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Increase in membrane permeability of electrolytes and betacyanin in beet root disc by fenitrothion

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Abstract. Effect of fenitrothion (phosphorothioic acid, 0,0-dimethyl 0–4-nitro-*m*-tolyl ester), an organophosphorous insecticide, on membrane permeability employing the leakage of betacyanin and electrolytes as the criteria were studied in beet root (*Beta vulgaris*) discs. The leakage of both betacyanin and electrolytes increased with increasing concentrations (10-150 ppm) of fenitrothion in the incubation medium. At 0.33 mM the increase in electrolyte leakage was approximately linear for the first 6 h, while the increase in betacyanin leakage started with a lag of about 2 h. Long term incubation (24 h) showed a biphasic nature (in the semilog plot) for the increase in betacyanin leakage, while the increase in electrolyte leakage appeared more complex. In the control sample, the Arrhenius plots (25-50°C) of leakage showed a break at 40°C. In treated samples no breax was observed, but the slope decreased (for both electrolyte and betacyanin leakage) as compared to the respective slopes in the control in the temperature region greater than 40°C. The results are discussed in terms of the possible effect of the insecticide on the active transport in plant membranes.

Keywords. Membrane permeability; beet root discs; fenitrothion; betacyanin; electrolyte leakage.

Introduction

In the present day agriculture organophosphorous insecticides are used extensively. The primary target of the insecticides is acetylcholinesterase. Recent studies with liposomes and erythrocyte membranes, however, have shown that these insecticides affect permeability of water, glycerol and proton into these membranes (Antunes-Madeira and Madeira, 1979, 1980, 1981). These observations prompted us to examine if the membrane permeability in plants is also affected by organophosphorous insecticides. To test this hypothesis, we have chosen beet root tissue as a simple plant material and have examined the effects of fenitrothion, a very common organophosphorous insecticide, on the permeability of electrolytes and betacyanin.

Materials and methods

Betacyanin leakage as measured spectrophotometrically and ion leakage measured by conductivity changes were used as criteria in studying the membrane permeability (O'Brein and Prendeville, 1979; Reid *et al.*, 1980). The experimental procedure used was similar to that of Poovaiah (1979) and Poovaiah and Leopold (1976). Fresh beet roots were obtained from a local market and maintained at 4°C until used. Discs of 1 mm thickness and 5 mm in diameter were cut with a cork borer and a gel slicer. The discs were repeatedly washed with double distilled water before incubation. Twenty discs were incubated in 20 ml of double distilled water in a 50 ml beaker at 32°C. Fenitrothion was dissolved in a minimum amount of methanol, and the final concentration of methanol in the incubation medium was less than 1%, and the control received the same amount of methanol. Water baths set up at different temperatures were used for varying the temperatures of incubation. Betacyanin leakage was measured by monitoring the change in absorbance at 540 nm using a Gilford spectrophotometer and ion leakage using a conductivity bridge (MHOS pH meter, Model PE-131, Elico, India).

Results

Effects of concentration

Figure 1 shows the effect of fenitrcthion on ion leakage and betacyanin leakage from beet root discs which were incubated for 12 h at room temperature with various concentrations of the insecticide. The values shown in the figure are obtained by subtracting the control values (without fenitrothion) from the values of

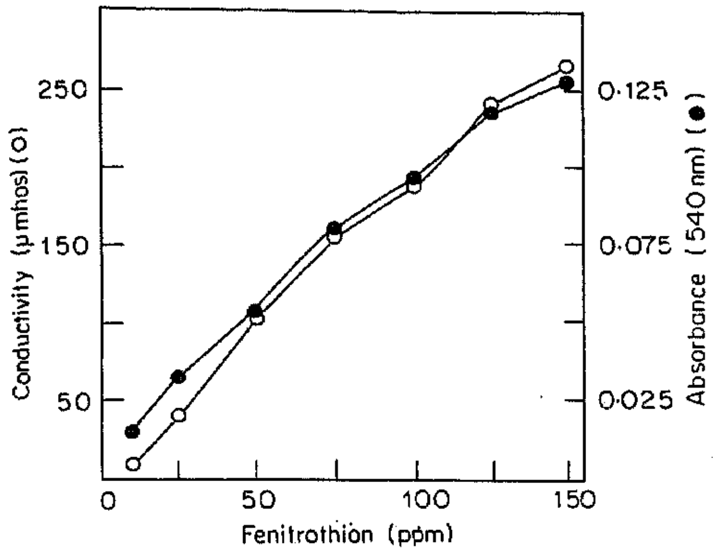

Figure 1. Effect of fenitrothion on the efflux of betacyanin and electrolytes from beet root discs.

Leakage of betacyanin and electrolytes were, measured at 12 h after treatment with different concentrations of fenitrothion.

treated samples. The result shows that the insecticide markedly increased the leakage of both ions and betacyanin. In the concentration range studied the effects were parallel to each other. The apparent difference observed at low concentrations should not be considered, because data obtained from several experiments showed no significant difference between the two effects.

Kinetics of leakage

To examine the kinetics of betacyanin and ion leakage the discs were incubated in solution of 100 ppm of fenitrothion and individual replicates (1 ml) were removed at intervals for the assay of betacyanin. A short term time course upto 6.5 h showed a linear increase in conductivity, whereas there was an initial lag period of 2 h in the case of betacyanin leakage to be observed (figure 2).

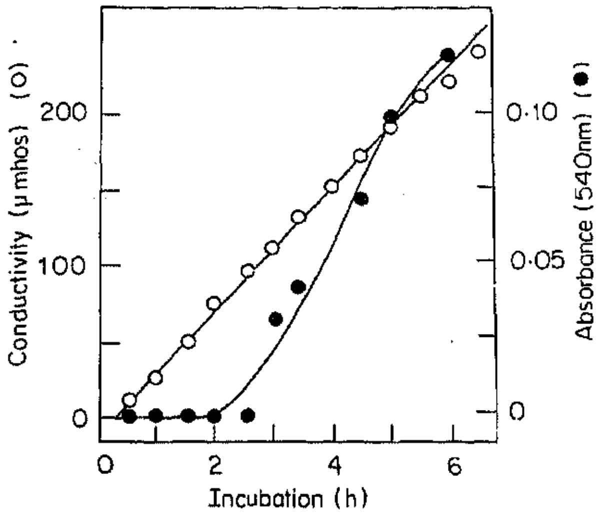

Figure 2. Short term time course of betacyanin and ion leakage from beet root discs.

Beet root discs were incubated in 100 ppm of fenitrothion at room temperature upto 6.5 h. At intervals, the betacyanin and ion content (relative) of the solution were measured. Control values were subtracted from the experimental values at each time interval.

A long-term time course carried out for 24 h with 100 ppm insecticide and data recorded during 3 to 24 h showed, however, an apparent parallelism between the two effects (figure 3). When these data were plotted on a semilog graph, a biphasic nature was revealed with a break at the 12 h, while the ion leakage appeared more complex (figure 3, inset).

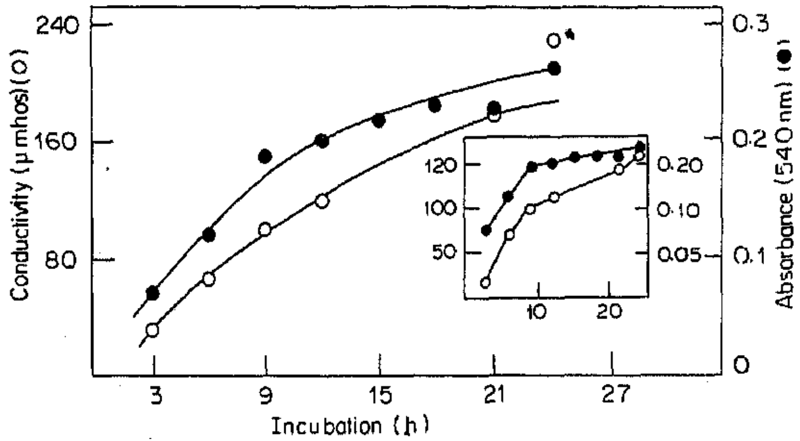

Figure 3. Long term time course of betacyanin and ion leakage. Leakage of electrolytes and betacyanin were measured at 3 h intervals for 24 h with 100 ppm fenitrothion. Control values were subtracted from the experimental values at each time interval.

Inset–Semilog plot of data from figure 3 showing the kinetics of electrolytes and betacyanin leakage.

Temperature dependence of fenitrothion effects

Discs bathed in solutions containing 100 ppm fenitrothion and incubated for 6 h at various temperatures did not show any effect on leakage below 25°C; the effects were observed when the discs were placed in solutions at 25°C and above. Arrhenius plots of conductivity and betacyanin leakage showed a break point at 40°C in the case of control samples (figure 4). Fenitrothion treated samples showed no break in the Arrhenius plots of betacyanin and ion efflux in the

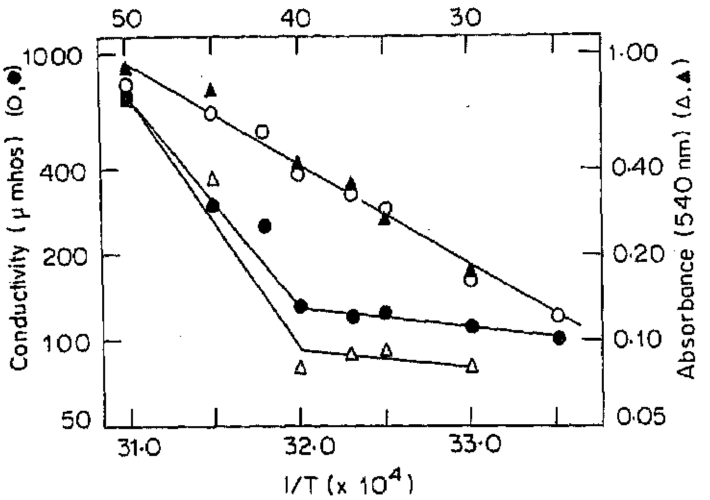

Figure 4. Arrhenius plot of fenitrothion effects on betacyanin and ion leakage.

Beet root discs were incubated in double distilled water with and without 100 ppm fenitrothion at different temperatures (25-50°C) for 6 h treatment.

O, Control; ●, 100 ppm for conductivity; Δ, Control; ▲, 100 ppm for absorbance at 540 nm.

temperature range used. The results also showed a decrease in the slope in case of the treated samples as compared to the respective slopes of the controls.

Discussion

The primary goal of the present study was to find out whether fenitrothion had any effect on membrane permeability in plants. The results demonstrated clearly that permeabilities of both ions and betacyanin in beet root cells were markedly increased. The parallel increase of ion leakage and betacyanin leakage at higher concentrations of insecticide (figure 1) immediately or after prolonged treatment (figure 3) is probably due to non-specific extensive damage that may have occured in the membrane structure.

The observation that there was a lag (~ 2 h) in the betacyanin leakage (figure 2), is not unexpected because both the plasmalemma and the tonoplast membranes should be altered for an effect on betacyanin leakage to be observed, (betacyanin pigments are located inside the vacuole), whereas a change in the plasmalemma alone may affect the permeability of the ions. The biphasic character of the increase in betacyanin leakage is (figure 3 inset) also consistent with the involvement of the two membranes in betacyanin leakage. The complex kinetics of ion leakage is not unexpected, because conductivity is a measure of leakage of ions of various kinds and the effects of the insecticide may not be identical for each ion.

The mechanism of the insecticide action on membrane permeability is not known. The absence of the insecticide effect at low temperature suggests that the passive transport of ions and of betacyanin is not a major site of action of the insecticide at the concentration used (100 ppm) during the period of incubation (6 h). In that case, it will be interesting to examine the insecticide effects on active transport of certain specific ions. A break (around 40°C) in the Arrhenius plots of the control samples suggests that active transport is primarily responsibile for the leakage; the enzymes involved become inactive at about 40°C resulting in an increase in activation energy. In the presence of the insecticide, the activation energy is somewhat lowered as indicated by a decreased slope, and the absence of a break in the line, in the temperature region studied, suggests a shift in the transition temperature beyond this range.

It should be noted that the concentration of fenitrothion and other organophosphorous insecticides used in the solution sprayed in the field is in the range of 100 to 500 ppm (amount used varies between 0.4 to 2 lb per acre); the concentration (100 ppm) used for most of the experiment in the present study is well within this range. This raises a concern on the use of insecticides at the present prescribed doses in terms of plant growth and yield. In fact, recent studies have shown that methyl parathion, another widely used organophosphorous insecticide, decreased the yield (diameter and weight) of lettuce (Sances *et al.,* 1981). A decrease in transpiration and net photosynthesis has also been noted in this report. Saroja and Bose (1982) have shown that methyl parathion (200 to 400 ppm) inhibits severely the growth characteristics of *Chlorella,* and Anbudurai *et al.* (1981) have shown that the rate of electron transport (Hill reaction) in isolated chloroplasts of higher plants is inhibited by methyl parathion with I_{50} around 50 ppm. All these observations suggest the necessity of more careful application of insecticides.

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