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Genotoxicity of pesticides to onion root tip Meristematic cells

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Abstract
Plants are direct recipients of agro-toxics and therefore important materials for assessing environmental chemicals for genotoxicity. Three doses, representing ¼, ½ and EC50 of all the eight pesticides were assessed for cytotoxic and genotoxic effects. The genotoxic effects of onion root tip cells to some common pesticides and the chromosome aberration assay were carried out after 24 h exposure. Cytotoxicity was inferred when the Mitotic index (dividing cells/1000 scored) of treated cells was ½ negative control. All the pesticides were found to be toxic. Genotoxicity was measured by analyzing 30 to 100 anaphase-telophase cells per dose of chemical for chromosome fragments, bridges, vagrant chromosome, c-anaphase, multipolarity and stick chromosomes and comparing the percentage of aberrant cells at each dose with that of the negative control. With the exception of Dieldrin, all the other pesticides were found to be genotoxic. The C-anaphase and Stick chromosomes and all the types of aberrations were predominated which was evidence of the action of the pesticides on the mitotic spindle and the coiling of chromosomes during anaphase to telophase.

Keywords: genotixicity, perticides, cytotoxicity, chromosomal aberrations

1. Introduction
The use of pesticides in modern agriculture has greatly improved yield through inhibition of disease causing organisms and by acting against pest in the fields and during storage of agricultural products [8, 14]. The mutagenic and carcinogenic action of herbicides, insecticides and fungicides on experimental animals is well known and several studies have shown that chronic exposure to low levels of pesticides can cause mutations and/ or carcinogenicity [3, 15]. Pesticide residues can be present in fruit and vegetables and represent a risk for human health. Several studies have shown that chronic exposure to low levels of pesticides can cause birth defects and that prenatal exposure is associated with carcinogenicity. Pesticides residues known to persist in soil, water and food and have posed problems all over the world [13].
Over the past decade, issues of animal use and care in toxicology research and testing have become one of the fundamental concerns for both Science and Ethics. Emphasis has been given to the use of alternatives to mammals in testing, research and education [10]. Plant genotoxicity assays are relatively inexpensive, fast, give reliable results and chemicals which cause chromosomal aberration (CA) in plant cells also produce CA in cultured animal cells that are frequently identical [6, 7]. The Allium cepa assay is an efficient test for chemical screening and in situ monitoring for genotoxicity of environmental contaminants and has been widely used to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberrations in root meristems of A. cepa [5, 7]. In the present study, eight pesticides were assessed for inhibition of cell division (toxicity) and genotoxicity in the A. cepa root tip meristematic cells.

2. Materials and Methods
Onion seeds (**Allium cepa** var. Co. 1 variety) purchased from Tamilnadu Agricultural University, Coimbatore were used in the present study. The pesticides, were used in the present study. Dimethoate, Oxydemeton, Quinalphos, Oxadiargyl, Toxiphan, Malathion, Heptachlor and Dieldrin used were purchased from the Tamil Nadu Agricultural University, Coimbatore.

2.1 Preliminary seed germination experiment to select doses of pesticide
Preliminary dose selection experiment was conducted for each chemical with concentration ranges between ten times above and below the manufacturers recommended dose (% solution in water). However, in cases where no inhibition of germination was observed, higher doses were tested. For each test, 100 onion seeds were spread on a filter paper moistened with a specific concentration of the pesticide in a petri dish and kept for 3 days at room temperature to germinate. The number of seeds that produced a radicle were recorded at the end of the three days and compared to the number of seeds that germinated in the concurrent water treated negative control to derive the percentage germination at each concentration. The EC50 for each pesticide was determined from the curve of percentage germination against dose.

2.2 Genotoxicity assay
A. cepa (onion) seeds were germinated in petri dishes containing pesticide-soaked filter paper (test) and water soaked filter paper (negative control). In this study, a discontinuous treatment protocol was used. Seeds were spread on water moistened filter paper in a petri dish until

they germinated and the radicles reached a length of about 5 cm. Germinated seeds were transferred onto filter paper kept moistened in a petri dish with specific concentration of pesticide for 24 h (acute treatment) at room temperature. At the end of the 24 h exposure, two root tips from two seeds per dose were collected at random and assessed. Three concentrations of each pesticide representing the ¼ EC50, ½ EC50 and EC50, as determined in the preliminary dose selection experiments were tested, together with a concurrent negative control which was water.

2.3 Root harvest and slide preparation

Root tips, 1 - 2 cm long were cut from the germinated seeds and placed in a small glass specimen bottle and fixed in acetic alcohol (ethanol : glacial acetic acid in 3:1 ratio) for 24 h in a fridge at 4 - 6°C. The root tips were washed twice with ice cold water for 10 min each and allowed to dry in a watch glass. A solution of 1 N HCl preheated to 60°C was added to the root tips in the watch glass for 10 min and the HCl was discarded. The HCl treatment was repeated a second time. Two root tips were transferred singly to a clean microscope slides and cut 2 mm from the growing tip. The tips were kept and the rest was discarded. Acetocarmine stain was added to each slide to cover the root tip for about 10 min. A glass cover slip was placed on the root tip and tapped gently with a pencil eraser to spread the cells evenly to form a monolayer to facilitate the scoring process for normal and aberrant cells in the different stages of the cell cycle.

2.4 Scoring of slides and Data analysis

The slides were viewed under the light microscope (Olympus) using the 100X objective lens with oil immersion.

2.5 Mitotic index

On one slide for each treatment, a total of 2000 cells, classified into interphase or dividing cell (Prophase, Metaphase, Anaphase and telophase) were scored. The mitotic index (MI) was expressed as the number of dividing cells per 1000 cells scored.

2.6 Cytotoxicity

The mitotic indices of the treated cells at each dose of each pesticide were compared with that of the negative control group. A dose of pesticide was treated cytotoxic if the mitotic index of treated cells was ≤ ½ of the mitotic index of the concurrent water treated cells.

2.7 Genotoxicity test

A total of 30 to 100 anaphase and telophase cells were examined for chromosome aberration per dose of each pesticide from one slide. The following categories of aberrations were observed and scored: Chromosome fragments, bridge, vagrant chromosomes, C-anaphase, multipolar anaphases and telophases and stick chromosomes. The percentage of anaphase telophase cells with aberrations at each dose of each pesticide was compared with that of the negative control.

3. Results and Discussion

Cytotoxicity of the pesticides

The results of the cytotoxicity assay are presented in Table 1. All the eight pesticides were toxic at one or more of the three concentrations tested.

Table 1: Determination of the mitotic index among 2000 cells scored following 24 h exposure of onion root tip cells to three different concentrations each of eight pesticides. The Data are the mean value of three different experiments.

Test Compound	Con. of Soln. (%)	Cells In Division Stages Per 2000 Cells Scored						MI	MI As % of Control
		Inter	Pro	Meta	Ana	Telo	Total		
Water	0	1686	69	31	21	31	152	76	100
Dimethoate	0.0115	1892	62	13	11	22	108	54	71
	0.023	1948	37	3	4	8	52	26	34
	0.046	1970	16	2	4	8	30	15	20
	0.025	1904	49	16	4	27	96	48	63
Oxydemeton	0.05	1914	52	23	4	7	86	43	57
	0.1	1976	7	3	4	10	24	12	16
	1.67	1916	59	3	12	10	84	42	55
Quinalphos	3.38	1928	55	4	6	7	72	36	47
	6.67	1966	25	2	2	5	34	17	22
	1.02	1896	58	12	12	22	104	52	68
Oxadiargyl	2.12	1944	30	3	9	14	56	28	3
	4.24	1976	12	6	2	4	24	12	16
	15	1962	18	12	3	5	38	19	25
Toxiphan	30	1968	16	4	4	8	32	16	21
	60	1948	19	10	10	13	52	26	34
	0.046	1964	16	4	8	8	36	18	24
Malathion	0.092	1982	10	2	2	4	18	9	12
	0.18	1992	5	0	1	2	8	4	0.05
	5.91	1940	28	8	12	12	60	30	40
Heptachlor	11.03	1958	23	3	7	9	42	21	28
	22.06	1970	19	4	4	3	30	15	20
	0.035	1924	45	22	3	6	76	38	50
Dieldrin	0.07	1970	13	9	2	6	30	15	20
	0.14	1994	4	0		2	6	3	0.04

MI = Mitotic index (number of cells in division stages out of 1000 cells); Pro = Prophase; Meta = Metaphase; Ana. = Anaphase; Telo.= Telophase.

Genotoxicity of the pesticides

The result of the determination of the genotoxic effects of the pesticides are presented in Table 2.

Table 2: Genotoxic effects of pesticides to onion root tip cells after 24 h exposure. The Data are the mean value of three different experiments.

Test Compound	Con. of Solution (%)	MI	A – T Scored	Aberrations Observed In Anaphase-Telophase Cells Scored					
				Fragment %	Bridge %	Vag Rant %	C-Ana Phase %	Multi Polarity %	Stick Chromosomes %
Water	100	76	83	0	1.205	0	0	0	0
	0.0115	54	55	0	9	0	16.36	0	9.09
Dimethoate	0.023	26	45	0	0	0	0	0	0
	0.046	15	32	0	0	0	0	0	50
	0.025	48	66	0	0	0	0	0	1.52
Oxydemeton	0.05	43	94	1.06	1.06	0	0	0	2.13
	0.1	12	35	0	0	0	0	0	0
	1.67	42	55	0	7.27	0	16.36	0	0
Quinalphos	3.38	36	32	0	0	3.13	34.38	0	0
	6.67	17	36	0	0	0	22.22	0	0
	1.02	52	54	0	0	0	22.22	0	0
Oxadiargyl	2.12	28	45	0	0	0	0	0	0
	4.24	12	42	0	0	0	16.67	0	33.33
	15	19	60	0	0	0	25	10	35
Toxiphan	30	16	42	0	0	0	16.67	0	16.67
	60	26	66	0	0	0	54.55	12.12	9.09
	0.046	18	20	0	0	0	50	0	0
Malathion	0.092	9	20	0	0	0	75	0	0
	0.18	4	10	0	0	0	0	0	0
	5.91	30	69	0	0	0	30.43	8.7	4.35
Heptachlor	11.03	21	35	0	0	2	54.29	0	40
	22.06	15	32	0	0	0	25	0	0
	0.035	38	25	0	0	0	0	0	0
Dieldrin	0.07	15	18	0	0	0	0	0	0
	0.14	3	15	0	0	0	0	0	0

MI = Mitotic index (number of cells in division stages out of 1000 cells); A - T (anaphase and telophase cells).

Dimethoate, Oxydemeton, Quinalphos, Oxadiargyl, Toxiphan, Malathion and Heptachlor were genotoxic at one or more doses of pesticide tested. Dieldrin, however was not genotoxic as the cells observed were in late telophase. It has to be noted that Malathion and Dieldrin were very toxic such that it was impossible to score 30 anaphase-telophase cells on a slide. For the pesticides that induced genotoxic effects, the C-Anaphase and Stick chromosomes classes made up 75% and above with the exception of one dose each of Oxydemeton and Quinalphos where the C- Anaphase and Stick chromosomes classes made up 50%. The most common types of aberrations observed were therefore, C-anaphase and stick chromosomes. Dimethoate, Oxydemeton and Quinalphos induced bridges twice the control value at one dose each. The formation of chromosomal bridges was not accompanied by the occurrence of chromosomal fragment. Only Toxiphan and Heptachlor induced multipolar anaphases and telophases.

The mitotic indices of onion root tips treated with all eight pesticides were reduced to half or less than half compared with the negative control. A depression of the mitotic index has been recorded by many investigators as a result of treatment with pesticides [1, 11, 2]. In addition, seven of the pesticides namely, Dimethoate, Oxydemeton, Quinalphos, Oxadiargyl, Toxiphan, Malathion, Heptachlor also exhibited genotoxic effects to onion root tip cells exposed for 24 h.

The commonest types of genotoxic effects observed were C-anaphase and Stick chromosomes which together accounted for 50% and above. The presence of c-metaphase cells was evidence of the action of the pesticides concerned on the mitotic spindle (Matsumoto, *et al*., 2006). The stick chromosomes have resulted in the abnormal uncoiling of chromosomes during anaphase to telophase [12]. The seven pesticides are thus more likely to be aneugenic than clastogenic. Bexadust with the active ingredient as gamma benzene hexachloride (BHC) was genotoxic to A. cepa root tip cells [4].

4. Conclusion

All the pesticides used in the present study were toxic to onion root tip cells with the exception of Dieldrin. The pesticides were also genotoxic, inducing mostly C-anaphase and Stick chromosomes. Types of aberration which was evidence of the action of the pesticides on the mitotic spindle and the coiling of chromosomes during anaphase to telophase. The study has further demonstrated that the usefulness of the A. cepa chromosome aberration assay in assessing the genotoxicity of environmental chemicals and pollutatns.

5. References

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