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Recovery from Methyl Parathion-induced Damage of the Photosynthetic Apparatus in *Chlorella protothecoides*

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Insecticides are being increasingly used to improve agricultural production. The once widely used organochlorine insecticides are now being replaced mostly by organophosphorus insecticides, because of their harmful effects on non-target organisms and carcinogenic nature. By 1968, the sales in the United States, of organophosphorus compounds were coming to exceed that of organochlorines (Tariff Commission, 1973). Methyl parathion (o,o-dimethyl-o-(p-nitrophenyl) phosphorothioate) is one of the most widely used organophosphorus insecticides in modern agriculture. The present day volume of production of all organophosphorus insecticides has reached approximately 150,000 tons per year. Of this approximately 60,000-70,000 tons per year are for methyl and ethyl parathion (Fest, 1977). Crop protection without phosphoric esters is today, uncommon. Recent observations indicate that the organophosphorus insecticides are not completely harmless to the non-target organisms such as plants. Methyl parathion has been reported to decrease the yield of lettuce (Sances et al., 1981). It inhibits photosystem II electron transfer of $H_2O \longrightarrow$ DCIP (or FeCN) reaction in thylakoids isolated from higher plants ((Anbudurai et al., 1981). It has also been shown that methyl parathion inhibits the growth (cell number, pigment and protein contents) of Chlorella when the insecticide is added to an exponentially growing autotrophic culture (Saroja and Bose, 1983a), or when the cells are inoculated with nutrient medium containing the insecticide (Saroja and Bose, 1982). It has been demonstrated further that the inhibition of growth is parallel to the inhibition of photosynthesis attributing inhibition of photosynthesis as the primary reason for inhibition of growth (Saroja and Bose, 1983a). Furthermore, the inhibition of photosynthetic electron transfer appears to be due to binding of the insecticide to certain proteinaceous molecule(s) located on the surface of the photosynthetic membranes (Saroja-Subbaraj and Bose, 1983b). More recently it has been observed that when Cholorella protothecoides cells are grown autotrophically in the prsence of methyl parathion (300 µM) for several weeks, the cells become adapted to the insecticide (Saroja-Subbaraj and Bose, 1983c). In the present study an attempt has been made to study whether the damages induced by the insecticide during short periods (1 hour to 3 days) of exposure were irreversible or, if the damage is reversible, what the extent and kinetics of revival would be as compared to the control cells.

MATERIALS AND METHODS

A stock culture of Chlorella protothecoides, supplied from the algal culture collection of the University of Indiana (Acc No. 25), was maintained in a nutrient medium containing 1g KH_2PO_4, 1g K_2HPO_4, 0.3g $MgSO_4$, 3mg $FeSO_4$, $7H_2O$, 1 ml of Arnon's A_5 solution and 10 mg thiamine hydrochloride per litre, plus 0.1% NH_4Cl as the nitrogen source (Senger and Oh-hama, 1976).

Nutrient medium (250 ml) in each of the two 500 ml flasks was inoculated with exponentially growing culture of Chlorella and grown autotrophically. Methyl parathion was added to one of the flasks so as to make the final concentration 300 µM while the other flask received an identical volume of the nutrient solution. Both the flasks were shaken reciprocally at a rate of 120 strokes per minute at 25°C and a light intensity of 3000 lux. The flasks were covered with cotton plugs.

After one hour 60 ml aliquot was removed from each flask and centrifuged. Supernatants were discarded and after resuspending each pellet in 20 ml of insecticide-free medium, both samples were centrifuged. supernatants were again discarded and each pellet was resuspended in 10 ml of insecticide-free medium. These washed cells were then transferred to 250 ml of insecticide-free medium in each of 500 ml flasks. From each flask (one containing cells treated with and washed off methyl parathion, the other washed control cells) 40 ml culture was withdrawn, 2 ml for determination of cell number, 3 ml for PCV measurement, 5 ml for pigment estimation and 30 ml for photosynthetic O_2 evolution studies. Washed sub-cultures were then grown for a further period of 20 days.

On the first day and the third day additional 60 ml aliquots were withdrawn from the methyl parathion containing culture and the cells were washed and resuspended in insecticide-free medium as described above. As before 40 ml was withdrawn from the resuspended cultures for cell count, pigment estimation and O_2 evolution. The washed subcultures were grown for the next 20 days.

Cell number was counted with a Neubauer double haemocytometer. packed Cell Volume (PCV) was determined in hematocrit tubes by centrifugation at 2000 x g for 5 min.

For pigment analysis, 5 ml aliquots were centrifuged at 2000 x g for 3 min to obtian a pellet. Chlorophylls were extracted from the cells with hot methanol and estimated according to the method of Holden (1965) using the extinction co-efficient given by MacKinney (1941).

RESULTS AND DISCUSSION

Cultures of Chlorella protothecoides treated with 300 µM methyl parathion for 1 hour, 1 day and 3 days were transferred to

Table 1

Cell growth (cell number and Chl content) after insecticide treatment for various periods

Days	1 hr Treatment		1 Day Treatment		3 Day Treatment	
	Control	Treated	Control	Treated	Control	Treated
0	14.5 (7.5)	14.6 (7.47)	15.0 (7.47)	10.05 (4.73)	19.8 (6.27)	9.8 (2.78)
2	16.8 (9.04)	13.5 (7.0)	23.8 (8.04)	15.3 (4.15)	30.8 (8.28)	13.7 (2.48)
4	32.8 (11.95)	31.8 (10.5)	44.5 (9.24)	38.0 (7.36)	31.8 (9.1)	19.8 (6.55)
8	136.0 (40.8)	134.0 (39.6)	95.6 (28.08)	92.6 (25.6)	67.0 (26.8)	64.0 (24.7)
13	187.0 (67.0)	187.5 (69.0)	167.0 (38.6)	167.5 (38.0)	166.0 (53.0)	160.0 (61.2)
20	250.0 (147.5)	237.0 (147.4)	337.5 (100.75)	337.4 (120.0)	341.0 (80.0)	348.0 (90.66)

Insecticide treatment and other conditions are described in Materials and Methods.

Each number represents the number of cells in millions/ml of the culture medium; each number in paranthesis represents Chl content in μg/ml.

insecticide-free media and changes in cell number, chlorophyll content, chlorophyll a/b ratio and photosynthetic O_2 evolution were observed. The results in Table 1 show that exposure to insecticide for 1 day and 3 days inhibited the cell number and chlorophyll content as compared to the control; exposure for 1 hour did not affect these parameters. During subsequent growth in insecticide-free medium the cells showed very little growth during the first two days. However, during the next 2 to 4 days the growth rates of the treated cells were faster than that of the control cells, and by the 8th day the cell number and chlorophyll content reached the same values as in the control. During the remaining period of observation the cell growth remained the same as in the control. When the cell number and the chlorophyll content expressed as percent control were plotted as a function of time (Fig.1), an inhibition (or a lag) until the second day and a rapid recovery thereafter were clearly observed. The magnitude of inhibition or lag depended on the duration of exposure of the cells to the insecticide. A similar pattern of inhibition and/or lag followed by rapid recovery was observed for packed cell volume also (fig. 2A).

In case of photosynthesis and Chl a/b ratio somewhat different pattern had been noticed (Figs. 2B and 3). Although the initial inhibition of photosynthesis and Chl a/b caused due to exposure to the insecticide was observed as expected, the recovery of these parameters started without lag or further inhibition. This is particularly noticeable when the rate of photosynthesis was expressed on Chl basis (Fig. 3B). In fact, in this case the recovery was compelte in 4 days and the rates exceeded slightly over the control rate on this day.

Our results suggest that Chlorella cells exposed to 300 mM methyl parathion for a short period up to 3 days are not irreversibly damaged. When the cells are removed from the the presence of the insecticide, they regain their normal functions in the course of time.

Cell number, PCV, chlorophyll content, chlorophyll a/b ratio and photosynthetic O_2 evolution increase and reach almost their control levels within a period of 8 days after their removal from the medium containing the insecticide. This observation indicates that the insecticide does not remain at least in active state inside the cells after they are washed and resuspended in insecticide-free medium.

The recovery of cell number and chlorophyll content is probably depended on the reversal of inhibition of photosynthesis, since photosynthetic O_2 evolution, showed a rapid and earlier recovery after the removal of cells from the medium containing the insecticide as compared to that of cell number, packed cell volume and chlorophyll content.

The inhibition of photosynthesis due to exposure to the insecticide occurred not only when photosynthesis was expressed on

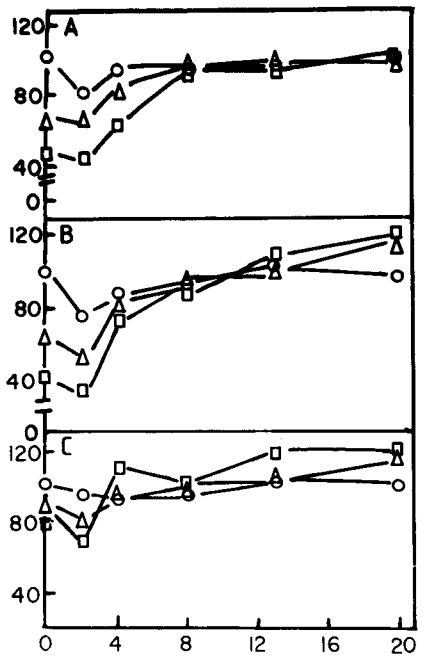

Fig. 1. Revival of cell growth of Chlorella protothecoides after insecticide treatment for 1 hr, 1 day and 3 days. A: Changes in cell number/ml, B: Chlorophyll content/ml, C: Chl/10^6 cells. The changes were expressed as % control. Details of treatment and other conditions are described in Materials and Methods.

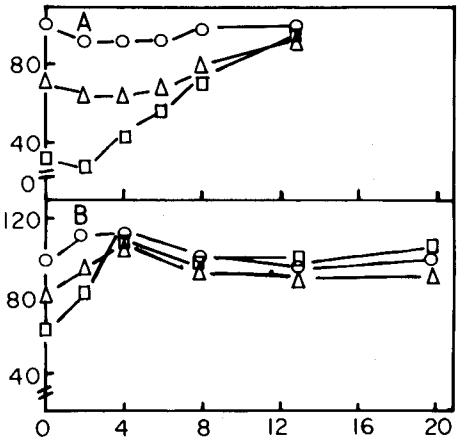

Fig. 2. Revival of PCV and Chl a/b ratio in Chlorella protothecoides after insecticide treatment for 1 hr, 1 day, and 3 days. The changes in PCV (A) and Chla/b ratio (B) are expressed as % control. Details of treatment and other conditions are described in Materials and Methods.

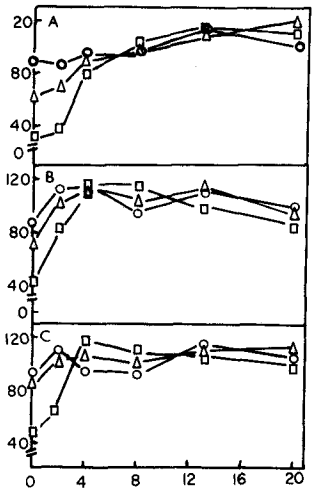

Fig. 3. Revival of the rate of photosynthesis in Chlorella protothecoides after insecticide treatment for 1 hr, 1 day and 3 days. The rate of photosynthesis was expressed in μmoles of O_2 evolved per ml (A), per mg Chl (B) and per 10^6 cells (C). Details of treatment and other conditions are described in Materials and Methods.

culture volume basis, but also on chlorophyll basis as well as on cell number basis. This indicates that the inhibition of photosynthesis on culture volume basis is not just due to inhibition of chlorophyll content and inhibition of cell number, but progressive damage in the photosynthetic apparatus otherwise has taken place during treatment with the insecticide. Decrease in Chl a/b ratio is also an indication of changes occurring in the structural organization of the photosynthetic apparatus (Bose et al., 1977). Complete recovery in the rate of photosynthesis indicates that the cells are capable of recovering from the damage on the photosynthetic membranes which occurred during insecticide treatment.

Powers et al. (1979) observed inhibition of cell number and ^{14}C uptake in DDE treated Exuviella baltica during the first 4 days of treatment. When the treated cells were transferred to DDE-free medium, cell number and ^{14}C fixation revived completely with lag periods proportional to the duration of DDE exposure. These observations along with the observations reported in the present paper suggest that recovery from the insecticide-induced damage of the photosynthetic apparatus is a general characteristic of photosynthetic organism.

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