Three successive treatments, each at 15 days interval, were made and the responses were recorded at intervals (0–48 h) after each treatment.

The chlorophyll *a* fluorescence was measured from the upper surface of the four fully open leaves from the growing apex using a Plant Efficiency Analyzer (Handy PEA, Hansatech Instruments, Norfolk, UK). Prior to the measurement, the measuring area was dark adapted for 10 min, after which fluorescence rise was induced by a saturating red light pulse (3,000 μE m^{-2} s^{-1}; Strasser et al. 1995). The OJIP fluorescence levels were recorded for 1 s. The fluorescence after 50 μsec (F_0), 2 ms (F_j), 30 ms (F_i), and 500 ms (F_m) were noted to calculate the required fluorescence parameters viz., 2 ms relative variable fluorescence (V_j), net rate of PS II closure (M_0), trapping probability (TR_0/Abs), electron transport probability (ET_0/TR_0), effective antenna size of active RC (Abs/RC), maximal trapping rate by PS II (TR_0/RC), electron transport in active RC (ET_0/RC), and effective dissipation of active RC (DI_0/RC) using the equations of Force et al. (2003).

The measurement of photosynthesis (P_N), transpiration (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were made from the first four fully opened leaves from the apex with the help of an Infrared Gas Analyzer (IRGA, PP systems, UK) under ambient light, temperature and CO_2 regime following the procedure described earlier (Mohapatra et al. 2010). The amount of dimethoate in the leaf tissue of the crop plant was measured at intervals by extracting the insecticide following the method described by AOAC International (1990) using a gas chromatograph (PE Sigma 2000). The total esterase activity was measured from the crude enzyme extract prepared from 1 g in 10 mL cool (4°C) 50 mM Tris–HCl buffer (pH 7.0). The mixture was centrifuged (10,000 rpm for 20 min at 4°C) and filtered through a 0.2 μm pore size membrane (Oxytech GmbH, Germany). One milliliter of filtrate was mixed with 1 mL of buffer having fluoresceine diacetate (FDA) to achieve a concentration of 1 μM in the assay mixture. The fluorescence emission was measured by a fluorescence spectrophotometer (Varian Analytical Instruments, Clayton, Australia) continuously for a period of 30 min at 520 nm on excitation of the sample at 482 nm. The excitation and emission band passes were 5 and 3 nm, respectively.

The samples were taken in triplicates each containing a group of 10 plants. No death of plants was reported during the experiment. The data obtained from each group was averaged and taken as a replicate. The experiment was done twice to verify the results. The data presented in tables and figures are thus the means of six replicates of two separate experiments. The means of each treatment have been compared using least significant difference (LSD) test. The biophysical parameters have been compared by regression analysis. P_N, E and g_s of subsequent treatments have been compared by F-test. In all comparisons $p < 0.05$ has been considered as the level of significance.

Results and Discussion

The photosynthetic activity (P_N), transpiration (E) and stomatal conductance (g_s) of *S. melangena* were inhibited but intercellular CO_2 concentration (C_i) was significantly increased within 6 h after insecticide application (Table 1). The rate of inhibition of photosynthesis was more than of transpiration causing an increase of E/P_N ratio up to 6 h and a decrease thereafter due to recovery of the physiological processes. There was a significant negative correlation between P_N and C_i ($r = 0.98$; $n = 18$), and E and g_s ($r = 0.96$, $n = 18$) during the 48 h observation showing the stomatal control of photosynthesis (Grassi and Magnani 2005; Vernay et al. 2008). However, a negative correlation between g_s and C_i as well as C_i and P_N indicated that a part of photosynthetic inhibition was of non-stomatal type (Wu et al. 2008) caused by the inhibition of photosynthetic electron transport and carbon assimilation enzymes by the insecticide (Mohapatra et al. 1996, 2010; Wu et al. 2008). The plant showed recovery of all physiological processes with prolongation of post treatment period beyond 6 h as a result of which the differences of photosynthesis and transpiration between the control and the treated after 48 h were insignificant ($t = 1.014$ and 1.502, $p \leq 0.05$ for P_N and E, respectively). The same was also observed with respect to C_i and g_s ($t = 0.973$ and 1.024, $p \leq 0.05$ for C_i and g_s, respectively). With each successive treatment the plant showed improvement of adaptation to insecticide treatment resulting in an increase in P_N and E, but decrease of g_s and C_i. After 24 h, photosynthetic activity of second and third insecticide application differed significantly but there was insignificant difference between intercellular CO_2 levels of the two treatments indicating almost complete recovery of stomatal activity but still reduced rate of carbon assimilation.

There was a rise in fluorescence after 50 μsec (F_0), 2 ms (F_j), and 30 ms (F_i) but a dip after 500 ms (F_m) (Fig. 1) up to 6 h of each treatment. Gradual reduction of F_0 was observed with subsequent treatments and the difference between F_0 of control and of third treatment was only 13%

Table 1 The P_N, E, g_s, E/P_N and Ci of *S.melangena* treated with field concentration of dimethoate

Time (hour)	P_N	E	g_s	E/P_N	Ci
	First treatment				
0	16.682^{a}	8.233^{a}	648^{a}	494^{a}	314^{a}
2	9.882^{c}	6.307^{c}	536^{c}	638^{c}	348^{b}
6	8.760^{d}	5.845^{d}	505^{c}	667^{d}	364^{c}
24	14.805^{b}	7.762^{b}	598^{b}	524^{b}	319^{a}
48	16.066^{a}	8.130^{a}	624^{a}	512^{ab}	317^{a}
	Second treatment				
0	16.703^{a}	8.213^{a}	647^{a}	492^{a}	303^{a}
2	10.795^{c}	6.927^{b}	578^{b}	642^{c}	336^{b}
6	9.283^{d}	6.012^{c}	516^{c}	648^{c}	351^{c}
24	14.888^{b}	7.832^{a}	597^{b}	526^{b}	305^{a}
48	16.028^{a}	8.150^{a}	623^{a}	508^{a}	308^{a}
	Third treatment				
0	16.402^{a}	8.133^{a}	633^{ab}	496^{a}	309^{a}
2	12.712^{b}	7.468^{c}	613^{b}	588^{b}	319^{ab}
6	12.482^{b}	7.297^{c}	617^{ab}	585^{b}	323^{b}
24	15.755^{a}	7.855^{b}	621^{ab}	499^{a}	308^{a}
48	16.462^{a}	8.185^{a}	641^{a}	497^{a}	313^{ab}

Note Means superscripted by same letter in a column of each treatment are not significantly different from each other at $p < 0.05$. Comparison among the means was made separately for each treatment

Abbreviations P_N, photosynthesis (μmol CO_2 m^{-2} s^{-1}); E, transpiration (mmol H_2O m^{-2} s^{-1}); g_s, stomatal conductance (mmol H_2O m^{-2} s^{-1}); E/P_N (rel units); C_i, intercellular CO_2 concentration (μmol mol^{-1})

after 6 h (compared to 47% on first treatment). There was also corresponding rise and stabilization of F_j and F_i and improvement of F_m with subsequent treatments. The decline of 2 ms fluorescence and a corresponding enhancement of fluorescence maximum confirmed gradual normalization of OJIP fluorescence rise. However, with each treatment, the fluorescence of all levels (O, J, I and P) of control and treated were significantly different from one another ($t = 4.028$, 3.952, 3.084 and 3.641 for O, J, I and P rise, respectively; $p \leq 0.05$) after 6 h indicating that the insecticide has still significant adverse effect on the photoelectron transport processes of the crop.

The derived fluorescence parameters viz., 2 ms relative variable fluorescence (V_j), net rate of PS II closure (M_0), effective dissipation of active RC (DI_0/RC), maximal trapping rate by PS II (TR_0/RC) and effective antenna size of active RC (Abs/RC) showed nearly the same pattern of change in all three treatments during 48 h observation period (Table 2). The correlations between V_j, M_0 and DI_0/RC with the post treatment period were significantly positive during first 6 h in the first and second treatments but insignificant in the third treatment indicating recovery of these parameters ($r = 0.38$, 0.27, 0.32, respectively; $n = 18$) caused by evenness in the photosynthetic electron transport of the plant. Maximal trapping rate and effective antenna size of active RC changed remarkably during the same period but there was no significant correlation between the recovery period and the fluorescence parameters. Variable fluorescence (F_v), trapping probability (TR_0/Abs), electron transport probability (ET_0/TR_0), and electron transport in active RC (ET_0/RC) decreased significantly up to 6 h of first two treatments but insignificantly in the third one. Further prolongation of recovery, however, caused increase in these parameters in case of each treatment. With the normalization of OJIP rise, the fluorescence parameters, except the variable fluorescence and electron transport (in RC) recovered to the control level.

It has been reported by Lazar (2003) that increase of F_j is caused by increased level of reduced Q_A and stabilization of F_j (or V_j and M_0) is an indication of more orderliness in the PS II—PS I electron flow. This is supported by the gradual lowering of F_0, dissipation and PS II trapping rate with subsequent treatments. It proves that there was activation of dissipation process during the period of stress as a protective mechanism of photosynthesis. Absence of any definite pattern of change of the trapping rate of PS II

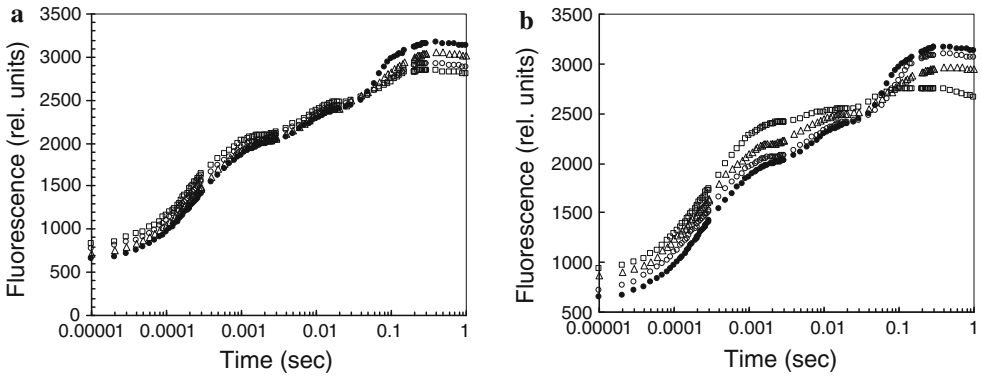

Fig. 1 The OJIP fluorescence rise of dark adapted *S. melangena* leaves as a response to three successive application of dimethoate. These are the responses measured 2 h (**a**) and 6 h (**b**) after spray application of the insecticide. Legends: *Filled circle* control; *Square* First treatment; *Triangle* Second treatment; *Circle* Third treatment

Table 2 The pattern of change of OJIP fluorescence parameters of *S. melangena* on repeated treatment with dimethoate at field concentration (1.419 mg g^{-1}fr wt)

Time (hour)	V_j	M_0	DI_0/RC	TR_0/RC	Abs/RC	Fv	TR_0/Abs	ET_0/TR_0	ET_0/RC
	First treatment								
0	0.506[a]	1.057[a]	1.339[a]	2.090[a]	2.798[a]	2358[e]	0.747[d]	0.494[d]	1.033[d]
2	0.600[b]	1.420[b]	1.525[b]	2.365[b]	3.607[c]	1858[b]	0.656[b]	0.400[b]	0.946[bc]
6	0.797[c]	1.617[d]	1.658[c]	2.028[a]	3.361[b]	1644[a]	0.603[a]	0.203[a]	0.411[a]
24	0.617[b]	1.497[c]	1.467[b]	2.425[b]	3.559[c]	1975[c]	0.682[c]	0.383[b]	0.929[b]
48	0.541[a]	1.134[a]	1.393[a]	2.095[a]	2.918[a]	2288[d]	0.718[e]	0.459[c]	0.961[c]
	Second treatment								
0	0.509[a]	1.048[a]	1.331[a]	2.057[a]	2.739[a]	2366[e]	0.751[c]	0.491[c]	1.009[b]
2	0.558[ab]	1.290[b]	1.455[ab]	2.312[b]	3.364[c]	2005[b]	0.687[b]	0.442[b]	1.022[b]
6	0.606[c]	1.277[b]	1.516[b]	2.105[a]	3.192[b]	1955[a]	0.659[a]	0.394[a]	0.828[a]
24	0.571[bc]	1.388[c]	1.426[ab]	2.430[b]	3.464[c]	2104[c]	0.701[b]	0.429[b]	1.042[b]
48	0.517[a]	1.142[a]	1.360[a]	2.210[ab]	3.005[b]	2301[d]	0.736[c]	0.483[c]	1.068[b]
	Third treatment								
0	0.507[a]	1.062[a]	1.335[a]	2.095[a]	2.797[a]	2369[c]	0.749[c]	0.493[b]	1.033[a]
2	0.532[a]	1.144[ab]	1.393[ab]	2.153[ab]	2.999[b]	2191[a]	0.718[ab]	0.468[a]	1.008[a]
6	0.527[a]	1.125[ab]	1.411[b]	2.134[ab]	3.010[b]	2193[a]	0.709[a]	0.473[a]	1.009[a]
24	0.541[a]	1.234[b]	1.367[a]	2.282[c]	3.119[b]	2267[b]	0.731[bc]	0.459[a]	1.047[a]
48	0.519[a]	1.142[ab]	1.342[a]	2.199[bc]	2.951[a]	2348[c]	0.745[c]	0.481[b]	1.057[a]

Note Means superscripted by same letter in a column of each treatment are not significantly different from each other at $p < 0.05$. Comparison among the means was made separately for each treatment

Abbreviations V_j, 2 ms relative variable fluorescence; M_0, net rate of PS II closure; DI_0/RC, energy dissipation; TR_0/RC, maximum trapping rate of active PS II; Abs/RC, effective antenna size of active RC; F_v, variable fluorescence; TR_0/Abs, PS II fluorescence yield of dark adapted leaf; ET_0/TR_0, electron transport probability; ET_0/RC, activity of RC

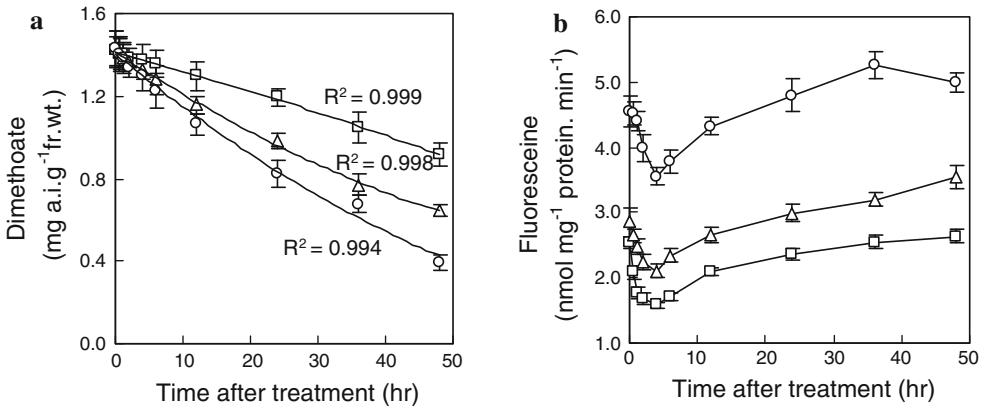

Fig. 2 Dimethoate content (**a**) and total esterase activity of the crude enzyme extract (**b**) of unwashed *S. melangena* leaves in response to repeated treatments. Treatments: *Square* First; *Triangle* Second; *Circle* Third

during treatments supported the view that this parameter did not provide much information on the adaptive response of plants to stress conditions (Force et al. 2003; Lazar 2003; Strasser et al. 2005).

Dimethoate content of unwashed leaves showed a time dependent decrease with each treatment. Significantly negative polynomial relationship between dimethoate content of leaves and post treatment time was observed (Fig. 2a). The reduction of the insecticide content in leaf tissue was found significant after 4 h in case of first treatment (LSD = 0.049 mg a.i.g^{-1} fr wt) whereas for other two treatments the reduction was significant after 2 h (LSD = 0.062 and 0.059 mg a.i.g^{-1} fr wt for second and third treatment, respectively). The rate of insecticide degradation increased with each successive treatment causing significant difference among treatments ($F = 28.3$, $p \leq 0.05$).

The activity of total esterase of leaf extract was found inhibited during first 4 h of treatment but the rate of reduction decreased with repetition of treatment (Fig. 2b). The correlations between the time after treatment and esterase activity were significantly negative for all

treatments ($r = -0.81$, -0.94 and -0.99 for first, second and third treatment, respectively; $n = 30$). The repetition of insecticide application enhanced the esterase activity of leaf samples resulting in higher level of activity after second and third treatments. After 6 h there was gradual recovery of the enzyme activity in each treatment. The rate of recovery increased with the repetition of treatment and could be well correlated with the insecticide content of the leaf tissue (-0.87, -0.91 and -0.82 in the first, second and third treatment, respectively; $n = 30$). Such insecticide stimulated activation of esterase has been reported by earlier workers (Kuo and Raushel 1994; Lai et al. 1994; Liu et al. 2001). This showed that repeated treatment of the crop with same insecticide may lead to the loss of efficiency of the toxicant to control the target pest, not only due to a probable increase in the tolerance of the target pest but certainly due to rapid degradation of chemical in the plant tissue. In addition, our observations confirmed that OJIP fluorescence parameters can be used as reliable indicators of the effect of organophosphorus insecticides on crop plants.

References

AOAC International (1990) Official methods of analysis. AOAC International, Arlington

Force L, Critchley C, van Rensen JJS (2003) New fluorescence parameters for monitoring photosynthesis in plant: I. The effect of illumination on fluorescence parameters of the JIP test. Photosynth Res 90:1–19

Grassi G, Magnani F (2005) Stomatal mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant Cell Environ 28:834–849

Kuo JM, Raushel FM (1994) Identification of the histidine ligands to the binuclear metal centre of phosphotriesterase by site-directed mutagenesis. Biochem 33:4265–4272

Lai K, Dave KI, Wild JR (1994) Bimetalic binding motifs in organophophorus hydrolase are important for catalysis and structural organization. J Biol Chem 269:16579–16584

Lazar D (2003) Chlorophyll *a* fluorescence rise induced by high light illumination of dark adapted plant tissue studied by means of a model of photosystem II and considering photosystem II heterogeneity. J Theor Biol 220:469–503

Liu YH, Chung YC, Xiong Y (2001) Purification and characterization of a dimethoate degrading enzyme of *Aspergillus niger* ZHY256, isolated from sewage. Appl Environ Microbiol 67:3746–3749

Megharaj M, Madhavi DR, Sreenivasulu C, Umamaheswari A, Venkateswaralu K (1994) Biodegradation of methyl parathion by soil isolates of microalgae and cyanobacteria. Bull Environ Contam Toxicol 53:292–297

Mohapatra PK, Schiewer U (2000) Dimethoate and quinalphos toxicity: Pattern of photosynthetic pigment degradation and recovery in *Synechocystis* sp PCC 6803. Arch Hydrobiol Suppl 134:79–94

Mohapatra PK, Schubert H, Schiewer U (1996) Short-term toxicity effects of dimethoate on transthylakoid pH gradient of intact *Synechocystis* sp PCC 6803 cells. Bull Environ Contam Toxicol 57:722–728

Mohapatra PK, Khillar R, Hansdah B, Mohanty RC (2010) Photosynthetic and fluorescence responses of *Solanum melangena* L. to field application of dimethoate. Ecotoxicol Environ Saf 73:78–83

Schäfer H, Hettler H, Fritsche U, Pitzen G, Röderer G, Wenzel A (1994) Biotests using unicellular algae and ciliates for predicting long-term effects of toxicants. Ecotoxicol Environ Saf 27:64–81

Strasser RJ, Srivastava A, Govindjee (1995) Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. Photochem Photobiol 61:32–42

Strasser RJ, Tsimilli-Michael M, Srivastava A (2005) Analysis of chlorophyll *a* fluorescence transient. In: Papageorgiou GC, Govinajee (eds) Chlorophyll *a* fluorescence—a signature of photosynthesis. Kluwer Academic Press, Dordrecht-Boston-London, pp 321–362

Vernay P, Gauthier-Moussard C, Jean L, Bordas F, Faure O, Ledoigt G, Hitmi A (2008) Effect of chromium species on phytochemical and physiological parameters in *Datura innoxia*. Chemosphere 72:763–771

Wauchope RD, Butter TM, Hoensby AG, Augustijn-Beckers PWM, Burt JP (1992) The SCS/ARS/CES pesticide properties database for environmental decision making. Rev Environ Contam Toxicol 123:1–32

Wu FZ, Bao WK, Li FL, Wu N (2008) Effect of water stress and nitrogen supply on leaf gas exchange and fluorescence parameters of *Sophora davidii* seedlings. Photosynthetica 46:40–48