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Effect of Methyl Parathion on Betacyanin Efflux from Beet Root Discs

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Organophosphorous insecticides have been shown to alter permeability properties of liposomes and erythrocytes (Antunes-Madeira and Madeira 1979; Antunes-Madeira et al. 1981; Machado et al. 1980). In this communication, we report the effect of methyl parathion (O,O-dimethyl O-p-nitrophenyl phosphorothioate), the most widely used organophosphorous insecticide(Fest 1977),on the permeability properties of beet root membranes.

MATERIALS AND METHODS

Small homogenous parenchymatous discs of 1cm diameter and 2 mm thickness were cut out of storage tissue of the common garden beet (Beta vulgaris). Care was taken to avoid those regions of storage root which were too near to the surface or too rich in conducting tissue.

Beet root discs were washed thoroughly with distilled water, separated into lots of four discs by random choice and incubated with various concentrations of methyl parathion solution at room temperature. In all the experiments, the active ingradient (technical grade) of methyl parathion was used.

Alterations in the membrane permeability were monitored by spectrophotometric determination of the amount of betacyanin pigment leaked into the ambient solution over 0.5 to 6.0 hr period by measuring the absorbance at 540 nm in a Gilford 250 spectrophotometer (Gilford instruments Inc. Oberlin, Ohio, USA). To measure the total betacyanin content, a batch of discs was crushed well with distilled water, centrifuged at 2000g for 5 minutes and the absorbance of the supernatant was measured at 540nm. The extraction with 0.1N HCl gave the same results. The results are the average of three experiments.

RESULTS AND DISCUSSION

Methyl parathion caused an increase in the betacyanin efflux from beet root discs. The rate of betacyanin efflux increased with increasing concentration of methyl parathion (Fig.1). A semilogrithmic plot of betacyanin efflux with respect to time indicated that

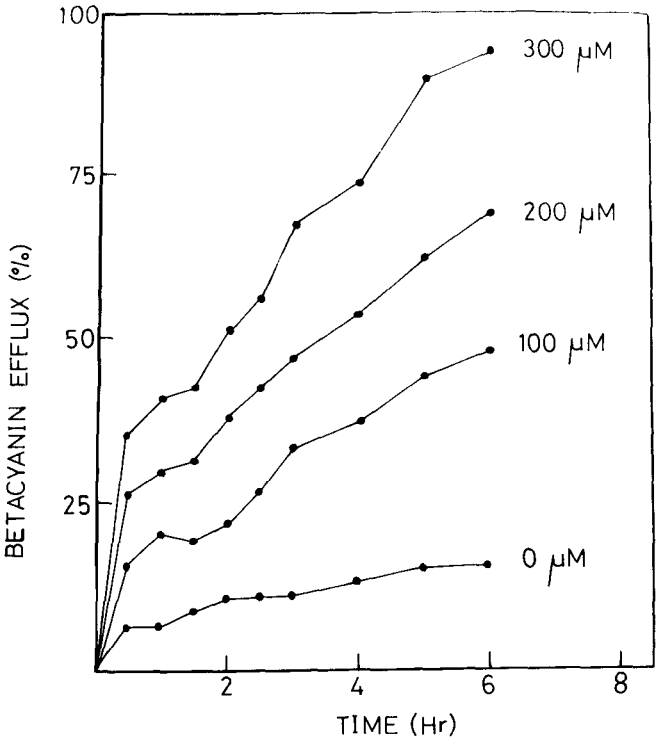


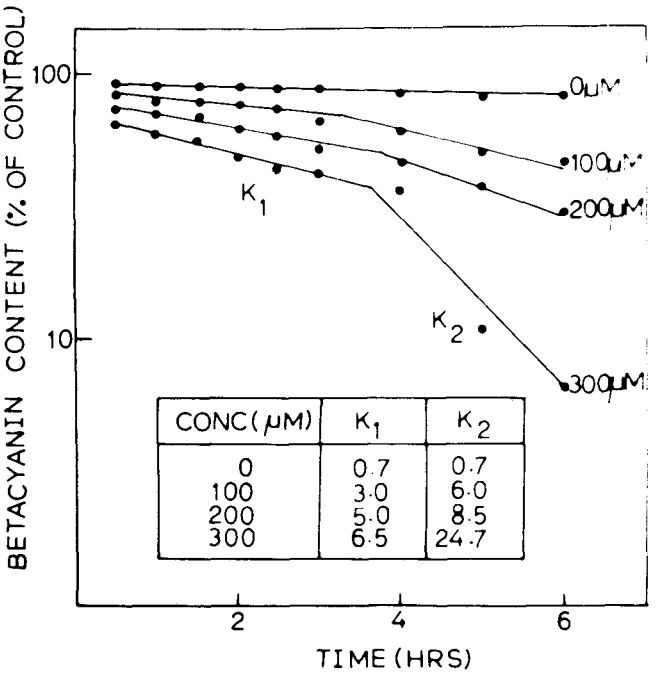
Figure 1. Time course of betacyanin efflux from the beet root discs due to treatment with various concentrations of methyl parathion.

the kinetics of methyl parathion induced efflux was exponential in nature (Fig.2). It, however, shows a biphasic pattern. The rate constant of the efflux during the first 3 hr (K_1) was found to be lower than that for the second 3 hr^{-1}(K_2).

The storage root of Beta vulgaris contains conspicuous amounts of the red purple coloured water soluble pigment, betacyanin, which is localized in the cell vacuoles (Siegal and Daly 1966). The tonoplast and plasmalemma are essentially impermeable to betacyanin. However, any treatment which alters the permeability properties of these membranes may result in a leakage

of betacyanin out of the cells. This effect can be monitored by measuring the betacyanin efflux from the tissue (Pooviah and Leopold 1976; Pooviah 1976; Seigel and Daly 1966; Reid et al. 1980).

In our experiments the efflux of betacyanin from beet root discs has been enhanced by methyl parathion. The biphasic pattern (Fig.2)of the efflux kinetics can be



CONC(μM)	K_1	K_2
0	0.7	0.7
100	3.0	6.0
200	5.0	8.5
300	6.5	24.7

Figure 2.Kinetics of betacyanin efflux from beet root discs due to treatment with various concentrations of methyl parathion. The insert shows the numerical values for the rate constants calculated from the slope values.

explained at the tissue level by the thickness of the discs which consist of atleast 20 cells. The first phase (K_1) may be due to the efflux from the cells near the surface of the disc, which are easily accessible to methyl parathion. The second phase (K_2) is probably due to betacyanin efflux from both the superficial as well as deeper layers on which the methyl parathion effect is delayed due to the time taken by methyl parathion to diffuse through the discs. Mercuric chloride treatment has been shown to have a similar effect (Thalouaran and Heller 1979).

Ethephon (2, chloroethyl-phosphoric acid) greatly stimulated the leakage of betacyanin from beet root discs (Pooviah 1976) and the stimulation was attributed to the decrease in the pH of the ambient solution brought about by the addition of ethephon (Reid et al. 1980). But the increased efflux of betacyanin by methyl parathion reported here was not due to the change of pH because pH remained unchanged with all the concentrations of methyl parathion used (pH 6.5). It has been reported that organophosphorous insecticides can bring about alterations in the permeability properties of liposomes and erythrocytes (Autunes-Madeira and Madeira 1979; Antunes-Medeira et al. 1981; Machado et al. 1980) to electrolytes. This is attributed to the increase of the fluidity of the membrane lipids promoted by organophosphorous insecticides.

We conclude that methyl parathion, an organophosphorous insecticide, enhanced the betacyanin efflux by altering the membrane permeability, which is consistent with previous reports that organophosphorous insecticides alter membrane permeability properties of liposomes and erythrocytes (Antunes-Madeira and Madeira 1979; Antunes-Madeira et al. 1981; Machado et al. 1980).

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