APPENDIX

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EVALUATION OF CLASTOGENICITY OF THREE PESTICIDES IN THE GRASSHOPPER TEST SYSTEM

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Abstract Clastogenic effects of three pesticides , borax, copper sulphate and weed-azol — TL have been tested for the first time on meiotic cells of a grasshopper, *Oxya velox* Different concentrations, and different exposure times have been employed Each chemical induced varieties of chromosomal anomalies In general, effects were dose and time dependent Of three pesticides, comparatively borax produced least effect and weedazol — TL the highest The results revealed the mutagenic property especially of copper sulphate and weedazol — TL in the present test system Further studies involving other test systems and restricted use are suggested

Key Words . Clastogenicity, Pesticides, Grasshopper

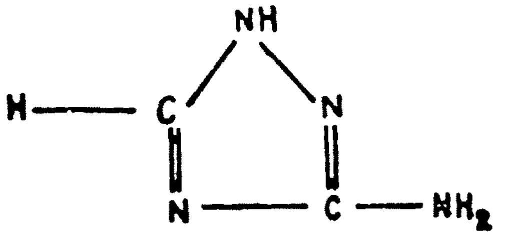
INTRODUCTION

Pesticides form one of the major areas of human exposures to environmental contaminants and genotoxicity of a number of these chemicals has been studied in plants and animals (Sieberf, *et al* 1970 , Epstein and Legator, 1971 Ahmed and Grant, 1972 , Wild, 1975 Manna and Bardhan, 1977 , Shirasu *et al*, 1980 , Amer and Fahmy, 1982 , Behera and Bhunya, 1980 , and Bhunya and Behera, 1984) Borax commonly used in the manufacture of glass, enamels, medicinal soaps, as food preservative as well as a weedicide has been found to increase puffing frequency in salivary gland chromosome of *Drosophila melanogaster* (Drozdovskaya, 1974) Copper sulphate, used as weedicide and fungicide, is less known for its mutagenic activity Weedazol — TL is used as herbicide and cotton defoliant and has been reported to be non-mutagenic in bacterial test systems (Shirasu *et al*, 1976 , Bampford *et al*, 1976) There is no record in the literature on the mutagenic effect of these three pesticides in insect test system We report here the clastogenic effects of borax, copper sulphate and weedazol — TL (Amitrol) on the meiotic cells of a grasshopper, *Oxya velox*

MATERIALS AND METHODS

Test animal Locally collected adult male individuals of a grasshopper, *Oxya velox* (Acrididae, Orthoptera)

Test chemicals Analytical grade of borax (sodium salt of boron, $Na_2B_4O_7, 10H_2O$) and copper sulphate ($CuSO_4$) donated by BDH and commercial grade of weedazol — TL (Amitrol as active ingredient) donated by Amchem, USA, were used The structural formula of amitrol is as follows



Structure of amitrole

Solutions of different concentrations of borax, copper sulphate and weedazol — TL were prepared in glass double distilled water Equal volume of g d d water served as controls

Different doses of each chemical were injected intra-abdominally at the rate of 0,05 ml/individual and animals were sacrificed at different time intervals (Table-1)

Tissue collection, processing, slide preparation and staining

The testes of the treated specimens were dissected out and their fatty coverings were removed under normal saline (0 67%) Fixation was done in acetic acid-alcohol mixture (1 3) After a minimum time lapse of three hours the material was squashed on dry albuminized slides following the technique of Smith (1943)

In total, 992 cells of different meiotic stages were examined in the control series and overall frequency of aberration has been calculated to be 0 30%. This pooled control value has been considered for all treated values

TABLE 1

Different doses and hours of exposure employed to study the cytogenetical effects of pesticides on *Oxya velox*

Sl No	Name of pesticide	Dose used % Soln	Hour of exposure
1	Borax	0.25	6, 12, 24
		0 50	6, 12, 24
		1 00	6, 12, 24
2	Copper Sulphate	0 125	6, 12, 24
		0 25	6, 12, 24
		0 50	6, 12
3	Weedazol — TL	0 25	6 12, 24
		0 50	6, 12, 24
		1 00	6, 12, 24

Statistical analysis All the data scored were statistically analysed to see whether the results in the treated series deviated significantly from the control one For statistical analyses X^2 test was employed

RESLUTS

All three pesticides produced two types of clastogenic effect - (1) General physiological effect like stickiness, clumpiness, chromatin and centromeric stretching uneven accumulation of the chromatin material, pseudobridge formation, laggards etc , and (2) Structural changes like chromatid gaps and breaks, chromosome gaps and breaks, centromeric fission, extreme case of chromosomal fragmentations and isochromatid gaps (Figs 1 - 10)

Quantitatively 0 25%, 0 50% and 1,00% solutions of borax induced 1 09%, 0 89% and 1 73% of aberration respectively and irrespective of doses the chemical induced 1 13%, 1 34 and 1 23% aberrations (Tables 2-3) Copper sulphate solutions, 0 125%, 0 25% and 0 50% induced 1 07%, 1 56% and 1 61% aberrations respetively and irrespective of doses the chemical induced 1 56%, 1 37% and 1 37% aberrations after 6, 12 and 24 hours exposures respectively (Tables 2-3) Weedazol — TL induced 2 70%, 5 68% and 10 09% aberrations treated with

0,25%, 0 50% and 1 00% solutions respectively and irrespective of doses the chemical induced 3 28%, 6 49% and 7 24% aberrations after 6, 12 and 24 hours exposure (Tables 2-3) Of all stages, anaphse — 1 cells were most susceptible to all the chemicals

The results were in general dose and time dependent (Figs 11-13)

COMMENTS

As mentioned elsewhere no study on the effect of borax, copper sulphate and weedazol — TL on the spermatocytic chromosomes of grasshopper has been carried out previously by any other worker A numbei of metallic compounds have been reported to be cytotoxic and genotoxic (Levan, 1945 , Sharma and Sharma, 1960 , Manna and Parida, 1965 , Parizek, 1960 , Sampson et al, 1965 , Suter, 1975) Borax, as a micronutrient of plants is related to the protein synthesis and respiration (Audus, 1976) Essentiality of boron for the continuation of DNA synthesis and mitotic activity in *Cucurbita pepo* has been reported by Cohen and Albert (1974) Drozdovskaya (1974) reported sodium tetraborate induced puffing in the salivary gland chromosome of *Drosophila* Borax solution injection for several days to the Indian desert Gerbils (*Meriocus hurrians*) induced different types of degenerative changes in the testis tissue In the present test system borax has been found to induce chromosomal damages

The chemical $CuSO_4$ has been found to be more effective than borax Metallic salt like aluminium chloride has been tested on the meiotic chromosomes of a grasshopper, *Phloeoba antennata* by Manna and Parida (1965) and varieties of clastogenic effects have been reported to be produced In the present case also $CuSO_4$ has induced different type of chromosomal anomalies in the meiotic chromosomes of *Oxya velox* For physiological effects like stickiness and chromatin stretching Manna & Parida (1965) suggested that aluminium chloride induced changes in the nucleoprotein gels or caused denaturation of proteins Similar type of action of $CuSO_4$ can be envisaged in the present case • Very likelv the ions of the copper might have modified the properties of the nucleoprotein gel of the chromosome through the alteration of the cellular ionic environment Although erosion or corrosion of the chromatin material induced by aluminium chloride was reported by Manna and Parida (1965) in the present case, both borax and $CuSO_4$ did not prpduce such type of effect So it can be assumed that probably physiological effect varies from chemical to chemical depending on the pH of the solution From tne results it is apparent that $CuSO_4$ has genotoxic property in the present test system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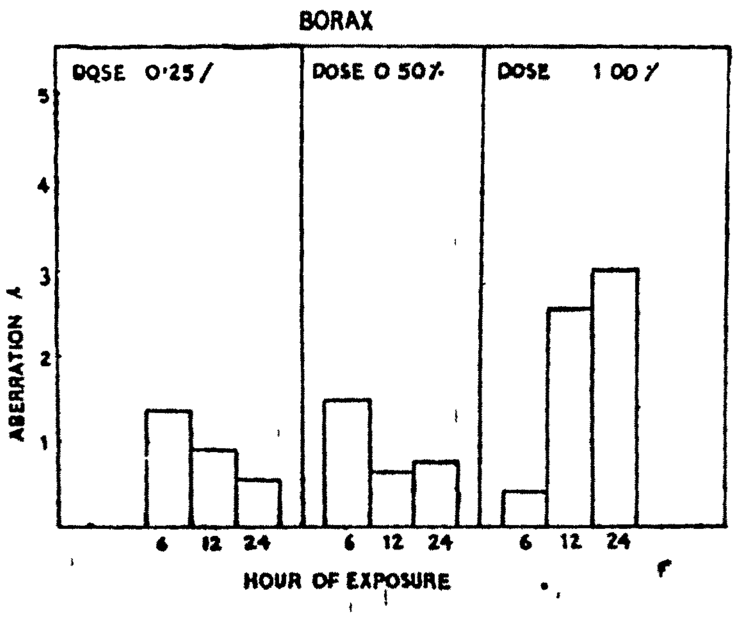

Fig 11 Histograms showing borax induced chromosomal aberration in *Oxya velox*.

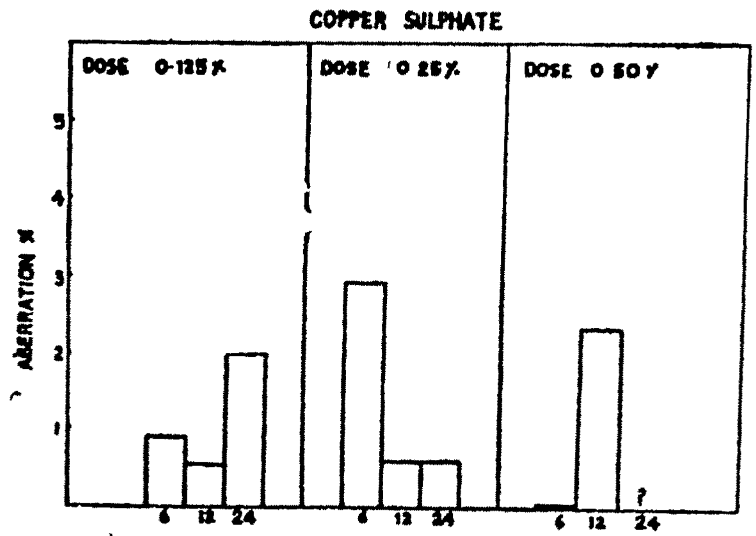

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Fig 12. Histograms showing $CuSO_4$ induced chromosomal aberrations

TABLE—2

Frequency distribution of chromosomal aberrations induced by different doses of borax, copper sulphate and weedazol—TL on Oxya velox

Chemical	dose	No of cells studied	No of Aberration	% of Aberration	X^2 Value
Borax	0 25	971	10	1 09	
	0 50	897	8	0 86	
	1 00	1046	18	1 73	
	Total	2914	36	1 23	83 91
Copper Sulphate	0 125	928	11	1 07	
	0 25	1022	16	1,56	
	0 50	680	11	1 61	
	Total	2630	38	1 44	113 58
Weedazol—TL	0 25	1182	32	2 70	
	0 50	756	43	5 68	
	1 00	852	86	10 09	
	Total	2790	161	5 77	2761 28

All X^2 values are significant at 0 01 level ($P<0\ 01$)

TABLE—3

Summary and statistical evalution of the chromosome aberration data in Oxya velox

Chemilal	Hour of exposure	Dose %	No of cells examined	Total no of aberration	%of aberrarion	X^2 Value
Borax	6	0 25	291	5	1 71	
		0 50	333	5	1 50	
		1,00	433	2	0 46	
	Total		1057	12	1 13	24 33
	12	0 25	317	3	0 94	
		0 50	301	2	0 66	
		1 00	348	8	2 29	
	Total		966	13	1 34	34.79

	24	0 25	363	2	0 55	
		0 50	263	1	0 38	
		1 00	265	8	3 01	
		Total	891	11	1 23	25 67
Copper Sulphate	6	0 125	221	2	0 90	
		0 25	401	12	2 99	
		0 50	266	–	—	
		Total	888	14	1 56	47 81
	12	0.125	364	2	0 54	
		0 25	309	2	0 64	
		0 50	414	11	2 65	
		Total	1087	15	1 37	41 87
	24	0 125	343	7	2 04	
		0 25	312	2	0 64	
		0.50	–	–	—	
		Total	655	9	1 37	24 88
Weedazol—TL	6	0 25	341	5	1 46	
		0 50	268	7	2;61	
		1 00	273	17	6 22	
		Total	882	29	3 28	260 82
	12	0 25	386	6	1 55	
		0 50	265	17	6 41	
		1 00	180	31	17 22	
		Total	831	54	6 49	1056.26
	24	0.25	455	21	4 61	
		0 50	223	19	8 52	
		1 00	399	38	9 52	
		Total	1075	78	7 24	1719 25

All X^2 Values are significant at 0 01 level ($P < 0\ 01$), Among three pesticides, weedazol — TL has been found to be most effective since it produced the highest frequency of aberratisns (Table 2) Behera and Bhuyan (1979, 1981) have reported weedazol — TL to be genoloxic in the bone marrow chromosomes of *Mus musculus*

All the three pesticides have produced highef percentage of gaps than that of breaks A number of chemicals has been reported to produce more gaps than breaks in grassoopper meiotic chromosomes (Manna and Bhuyan, 1972 , Manna and Parida, 1965 , Manna & Mukherjee, 1966 , Manna, 1973) and in mouse bone marrow chromosomes (Behera and Bhunya 1980 , Bhunya and Behera, 1984 , Manna and Das, 1973 , Manna and Bardhhan, 1977).

Of the three pesticides borax and copper sulphate produced only chromatid type aberration and weedazol – TL produced both chromatid and chromosome type aberration Such type of results indicate that borax and copper sulphate acted following G_1 phase whereas, weedazol—TL acted both at G_1 and the following G_1 stage of the cell cycle

The results were mostly dose dependent The decrease of aberration with lower dose is indicative of the probablity due to non-availability of the critical concentration of the genetically reactive metabolites at the target molecules (DNA and protein)

The gentoxic nature of borax (week), copper sulphate and weedazol-TL are evident from the present results Cautious handing and restricted use especially of $CuSO_4$ and weedazol-TL are suggested

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