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Endosulfan Induced Biochemical Changes in Germinating *Cicer arietinum* Seeds

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Endosulfan (a cyclic sulfurous acid ester of cyclodiene group), commonly used as a broad spectrum chlorinated insecticide, was tested for its potential to produce impairments on seeds germination. Endosulfan (10ppm) when incorporated in the medium lowered viability (22%) and delayed germination (24 hrs). A concentration dependent inhibition in root (16–45%) and shoot (13–43%) growth was observed when seeds were exposed to 0.01, 0.10, 1.0 and 10.0 ppm of the insecticide. At 10ppm the mobilization and utilization of seed reserves was affected leading to disturbances in cell wall components and auxin level. Insecticide (10ppm) treated seedlings picked up more of ^{59}Fe.

Key words : **Germination, Endosulfan,** *Cicer arietinum* **Phytotoxicity**

Introduction

Most of the information on phytotoxicity of pesticide has come from studies on herbicides (Menn & Still 1977) very few reports pertain to the effect of insecticide on chemical constituents formed during germination and development. Dalvi and Salunkhe (1975) and Agarwal and Beg (1979) have proposed germinating seeds as a model system for assessing the toxic-cological potential of pesticides. This communication deals with the demonstration of growth inhibitory activity of endosulfan (a cyclic sulfurous acid ester of cyclodiene) on germinating Bengal gram.

Material and Methods

Preparation of Plant Material

C. arietinum seeds procured from local grocery stores (variety unknown) after surface sterilization with 0.1% (wt/vol) calcium hypochlorite were imbibed overnight at 10 ± 2°C. After 48 hrs of germination on cotton bed in dark at 28±2°C, the seedlings were implanted on 1% (wt/vol) agar bed containing 10ppm endosulfan.

Chemical and Enzyme Assay

Tissues were processed for analysis of sugars according to Azhar et al. (1972). Total

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sugars, reducing sugars, hexoses, ketosugars and pentoses were estimated according to the methods of Montgomery (1967), Nelson (1944) and Somogyi (1945), Roe (1955), Roe and Papadopoulos (1954), and Tracey (1950) respectively, protein was estimated according to Lowry et al. (1951) after precipitation with 5% trichloro acetic acid. Amylase (E.C. 3.2.1.1.) phosphorylase (E.C. 2.4.1.1.) and invertase (E.C. 3.2.1.26.), were assayed according to Bernfeld (1955), Taussky and Shorr (1953) and Straus (1962) respectively. Tissue homogenates (10% wt/vl.) prepared in chilled (0—4°C) distilled water were used as source of enzymes.

Isolation and Fractionation of Cell Wall

Cell wall was isolated according to Selvendran (1975) and fractionated by the modified procedures of Dever et al. (1968).

Extraction of Indole Acetic Acid (IAA)

Indole acetic acid was extracted according to Agarwal and Beg (1982).

Residue Analysis

n-Hexane-isoporpanol mixture (2 : 1 wt/wt) was used for the extraction of endosulfan as advocated by Maier-Bode (1968). Endosulfan was estimated in extracts by gas-liquid chromatography (GLC) using an electron capture detector (^3H) (Varian Aerograph Series 2400).

Uptake of ^{59}Fe

^{59}Fe as ferric citrate was procured fro Isotope Division, BARC, Bombay.

Fifteen plants each of 5 and 7 days old controls and endosulfan exposed seedlings were kept in Kreb's Ringer's phosphate buffer *p*H 7.4, containing ^{59}Fe (50μg 0.5 ml diluted to 500 ml with KRP buffer) as ferric citrate. Five plants each were withdrawn at 0, 2.5 and 5.0 hrs respectively, washed thoroughly with KRP buffer (free from ^{59}Fe) and processed for weighing and counting. The samples were counted for ^{59}Fe, directly in a Packard 5230 Autogamma scintillation spectrometer.

Statistical Analysis

Calculations for mean ± S.E., student 'T' test and 'P' values were done according to Fischer (1938)

Results and Discussion

From the extensive biochemical and morphological changes noticed in the present study it would appear that seeds are very vulnerable to the toxic activity of chemicals in the post imbibition phase. Endosulfan (10ppm) treated seeds showed 78% germination at 24 hr and 87% at 48 hrs. No further change was observed after this time interval. Endosulfan caused a concentration dependent inhibition in root and shoot growth (table 1). Though a strict quantitative

Table 1 *Effect of different concentration of endosulfan on* C. arielinum *seedlings*

Concentration (ppm)	Length (cm)					
	4th day		6th day		10th day	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	9.5±0.30	3.5±0.10	14.0±1.0	8.0±0.90	16.0±1.20	14.0±1.3
0.01	7.0±0.41	3.3±0.25	13.0±0.80	10.0±1.02	16.3±1.53	14.5±1.50
0.10	5.0±0.20	3.4±0.40	9.0±0.90	6.5±0.61	16.2±1.18	14.2±1.46
1.0	4.0±0.15	3.0±0.18	8.0±0.56	6.5±0.55	15.0±1.33	12.5±1.13
10.0	3.0±0.03	2.6±0.23	6.0±0.49	4.5±0.36	9.0±0.83	7.0±0.60

relation could not be established, lowest growth was found at highest endosulfan concentration. *C. arietinum* seeds exposed to 10ppm endosulfan exhibited a reduced growth in root and shoot at all the stages. The inhibition eventually resulted in a dwarf plant. There was no evidence of recovery from the toxic effect of endosulfan at 10ppm concentration unlike that observed at 0.01 and 1ppm. Endosulfan exposure even at low concentrations retarded the germination and seedling growth at early periods of germination in agar pots. It is interesting to note that the degree of inhibitions caused by endosulfan declined sharply at later stages of germination so much so that in some cases seedling growth even matched the control values. The only exception was the case of seeds treated with 10ppm endosulfan which showed a persistent inhibition throughout the period of study. Recovery from inhibition may be facilitated by a relatively quick disposal of the small amounts of the insecticides or due to its inactivation after its binding to biomacromolecules (Menn 1978). This view is supported by the fact on exposure to high concentrations of endosulfan (10ppm) the inhibitory effect is persistent throughout, probably, on account of the fact that the insecticide could saturate the binding sites.

At 24 hrs of exposure there was considerable depletion of starch reserves. However, at subsequent stages the depletion was comparable to that in control. The depletion of starch is accompanied by triggering of amylase and release of soluble sugars on the first day of germination, the amount being much more than in controls (table 3). The

Table 2 *Levels of starch, amylase, phosphorylase and total sugars in cotyledons on 10ppm endosulfan treatment*

Days	Starch % dry wt.		Amylase Units/mg protein		Phosphorylase Units/mg protein		Total Sugars mg/g dry wt.	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
48 hrs	65±3.0	65±3.0	0.50±0.02	0.50±0.02	2.90±0.10	2.90±0.10	10.0±0.87	10.0±0.87
One	55±4.0	41±3.5	0.60±0.01	1.00±0.08	5.80±0.25	3.90±0.20	15.0±0.94	20.0±1.20
Three	47±3.2	38±2.0	1.60±0.12	1.95±0.15	6.50±0.39	4.00±0.23	19.0±1.39	25.0±1.63
Five	40±2.8	33±1.5	2.70±0.18	2.25±0.13	8.40±0.54	6.50±0.67	28.0±1.68	27.0±1.24
Seven	35±1.7	31±2.5	2.90±0.11	2.60±0.16	9.80±0.83	12.3±0.99	39.0±2.97	26.5±2.31

Table 3 *Effect of endosulfan on ^{59}Fe uptake by the root system*

Treatment time (hrs)	Endosulfan Concentration	Cpm/g fresh wt.	
		5 day old	7 day old
1.0	Control	958.85±65.39	2607.60±60.34
	endosulfan (10ppm)	1373.10±90.38[b] (+43)	3118.61±52.91[a] (+20)
2.5	Control	1552.04±50.63	3906.32±90.43
	endosulfan (10ppm)	1747.60±73.60[c] (+13)	4583.18±83.20[a] (+17)
5.0	Control	2900.30±240.97	5192.79±152.39
	endosulfan (10ppm)	3224.76±190.81 (+11)	6027.18±132.85[a] (+16)

All values are mean ± S.E. from five to six observations.

Figures in parenthesis indicate percent change as compared to controls.

[a] $p<0.001$; [b] $p<0.005$; [c] $p<0.05$

increase in amylase activity was comparable in control and experimentals on fifth and seventh day. Phosphorylatic clevage is considered to be the chief route for the mobilization of starch in legumes at earlier stages of germination (Juliano & Varner 1969, Fernandez-Taroago & Nicolas 1976). In the present study however endosulfan treatment caused a lowering of phosphorylase activity from first to fifth day of germination. On seventh day the activity was found elevated. The role of phosphorylase is not very clear at present. It is reported to play some role in the synthesis of cell constituents in other species (Tsai & Nelson 1969). Endosulfan also caused inhibition of invertase activity (figure 1) thus restricting the amount of sucrose available for entry into the cell constituents. This also suggests less utilization of soluble sugars, as their level remained high in experimental seedlings than in controls (figure 2).

Plant growth is associated with change in cell wall polysaccharides (Nishitani et al. 1979). In the present study endosulfan treated chick pea seedlings showed a higher content of the free sugar reflecting a diminished utilization of reducing, ketoses, hexoses and pentoses (data not mentioned here) for the synthesis of polymers including cellulose and hemicellulose. The effect on pectin seems to be very drastic. Since IAA level are found to be lowered in pesticide exposed seedlings (figure 4) a certain degree of disturbance in polysaccharide metabolism is to be expected eventually reflected as modification in the cell wall structure (Nishitani et al. 1979). Dwarf plants often contain comparatively low amount of IAA (Jindal et al. 1974). Promotion of cell wall synthesis induced by auxin is a delayed effect compared with the effect of auxin on rate of cell enlargement.

One of the factors which is likely to

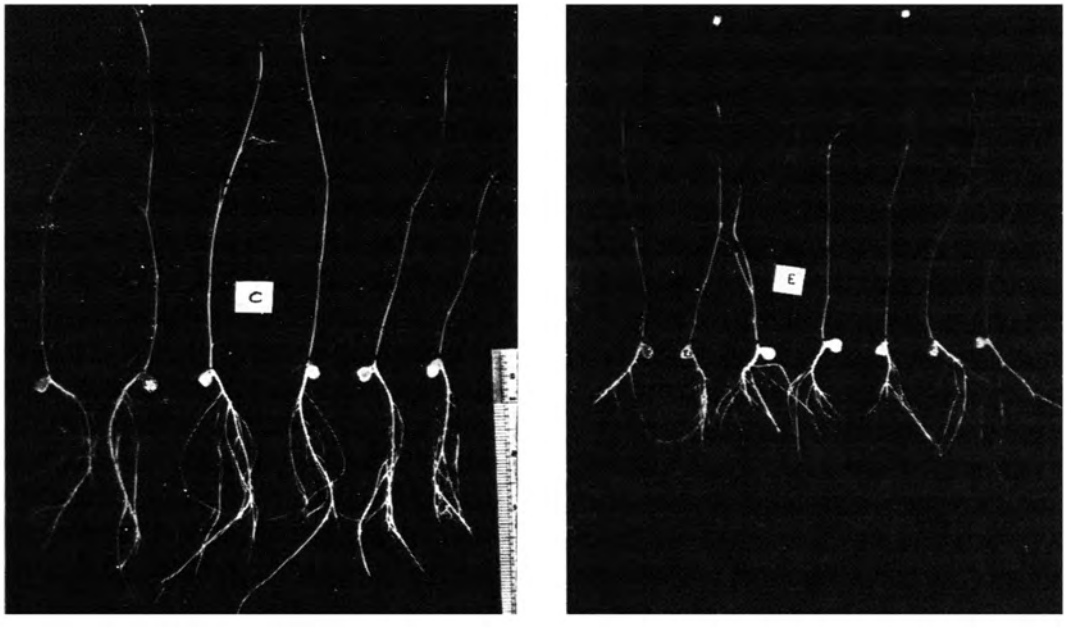

Figure 1 Invertase activity in control and experimental seedlings of germinating *C. arietinum* seeds

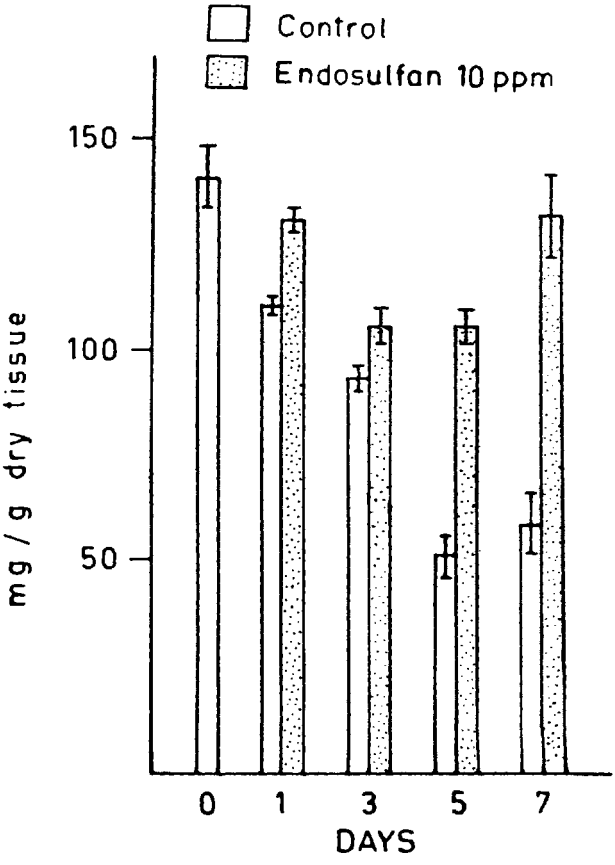

Figure 2 Total sugar content in control and experimental seedlings of germinating *C. arietinum* seeds

influence the biological response of xenobiotics is their uptake by the test system. Endosulfan appeared to accumulate in the seedling although no strict proportionality with time or concentration was observed. According to Finlayson and Mac Carthy (1965) the chemical nature and polarity of a compound determine its absorption through waxy non-living layers of plant surface. Thus lack of proportionality between the exposure level and the subsequent uptake by the growing seedlings may be due to the lipohilic nature of endosulfan and its limited solubility in water (Reynolds & Metcalf (1962.)

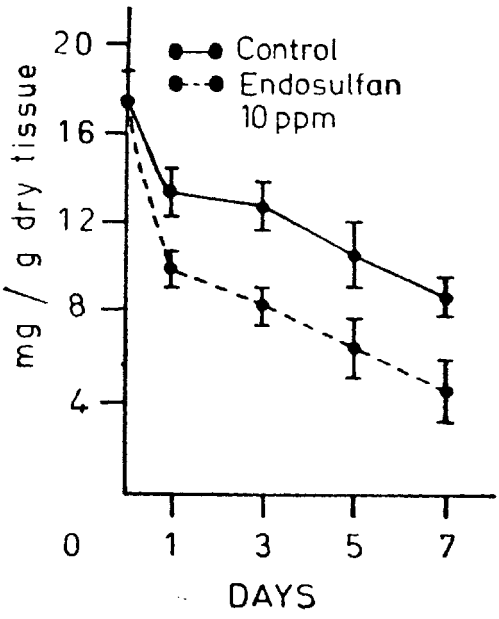

Figure 3 Pectin content in control and experimental seedlings of germinating *C. arietinum* seeds

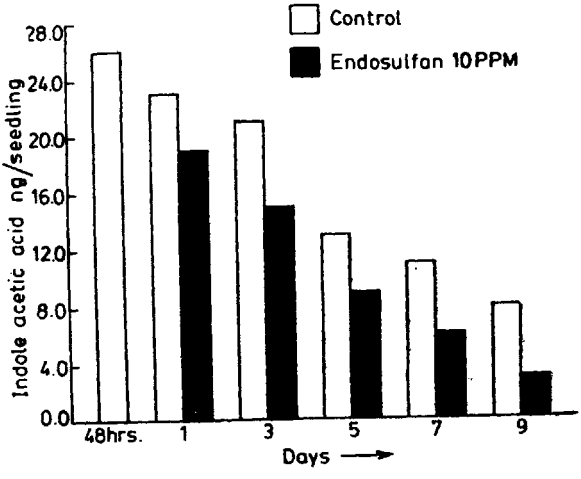

Figure 4 Indole-acetic acid levels in control and endosulfan exposed seedlings

Increase in α/β ratio as observed on 10th day of endosulfan exposure is perhaps due to preferential uptake of the α-isomer, as no other metabolite was detected during study. It can be assumed that once a certain amount of the compound gains entry (figure 5) into the plant membrane premeability is altered resulting in an enhanced uptake of the compound.

Table 2 shows that when five and seven days old control and 10ppm endosulfan treated seedlings were exposed to radioactive iron (^{59}Fe), a significant increase in ^{59}Fe uptake was found in endosulfan treated seedlings. Earlier Agarwal and Beg (1982) deduced evidence for the changes in membrane permeability by in vitro experiments. Here changes in the membrane properties are also reflected by ^{59}Fe uptake studies. The present approach thus helps to understand the possible biochemical loci of phytotoxic effects of environmental xenobiotics.

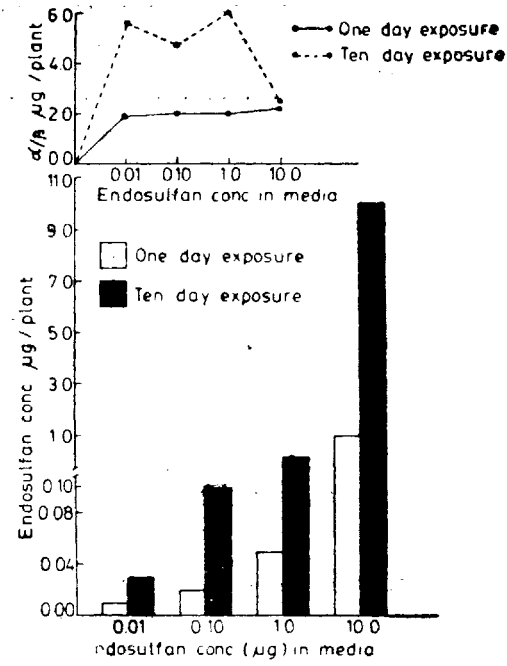

Figure 5 Residue levels in plants exposed to various concentrations of endosulfan

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