Threshold Dose of Cytogenetic Toxicity of Lindane, Malathion and Metacid in *Allium cepa* Root-tip Cells

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In order to have plenty of cheap food for the ever increasing population, large number of agrochemicals are these days being used. Pesticides are one such chemical which protect the standing as well as stored crops and cereals. Plant-based residues of most of these agrochemicals have been reported to be genotoxic (Stevens 1971, Amer and Farah 1974, Amer and Farah 1976, Mishra and Sinha 1979, Sinha *et al.* 1979, Fishbein 1984, Sinha *et al.* 1984, Sinha 1988).

Under the circumstance, it is being advocated that the use of chemical pesticides be stopped, and some alternative safer method be adopted. As the commercial exploitation of the latter technology is still in its developing phase, the use of chemical pesticides is bound to continue at least for a few more decades. One short-term solution to the problem is to use the pesticides at such dose on which they retain their pesticidal property but have little or no cytotoxic/genotoxic effect. This dose is called as threshold dose, and it can be inferred with confidence from dose-response curves well populated with accurate experimental points at low doses (Kumar and Sinha 1989). The aim of the present work is to determine such threshold dose of a few commonly used pesticides in a well-known test-system.

Material and methods

Experiments were performed using Malathion (O-O, dimethyl phosphorodithioate of diethyl mercaptosuccinate), Metacid (O-O, dimethyl-O, -p nitrophenyl phosphorothioate) and Lindane (γ -1, 2, 3, 4, 5, 6-hexachlorocyclohexane). The test-system selected for the purpose is mitotically dividing root-tip cells of onion (*Allium cepa*).

Onion bulbs (25-30 g) with 48 hrs old roots (grown over tap water) were allowed to grow for another 48 hrs on ten different concentrations of pesticides ranging from 0.1 to 500.0 ppm (because these pesticides are used commercially in the dose-range of 100-2000 ppm depending upon the pest). The roots were then thoroughly washed, fixed in aceto-alcohol and stained with acetocarmine squash method. The mitotic index and the rate of chromosomal abnormalities among the meristematic cells were considered as parameters for evaluating the relative cytotoxicity of the pesticides at various doses. The mitotic-index was expressed in terms of relative division rate (RDR) (Egami and Hyodo-Taguchi 1973):

$$\text{RDR} = \frac{\frac{\% \text{ of dividing cells in treated variant}}{100 - \% \text{ of dividing cells in control variant}} \times \frac{\% \text{ of dividing cells in control variant}}{\% \text{ of dividing cells in control variant}}}{100 - \% \text{ of dividing cells in control variant}} \times 100.$$

A similar ratio, the relative abnormality rate (RAR) was also calculated in the same manner.

Desired randomness in counting of the dividing cells was achieved by the method suggested by Bhalla *et al.* (1973). The control variant of bulbs were all along grown over normal tap water with one change after 24 hrs.

Fig. 1. Relative division rate (RDR) in root-tip cells of onion (*Allium cepa*) after treatment with different concentrations of malathion, metacid and lindane.

Fig. 2. Relative proportion (%) of mitophases (RPM) in onion root-tip cells after treatment with three pesticides.

Results and discussion

The relative division rate (RDR) and relative abnormality rate (RAR) were plotted as dose-response curves. All the three pesticides could retard the mitotic-index which was significantly so at doses above 25 ppm of Malathion, 20 ppm of Metacid and 50 ppm of Lindane (Fig. 1). Hence these doses may be deemed as threshold dose for the mitoinhibitory effect of the respective pesticides. Phase-wise distribution of dividing cells could indicate (Fig. 2) that the decreased RDR is chiefly due to decrease in prophase population, which may be due to pesticide induced arrest of cells in G_0 phase or a retardation in the pace of events during 'S' and/or G_2 phases. Metaphase and ana-telophase populations were less affected (Fig. 2). (Cummins 1969, Petersen *et al.* 1969).

Fig. 3. Relative abnormality rate (RAR) in dividing cells.

The pesticides could also induce abnormalities of both gross (fragmentation, clumping, polyploidy, multipolar spindle, multinucleate cells, disturbed cytokinesis etc.) and individual (anaphase bridge, laggards and chromosome break) nature (Table 1, Fig. 4). The relative abnormality rate (RAR), for both types taken together, appears to increase very sharply after about 25 ppm concentration of Malathion and Lindane, and about 50 ppm in case of Metacid (Fig. 3). These values are thus in close approximation of the threshold dose observed for mitotic inhibition. Decrease in the abnormality rate observed at 100 ppm and concentrations higher than it, is perhaps due to ceasure of mitosis caused by them. The gross type abnormalities were relatively more frequent than the individual ones at almost all the concentrations of the three pesticides. The reason may be that the gross type abnormalities are a conglomerate

Table 1. Frequency (% $\pm$ S.E.) of abnormal dividing cells and their types in pesticide-treated root-tips. (Ab-Anaphase bridge; Ig-Laggard; Cb-Chromosome break; f-Fragmentation; Ply-Polyploidy; Mps-Multipolar spindle; Bnu-Binucleate cell; Tnu-Trinucleate cell; Cl-Clumping; Dcy-Disturbed Cytokinesis; Pul-Pulverisation)

Dose in ppm	Malathion				Metacid				Lindane			
	Abnormal cells		No. of cells observed	Type	Abnormal cells		No. of cells observed	Type	Abnormal cells		No. of cells observed	Type
	% $\pm$ S.E.	(G/I)			% $\pm$ S.E.	(G/I)			% $\pm$ S.E.	(G/I)		
Control	0.19 $\pm$ 0.19 (1/519)*	lg-1 (0/1)	4329	lg-1	0.15 $\pm$ 0.15 (1/654)	lg-1 (0/1)	4861	lg-1	— (0/477)	— (0/1)	3949	Nil
0.1	— (0/440)	Nil (—)	4084	Nil	— (0/413)	Nil (—)	3426	Nil	— (0/560)	— (—)	4452	Nil
0.5	— (0/502)	Nil (—)	4236	Nil	— (0/608)	Nil (—)	4860	Nil	— (0/485)	— (—)	3840	Nil
1.0	0.17 $\pm$ 0.17 (1/577)	Tnu-1 (1/0)	4598	Tnu-1	0.21 $\pm$ 0.21 (1/475)	lg-1 (0/1)	3771	lg-1	— (0/543)	— (—)	4671	Nil
5.0	0.21 $\pm$ 0.21 (1/479)	lg-2 (0/2)	4174	lg-2	0.40 $\pm$ 0.28 (2/500)	Ab-1 (0/2)	4189	Ab-1	— (0/589)	— (—)	5060	Nil
10.0	0.31 $\pm$ 0.22 (2/652)	Bnu-1 f-1	5649	Bnu-1 f-1	1.01 $\pm$ 0.38 (7/695)	Cb-4 Tnu-1 Dey-2 (3/4)	4780	Cb-4 Tnu-1 Dey-2 (3/4)	0.74 $\pm$ 0.37 (4/544)	— (0/4)	4536	Ab-1 lg-3
25.0	0.19 $\pm$ 0.19 (1/529)	lg-1 (0/1)	4970	lg-1	0.70 $\pm$ 0.40 (3/431)	Cb-2 Cl-1 (1/2)	4652	Cb-2 Cl-1 (1/2)	0.40 $\pm$ 0.27 (2/528)	— (1/1)	4004	Cb-1 f-1
50.0	1.50 $\pm$ 0.61 (6/399)	lg-2 f-2 Ply-1 (4/2) Dey-1	5959	lg-2 f-2 Ply-1 (4/2) Dey-1	0.61 $\pm$ 0.30 (4/653)	Cb-1 Ab-1 lg-2 (0/4)	5751	Cb-1 Ab-1 lg-2 (0/4)	2.60 $\pm$ 0.71 (13/499)	— (—)	4765	Cb-4 f-2 Bnu-1 Tnu-3 Dey-3 (9/4)
100.0	3.47 $\pm$ 1.08 (10/288)	Ab-2 f-5 Cl-1 Bnu-1 (8/2) Dey-1	5240	Ab-2 f-5 Cl-1 Bnu-1 (8/2) Dey-1	44.11 $\pm$ 8.50 (15/34)	f-14 pul-1 (15/0)	2896	f-14 pul-1 (15/0)	4.90 $\pm$ 1.04 (21/429)	— (—)	4352	Ply-2 Mps-3 Bnu-6 Tnu-4 (21/0) Dey-6
250.0	1.01 $\pm$ 0.50 (4/395)	Bnu-4 (4/0)	4896	Bnu-4 (4/0)	3.85 $\pm$ 2.18 (3/78)	Bnu-3 (3/0)	1711	Bnu-3 (3/0)	2.93 $\pm$ 1.02 (8/273)	— (—)	4128	lg-2 f-3 ply-1 (6/2) Dey-2
500.0	completely ceased	ceased	Division	completely ceased	1.16 $\pm$ 0.66 (3/259)	f-2 Ply-1 (3/0)	5358	f-2 Ply-1 (3/0)	2.76 $\pm$ 1.11 (6/217)	— (—)	3848	Cb-1 f-1 Tnu-2 Dey-2 (5/1)

* No. of abnormal cells/Total No. of dividing cells.
G/I=No. of gross/No. of individual type abnormalities.

Fig. 4. Chromosomal abnormalities in treated root-tip cells of onion. A, laggard (lg). B, anaphase bridge (Ab). C, multipolar spindle (mps). D, a polyploid cell in anaphase. E, fragmentation (f). F, arrested cytokinesis with metaphase configuration of chromosomes in daughter cells. G, H, unequal karyokinesis leading to bi-(G) and trinucleate (H) cells.

of wide spectrum changes induced by variety of disturbances in the internal milieu of the cell, whereas, the individual types are caused by very specific mechanism. No specificity in the category of abnormality and treatment dose was observed (as exhibited by G/I ratio in Table 1). Use of these pesticides at their cytogenotoxic sub-threshold doses is advised.

Summary

Cytotoxic threshold dose of three commonly used pesticides (Malathion, Metacid and Lindane) was determined using relative division and abnormality rates in meristematic cells of *Allium cepa* root tip by using dose-response curves populated with varied number of smaller doses. The results obtained show such dose to be 25, 20 and 50 ppm for Malathion, Metacid and Lindane respectively.

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