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Phytotoxicity of Methyl Parathion with Special Reference to Photosynthesis in Wheat Seedlings

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Methyl parathion (o-o-dimethyl-o-nitrophenyl phosphorothioate), the most widely used organophosphorous insecticide (Fest 1977), is reported to constitute 33% of the organophosphorous insecticides produced in India. *(A Status report of R & D work done in India by Industrial Toxicology Research Centre, Lucknow. India. pp.69, 1978).* Methyl parathion has been shown to inhibit photosystem II (PSII) electron transport activity (Anbudurai et al. 1981), decrease yield in lettuce (Sances et al. 1981) and affect permeability properties of beet root membranes (Anbudurai et al. 1986). Further, this has also been shown to alter the pigment composition in the green alga **Chlorella protothecoides** (Saroja and Bose 1982). Its persistance in soil could be hazardous to the plant systems. It is to be noted that residual methyl parathion is known to persist in the rice fields, wherein water persists throughout the period of cultivation (Adhya et al. 1981). In this context it would be interesting to know about the effect of methyl parathion on germination and physiology of crop plants which grow under flooded irrigation conditions. For the present study wheat (**Triticum vulgare** L.) seedlings which require similar irrigation conditions as those of rice were chosen to investigate the phytotoxicity of methyl parathion.

MATERIALS AND METHODS

For our experiments, wheat seedlings were used. Seeds were surface sterilized, water soaked and then grown (50 seeds/petriplate of 15 cm diameter) in the presence of various concentrations of methyl parathion (MP) (20,40,60,80 and 100 μM) and in the absence of methyl parathion (control) over three layers of filter papers on petridishes and at a light intensity of 15 w/m.^{2} Initially 30ml of respective concentrations of the pesticide was added to the experimental plants and for control 30 ml of distilled water was added. From the second day onwards only distilled water was added regularly to all.

Photosynthetic pigments were extracted in 80% acetone and the chlorophylls and carotenoids were estimated according to Arnon

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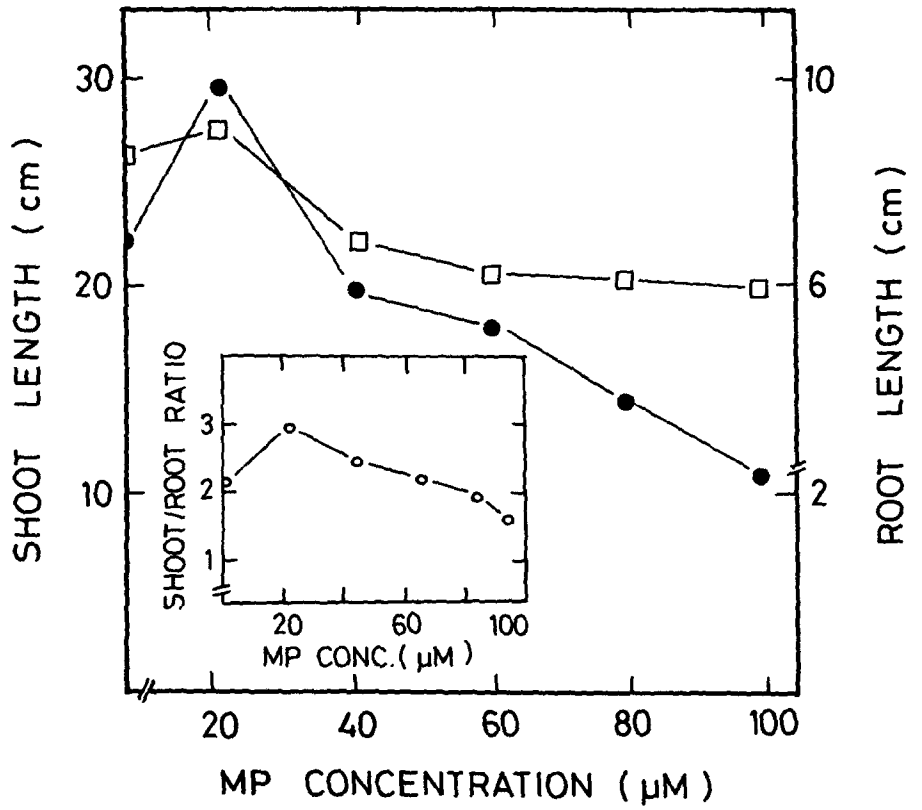

Figure 1. Effect of various concentrations of methyl parathion (MP) on shoot (□——□) and root (●——●) elongation of wheat seedlings. Inset of the figure shows the changes in the shoot by root ratio in methyl parathion treated seedlings.

(1949) and Ridley (1977) respectively. Thylakoid membranes were isolated from the leaves of ten day old seedlings of wheat with ice chilled isolation mixture containing 20 mM Tris-HCl pH 7.6, 5mM $MgCl_2$, 10 mM NaCl and 400 mM sorbitol by hand grinding using a pestle and mortar and the homogenate was filtered through three layers of cheese cloth and centrifuged at 5000xg for five minutes in a Sorval RC 5B centrifuge. The pellet was washed in the isolation mixture once and resuspended to a final chlorophyll concentration of 1-2 mg/ml in the isolation mixture for further use. Photosystem II activity was measured spectrophotometrically by DCIP (2,6-dichlorophenol indophenol) reduction method at 590 nm in a Hitachi-557 dual wavelength spectrophotometer, adapted for side illumination of the sample cuvette with red actinic light (> 620 nm) at a photon flux density of 650 $\mu E/m^2/s$. The photomultiplier was protected with corning CS

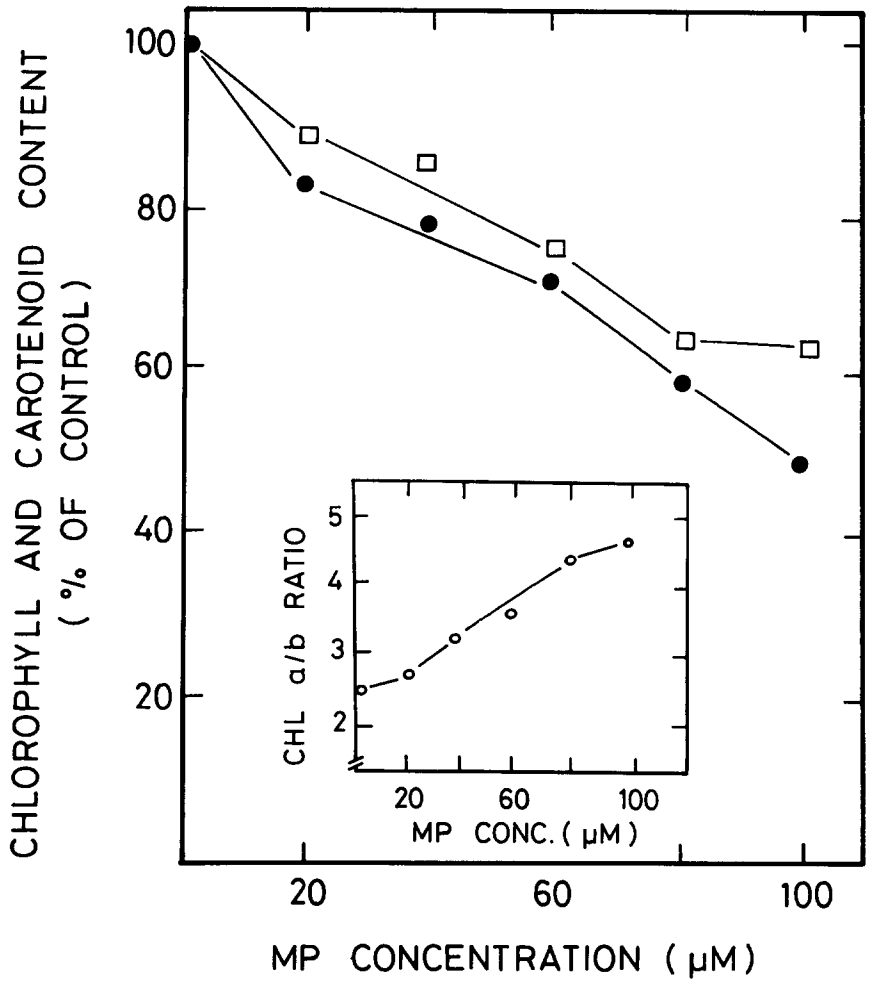

Figure 2. Effect of various concentrations of methyl parathion (MP) on the chlorophyll (□———□) and carotenoid (●———●) contents in wheat seedlings. Inset shows the changes in the chlorophyll a/b ratio in wheat seedlings due to methyl parathion treatment.

4-96 blue filters. The reaction mixture in a final volume of 3 ml contained thylakoid membranes equivalent to 25 μg chlorophyll/ml in 20 mM Tris-HCl pH 7.6, 100 mM sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 5 mM NH_4Cl and 0.05 mM DCIP.

RESULTS AND DISCUSSION

Methyl parathion inhibited the shoot and root elongation in wheat seedlings (Fig.1.). Inhibition of shoot and root elongation increased with increasing concentration of methyl

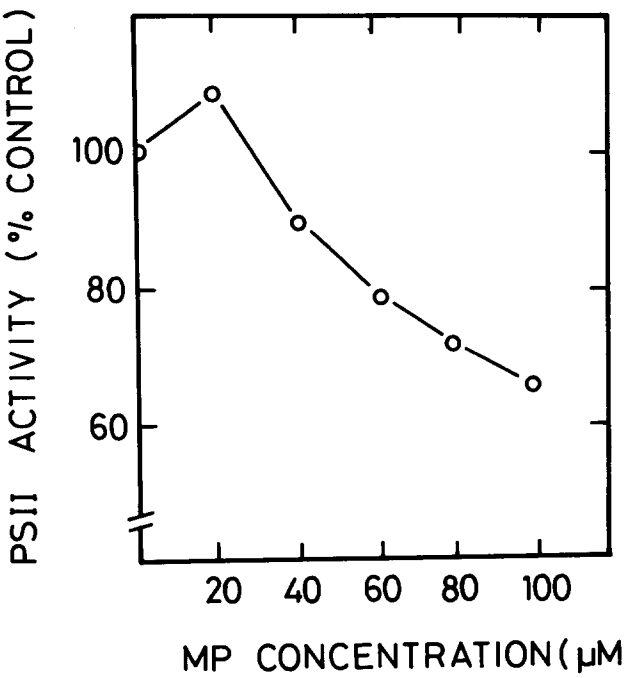

Figure 3. PSII activity ($H_2O \longrightarrow$ DCIP) in the isolated thylakoid membranes of methyl parathion (MP) treated wheat seedlings. The rates are expressed as per cent of control. The control (100%) rate was 210 μmoles DCIP reduced/mg. chlorophyll/hr.

parathion treatment. Inhibition of root elongation was greater than that of shoot as indicated by the increased shoot/root ratio (inset of Fig.1.). However, there was a notable increase in shoot and root elongation at lower concentration (20 μM) of methyl parathion treatment. The amount of photosynthetic pigments (chlorophylls and carotenoids) also decreased in the methyl parathion treated seedlings (Fig.2.). The decrease in carotenoid content was higher than that of chlorophyll. The chlorophyll a/b ratio increased steadily with increasing concentration of methyl parathion treatment (inset of Fig.2.). The increase in chlorophyll a/b ratio suggests a possible decrease in chlorophyll b content in the methyl parathion treated seedlings. All the chlorophyll b are known to be associated with the LHC (Light Harvesting Chlotophyll) (Thornber 1975) and most of the LHC are known to be associated with PS II (Melis and Anderson 1983). Hence the reduction in the amount of chlorophyll b (increased chlorophyll a/b ratio) indicates a drastic reduction in the amount of LHC in the chloroplasts of methyl parathion treated seedlings. The rate of photosystem II (PS II) electron transport activity ($H_2O \longrightarrow$ DCIP) in isolated chloroplast

membranes has decreased with increasing concentration of methyl parathion treatment (Fig.3.). Earlier studies with herbicide treated plants show that the reduction in the carotenoid content could lead to the destabilisation of LHC II (Plumley and Schmidt 1987; Dahlin 1988). Marked decrease in carotenoid content (Fig.2.) could lead to the destablisation of LHC II in methyl parathion treated seedlings. The notable increase in chlorophyll a/b ratio (Inset of Fig.2.) also indicates drastic decrease in the LHC content in methyl parathion treated seedlings. Studies with chlorophyll b - less mutants (altered LHCP) (Melis and Thielen 1980) and herbicide treated plants (Dahlin 1988) (lower amount of proteins of the light harvesting complex - LHC II) suggest that the LHC II plays an important role in the organisation of the chloroplast membranes and the light harvesting efficiency of PS II (Lam et al. 1983; Melis and Anderson 1983). Therefore, it is logical to assume that the reduction in the LHC II, could be the reason for the reduction in the PS II activity in methyl parathion treated seedlings.

Previous studies with organophosphorous insecticides have shown that they alter the permeability properties of erythrocyte membranes (Antunes-Madeira et al. 1981) and the beet root membranes (Anbudurai et al. 1986). Methyl parathion, the most widely used organophosporous insecticide, has been shown to block the photosystem II activity in isolated thylakoid membranes (Anbudurai et al. 1981) and pigment compostion in the green alga **Chlorella protothecoides** (Saroja and Bose 1983). This study shows that wheat seedlings grown in the presence of methyl parathion are prone to changes in the primary process of photosynthesis (electron transport activity). Further it can also be realised that that the structure and composition of the thylakoid membranes of tthe methyl parathion treated plants may be substantially altered. Further studies on thes lines may help to find out exact nature of interaction of methyl parathion with the photosynthetic apparatus of higher plants and also to elucidate the structure-function relationships between various components of the thylakoid membranes.

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