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Protection against the effects of common insecticides on *Allium cepa* L.

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Abstract
Independently the five insecticides as used in this study produced a variety of chromosomal abnormalities. The independent chromosomal effects of the five insecticides Malathion, Dimecron, Dimethoate, Metasystox & Endosulfan as described have exhibited following specific chromosomal anomalies. Clumping, Scattering, Bridges, Fragments, Bridges with laggards, Laggards, Stathmoanaphase, Mis-orientation, Erosion. Specific chromosomal anomaly effects of the insecticides were altered by the protectants in two ways first omission of some specific anomalies and second addition of some new anomalies not shown earlier under independent effects. All the four protectants brought about reductions in the types of anomalies in differing magnitudes.

Keywords: *Allium cepa* L. insecticides, protection

Introduction
The *Allium cepa* (onion) plant has been grown and selectively bred in cultivation for at least 7,000 years. It is a biennial plant, but is usually grown as an annual. Modern varieties typically grow to a height of 15 to 45 cm (6 to 18 in). The leaves are yellowish- to bluish green and grow alternately in a flattened, fan-shaped swathe. They are fleshy, hollow, and cylindrical, with one flattened side. They are at their broadest about a quarter of the way up, beyond which they taper towards a blunt tip. The base of each leaf is a flattened, usually white sheath that grows out of a basal disc. From the underside of the disc, a bundle of fibrous roots extends for a short way into the soil. As the onion matures, food reserves begin to accumulate in the leaf bases and the bulb of the onion swells. Onions are cultivated and used around the world [1]. As a food item, they are usually served cooked, as a vegetable or part of a prepared savoury dish, but can also be eaten raw or used to make pickles.
Insecticides are pesticides that are formulated to kill, harm, repel or mitigate one or more species of insect. Insecticides work in different ways. Some insecticides disrupt the nervous system, whereas others may damage their exoskeletons, repel them or control them by some other means. They can also be packaged in various forms including sprays, dusts, gels, and baits. Because of these factors, each insecticide can pose a different level of risk to non-target insects, people, pets and the environment [2].

Methodology
In most of the cytological studies the experimental materials used are plants *Allium cepa*. The universality of the genetic material and its structural similarities in all living organisms, make plant materials ideal for experimental cytological studies. Hollaender (1976) [3] has pointed out the advantages of plant materials for cytological and mutagenic studies [3]. Plant materials are easy to regenerate, regeneration cycles, are easy to handle, require not much space, exhibit organizational chromosomal similarities with humans and the massive cytogenetic data pooled during the last many decades has proved beyond doubt that the results obtained through them have wide applicability.
The present study falls in line with others in using *Allium cepa* as the principle experimental material. Mishra (1973) [4] reported that the duration of mitotic cycle in *Allium cepa* is 16-17 hours with peak MI (mitotic index) between 11-12 hours which makes it as ideal for recovery experiments [4]. This study is involved two sorts of treatment materials– insecticides and protectants (Table-1). The choice of these materials depended on many factors but the most important considerations were, (1) commonness of the use (2) local availability, and in the case of protectants (3) established protection potential and (4) relative harmlessness to plant and animal systems.

The complete study was planned in three phases. In the first phase the treatment materials (insecticides) were screened for their mitotic and chromosomal effects. The objectives of these studies were not only to know the effects on MI (mitotic index) and chromosomes but also to find out the peak toxicity concentration level prior to the lethal effect. In the second phase, the protective effects of the protectants were investigated. It was not thought as necessary to investigate the possible mitotic and chromosomal effects of the selected protectants as most of them have fairly worked out protection profiles, and even if any of them eventually enhances the deleterious effects, them these would be revealed during the protective effect screening. The last phase related to data analysis interpretations.

Root tips squashing was the main cytological technique adopted for this study. Root tips from peregrinated (72 hour) bulbs (care was taken to select the bulbs of the same size from the same variety of *Allium cepa*) were treated with different treatment materials. For mitotic and chromosomal studies root tips were stained with 9:1 mixture of aceto-orcein and 1N HCL and then mounted in 45% acetic acid in according to Tijo and Levan (1950) [5] and Sharma & Sharma (1957) [6]. Extreme caution was taken to avoid over heating of orcein-HCL micture.

The main parameters were (1) Mitotic Index, (2) Percent total chromosomal animaly. (3) Percent specific chromosomal anomaly. Mitotic indices (MI) were computed on the basis of following formula (Chaursia, 1976; Yadav, 1977) [7, 8].

$$\text{MI} = \frac{\text{Total number of cells in division}}{\text{Total numbers of cells observed}} \times 100$$

The total percent anomaly (PA) was computed on the basis of the following formula (Chaursia, 1976; Yadav, 1977) [7, 8].

$$\text{PA} = \frac{\text{Total number of cells showing chromosomal anomaly}}{\text{Total number of dividing cells}} \times 100$$

Percent specific anoalies (PSA) were computed on the basis of the following formula (Yadav, 1977; Parihar, 1983) [8, 9].

$$\text{PSA} = \frac{\text{Total number of cells showing the specific anomaly}}{\text{Total number of abnormal cells}} \times 100$$

Table 1: Experimental layout: MI, PA, PSA Effects of Insecticidal Treatments. Experimental Material = Allium Cepa

Treatment Duration	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	Total Readings
	2RT	2RT	2RT	2RT	2RT	2RT	2RT	2RT	2RT	2RT	2RT	2RT	
(X) 24 hours	10Re	10Re	10Re	10Re	10Re	10Re	10Re	10Re	10Re	10Re	10Re	10Re	120
(C) 24 hours	10Re	10Re	10Re	10Re	10Re	10Re	10Re	10Re	10Re	10Re	10Re	10Re	120

X = Specific treatment
C = Control in distilled water
B1-B12 = Bulb number
RT = Number of root tips examined
Re = Number of Readings taken

The second phase protection studies planned in terms of (1) Pre-treatment (2) Mid-treatment (3) Post treatment. In the pre-treatment mode, the treatment of protectant (PR) preceded the treatment of the insecticide (IC). The mode can be abbreviated as PR-IC.

In the Mid-treatment mode the insecticide treatment was sandwiched between the protectant treatments and this can be expressed as PE-IC-PR. In the post-treatment mode the insecticide preceded the protectant and this mode can be abbreviated as IC-PR. All these modes had sub-variants relating to the presence or absence of duration of recovery time. Thus each of these modes had sub-sets of (1) No Recovery (2) 24 hours Recovery and (3) 48 hours Recovery. The detailed experimental layouts for these modes are given in the tabulated forms as below (Table-2, 3, 4).

Table 2: Experimental layout: protection studies pre-treatment (PR-IC)

Experimental Set	Treatment Exposure Time	B1	B2	B3	B4	B5	Total Readings
(X)	PR 12 hour IC 24 hour	2RT	2RT	2RT	2RT	2RT	
	No Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	24 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	48 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re
		B1	B2	B3	B4	B5	
(C)	C 12 hours IC 24 hours	2RT	2RT	2RT	2RT	2RT	
	No Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	24 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	48 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re

X = Non control set
C = Control in distilled water
PR = Protectan
IC = Insecticide
B1-B5 = Bulb number
RT = Number of root tips examined
Re = Number of Readings taken

Table 3: Experimental layouts: protection studies mid-treatment (PR-IC-PR)

Experimental Set	Treatment Exposure Time	B1	B2	B3	B4	B5	Total Readings
(X)	PR 2 hour IC 24 hour PR2 hours	2RT	2RT	2RT	2RT	2RT	
	No Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	24 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	48 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re

	C 2 hours	B1	B2	B3	B4	B5	
(C)	IC 24 hours						
	C 2 hours	2RT	2RT	2RT	2RT	2RT	
	No Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	24 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	48 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re

X = Non control set
C = Control in distilled water

Table 4: Experimental layouts: protection studies post – treatment (IC-PR)

Experimental Set	Treatment Exposure Time	B1	B2	B3	B4	B5	Total Readings
(X)	IC 24 hour PR 12 hours	2RT	2RT	2RT	2RT	2RT	
	No Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	24 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	48 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re
		B1	B2	B3	B4	B5	
(C)	IC 24 hours						
	C 12 hours	2RT	2RT	2RT	2RT	2RT	
	No Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	24 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re
	48 hours Recovery	10Re	10Re	10Re	10Re	10Re	50Re

X = Non control set
C = Control in distilled water

Observation and result

The loading of biosphere with the insecticides can be regarded as the most alarming development for the future survival of mankind. Fundamentally man is a biological organism and is controlled by biological processes and laws [10].

Table 5: Mitotic & Chromosomal Anomalies induced by Malathion Treatment Duration = 24 hours

Concentration (PPM)	Total No. of cells	Total No. of dividing cells	Total No. of Abnormal cells	Mitotic Index	Percentage of Anomalies (%)	No. of Cells Showing Specific Anomaly								
						Clumping	scattering	Bridge	Fragments	Bridge & Laggards	Laggards	Stathmoanaphase	Misorientation	Erosion
1	632	80	47	12.65	58.75	9.30	55.13	-	35.55	-	-	-	-	-
C	559	64	1	11.44	1.56	-	-	-	8.33	-	-	-	-	-
5	755	163	47	21.58	30.06	1.38	55.20	-	43.40	-	-	-	-	-
C	494	58	-	11.74	-	-	-	-	-	-	-	-	-	-
10	1049	187	114	17.87	60.93	19.60	42.68	1.30	28.76	1.19	6.44	-	-	-
C	642	89	5	13.86	5.61	8.33	16.66	-	8.33	-	-	-	-	-

C = Control in distilled water

Thee concentrations (1, 5 and 10 ppm) of Malathion were tested. 1ppm, 5 ppm, and 10 ppm concentrations induce 58.75%, 30.06% and 60.93% abnormalities, respectively. The most common abnormalities induced and the inducing concentrations are given as below –

1 ppm = Scattering
5 ppm = Scattering
10 ppm = Scattering

The percentages of chromosomal scattering anomalies are higher in all three concentrations tested. Malathion treatment accelerates mitosis. However concentrations higher than 10 ppm deform the cells considerably and make chromosomal observations difficult. Although all the three concentrations reveal higher MI in companion to controls, however, the peak MI is attained at 5 ppm (Table-5).

Table 6: Miotic and chromosomal anomalies induced by dimethoate treatment duration = 24 hours

Concentration (PPM)	Total No. of cells	Total No. of dividing cells	Total No. of Abnormal cells	Mitotic Index	Percentage of Anomalies (%)	No. of Cells Showing Specific Anomaly								
						Clumping	scattering	Bridge	Fragments	Bridg & Laggards	Laggards	Stathmoanaphase	Misorientation	Erosion
2	619	129	74	20.84	57.36	-	11.47	7-26	-	4.16	49-79	5.83	11.45	-
C	511	90	-	17.61	-	-	-	-	-	-	-	-	-	-
4	680	140	8	20.58	57.14	21.00	14.00	18.00	1.00	6.00	37.000	2.00	-	1.00
C	618	83	-	13.43	-	-	-	-	-	-	-	-	-	-
6	781	144	70	18.43	48.61	40.00	40.00	-	-	-	60.00	-	-	-
C	691	102	-	14.76	-	-	-	-	-	-	-	-	-	-
8	737	147	86	19.94	58.51	5.71	27.85	5.35	1.66	9.04	40.82	3.04	-	6.49
C	716	86	-	12.01	-	-	-	-	-	-	-	-	-	-
10	639	107	69	16.73	64.08	2.59	18.31	11.80	6.30	5.00	39.68	10.90	-	5.38
C	590	85	-	14.41	-	-	-	-	-	-	-	-	-	-

C = Control in distilled water

Table-6 records observations on the chromosomal abnormalities induced by 2, 4, 6, 8 and 10 ppm concentrations of dimethoates which induce 57.36%, 57.14%, 48.61%, 58.51% and 64.08% anomalies respectively. The most common chromosomal abnormalities induced by the concentrations are given as below –

2 ppm = Laggards
4 ppm = Laggards
6 ppm = Laggards
8 ppm = Laggards
10 ppm = Laggards

Laggards are therefore, most frequent anomalies as observed in all the concentrations of Dimethoate. Treatment shows acceleration of mitosis. Concentrations higher than 10 ppm, however, deform the cells (Table-6).

Table 7: Miotic and chromosomal anomalies induced by dimecron treatment duration = 24 hours

Concentration (PPM)	Total No. of cells	Total No. of dividing cells	Total No. of Abnormal cells	Mitotic Index	Percentage of Anomalies (%)	No. of Cells Showing Specific Anomaly								
						Clumping	scattering	Bridge	Fragments	Bridge & Laggards	Laggards	Stathmoanaphase	Misorientation	Erosion
20	726	153	91	21.07	59.47	24.44	3.35	7.06	10.73	2.48	29.95	12.26	-	9.69
C	618	76	-	12.43	-	-	-	-	-	-	-	-	-	-
40	759	112	78	14.85	69.67	22.23	4.65	15.13	15.09	6.20	14.55	3.66	-	18.48
C	853	80	-	9.37	-	-	-	-	-	-	-	-	-	-
60	703	108	72	15.36	66.66	15.20	22.94	8.12	9.25	1.66	28.27	6.19	-	8.33
C	733	77	-	10-44	-	-	-	-	-	-	-	-	-	-
80	697	101	73	14.49	72.27	18.10	18.11	6.20	10.11	9.25	21.01	4.08	-	13.13
C	619	93	-	15.02	-	-	-	-	-	-	-	-	-	-
100	732	76	76	10.64	100.00	37.90	5.89	4.16	26.94	-	19.26	3.73	-	2.11
C	952	88	-	9.24	-	-	-	-	-	-	-	-	-	-

C = Control in distilled water

The effect of dimecron were tested in five concentrations i.e. 20, 40, 60, 80 and 100 ppm, induce 59.47%, 69.67% 66.66%, 72.27% and 100% chromosomal abnormalities, respectively. The most common anomalies induced are given as below –

20 ppm = Laggards
40 ppm = Clumping
60 ppm = Laggards
80 ppm = Laggards
100 ppm = Clumping

Thus, Laggards and clumping can be identified as the most common abnormalities induced by dimecron. Dimecron accelerates, mitosis in all the concentrations tested with peak MI (21.07%) at 20 ppm (Table-7).

Table 8: Miotic and chromosomal anomalies induced by metasystox treatment duration = 24 hours

Concentration (PPM)	Total No. of cells	Total No. of dividing cells	Total No. of Abnormal cells	Mitotic Index	Percentage of Anomalies (%)	No. of Cells Showing Specific Anomaly								
						Clumping	scattering	Bridge	Fragments	Bridge & Laggards	Laggards	Stathmoanaphase	Misorientation	Erosion
20	950	146	90	15.36	61.64	40.16	6.78	6.96	-	4.67	21.99	3.12	3.95	12.36
C	696	88	-	12.64	-	-	-	-	-	-	-	-	-	-
40	907	95	53	10.47	55.78	20.83	25.83	-	32.08	1.66	19.58	-	-	-
C	590	89	-	15.08	-	-	-	-	-	-	-	-	-	-
60	661	145	63	21.93	43.48	4.86	15.77	10.97	11.11	7.73	2.57	6.94	12.36	27.76
C	671	84	-	12.51	-	-	-	-	-	-	-	-	-	-
80	571	135	98	23.64	72.59	18.75	12.73	7.75	11.00	8.10	32.14	4.62	0.92	3.96
C	614	98	-	15.96	-	-	-	-	-	-	-	-	-	-
100	691	49	49	6.98	100.00	56.15	2.38	20.83	12.20	-	-	5.16	-	3.27
C	860	134	1	15.58	0.74	-	-	-	-	-	-	-	-	-

C = Control in distilled water

Five concentrations (20, 40, 60, 80 and 100 ppm) of Metasystox were tested. 20, 40, 60, 80 and 100 ppm concentrations induce 61.64%, 55.78%, 43.48%, 72.59% and 100% abnormalities, respectively. The most common anomalies are given below –

20 ppm = Clumping
40 ppm = Fragments
60 ppm = Erosion
80 ppm = Laggards
100 ppm = Clumping

Thus clumping, Fragments, erosion and laggards are the characteristic anomalies. Concentrations higher than 100 ppm deform the cells making abnormality observations difficult. Metasystox could not produce consistent results with respect to MI concentrations 20, 60 % 80 ppm accelerated mitosis while 40 and 100 ppm repressed it (Table-8).

Table 9: Miotic and chromosomal anomalies induced by endosulfan treatment duration = 24 hours

Concentration (PPM)	Total No. of cells	Total No. of dividing cells	Total No. of Abnormal cells	Mitotic Index	Percentage of Anomalies (%)	No. of Cells Showing Specific Anomaly								
						Clumping	Scattering	Bridge	Fragments	Bridge & Laggards	Laggards	Stathmoanaphase	Misorientation	Erosion
1	972	120	51	12.34	42.53	0.83	36.87	23.05	9.72	11.87	11.38	-	6.24	-
C	445	87	-	16.99	-	-	-	-	-	-	-	-	-	-
5	619	71	37	11.48	52.11	2.08	-	2.08	19.58	42.91	30.41	2.91	-	-
C	629	91	-	14.46	-	-	-	-	-	-	-	-	-	-
10	529	142	89	26.87	62.81	-	21.00	5.00	6.00	29.00	24.00	5.00	-	-
C	550	76	1	13.18	1.31	-	-	-	-	-	8.33	-	-	-

C = Control in distilled water

Endosulfan was tested in three concentrations i.e. 1, 5 and 10 ppm which induce 42.53%, 52.11% and 62.81% abnormalities respectively. The most commonly induced anomalies are given as below –

1 ppm = Scattering
5 ppm = Laggards
10 ppm = Laggards

Endosulfan treatment represses mitosis at 1 and 5 ppm. At 10 pp, however, mitosis is accelerated (Table-9).

A laggard is the abnormality most common, followed by scattering and clumping. Fragmentation is least common. Malathion and Dimethoate are characterized by single anomalies– scattering in the case of Malathion and laggards in the case of Dimethoate. Dimecron, Metasystox and endosulfan exhibited. Dimecron, Metasystox and Endosulfan exhibited, mixed anomalies, with clumping taking the highest values in Dimecron and Metasystox and laggards in the case of Endosulfan [11].

Table 10: Insecticide concentration showing highest percentage anomaly & specific anomaly treatment duration = 24 hours

Insecticide	Concentration (ppm)	Percent Anomaly (%)	Specific Anomaly (%)							
			Clumping	Scatterng	Bridge	Fragments	Bridge & Laggards	Laggards	Stathmoanaphase	Erosion
Malathion	10	60.93	-	42.68	-	-	-	-	-	-
Endosulfan	10	62.81	-	-	-	-	-	34.08	-	-
Dimethoate	10	64.08	-	-	-	-	-	39.68	-	-
Dimecron	100	100.00	37.90	-	-	-	-	-	-	-
Metasystox	100	100.00	56.15	-	-	-	-	-	-	-

A laggard is the abnormality most common, followed by scattering and clumping. Fragmentation is least common. Malathion and Dimethoate are characterized by single anomalies– scattering in the case of Malathion and laggards in the case of Dimethoate. Dimecron, Metasystox and endosulfan exhibited. Dimecron, Metasystox and Endosulfan exhibited, mixed anomalies, with clumping taking the highest values in Dimecron and Metasystox and laggards in the case of Endosulfan (Table- 10).

10 ppm concentrations of Malathion, Endosulfan and Dimethoate produced highest number of total anomalies while for Dimecron and Metasystox this concentration was 100 ppm (Table- 6). Since concentrations above 10 ppm in the case of Malathion, Endosulfan and Dimethoate and above 100 ppm, in the case of Dimecron and Metasystox, Deform the cells, these concentrations were selected for protection studies.

Conclusion

The independent chromosomal effects of the five insecticides Malathion, Dimecron, Dimethoate, Metasystox & Endosulfan as described have exhibited following specific chromosomal anomalies. Clumping, Scattering, Bridges, Fragments, Bridges with laggards, Laggards, Stathmoanaphase, Mis-orientation, Erosion. Specific chromosomal anomaly effects of the insecticides were altered by the protectants in two ways first omission of some specific anomalies and second addition of some new anomalies not shown earlier under independent effects. All the four protectants brought about reductions in the types of anomalies in differing magnitudes.

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