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CYTOTOXIC EFFECTS OF PESTICIDES IN SOMATIC CELLS OF *VICIA FABA* L.

The effects of pesticides (Endosulfan, Dieldrin, Aldrin) on cell division and chromosomal morphology of Vicia faba L. were studied. The results showed that the pesticides are mitodepressive in higher concentrations and mitopromotor in lower concentrations and induced a variety of chromosomal abnormalities such as stickiness, fragments, chromatid separation, disturbed metaphase, C-mitosis, laggards, precocious movement and late separation where lagging chromosomes were predominant. The concentration of 500 ppm or above, for all the pesticides used in the present study showed pronounced toxic effect. In remaining treatments, although the mitotic index was improved but less than that of absolute controls.

Introduction. Pesticides used in the modern agricultural practices represent a very large input of toxic chemicals in our environment [16]. Their usage has increased manifolds in disease control management without considering their harmful side effects on plants, animals and human beings [2]. Although the use of these chemicals has become essential, but their ingredients have induced acute toxic effects [3, 8]. The toxic effect of pesticides is not necessarily a result of direct application, some pesticides accumulate into the food to a toxic level and affect the public health [17, 14]. However, the potentiality of these pesticides as mutagenic or carcinogenic agent to non- target organisms has been reported [31]. Cytological investigations have been carried out to detect the harmful effects of agricultural chemicals on various crop plants [20, 29, 28, 19]. It was also reported that mutagens significantly affect the sex organs and alter fertility [25]. But no significant work has been done on the effect of these pesticides (endosulfan, dieldrin, aldrin) on cell division and chromosomes of *Vicia faba*. Therefore, the aim of the present study was to examine the cytotoxic effects of these chemicals on mitotic activity of *Vicia faba*.

Materials and Methods. Different concentrations (5, 50, 500 and 1000 ppm) of endosulfan, dieldrin and aldrin were used in the treatment of *Vicia faba*. The solutions were prepared in distilled water. Seeds were directly placed in test liquids and controls were placed only in the distilled water. The seeds were treated with test solution for 12 hours and then allowed a recovery period of 2 hours in distilled water [23]. The treated seeds of *Vicia faba* were put on wet filter paper in petridishes and kept in darkness at 22–25 °C. Root tips were obtained after germination and the experimental as well as the control root meristems were excised from the seeds. The root tips were cut carefully and fixed in acetoalcohol solution (1 acetic acid : 3 alcohol) for 24 hrs. The root tips were hydrolyzed in 1 NHCl for 10 minutes and squashed in 2 % acetocarmine for cytological studies. The slides were temporarily sealed, 10 root tip squashes were prepared for each variables and a minimum of 250 mitotic cells were examined from each slide and microphotographs of selected slides were taken. The mitotic index was calculated using the method of [28]. Chromosomal aberrations and their percentage in each treatment were also recorded.

$$\text{Mitotic index} = \frac{\text{No. of dividing cells}}{\text{No. of total cells}} \cdot 100$$

Table 1

Effect of pesticides on mitotic cell division of *Vicia faba* L.

Treatments	Concentration (ppm)	Number of cells observed	Number of actively dividing cells	Mitotic Index (MI), %	Metaphase, %			Anaphase, %		
					Normal	Abnormal	Total	Normal	Abnormal	Total
	Control	2629	618	23.51	45.82	–	45.82	54.18	–	54.18
Endosulfan	5	2679	592	22.10	44.19	1.05	45.24	52.98	1.78	54.76
	50	2468	538	21.80	25.79	17.96	43.75	39.89	16.36	56.25
	500	2373	408	17.19	17.69	19.42	37.11	14.67	48.22	62.89
	1000	2637	245	9.29	8.57	22.31	30.88	12.02	57.10	69.12
		2557.2 ± ± 58.41	480.2 ± ± 69.05	18.78 ± ± 2.07	28.41 ± ± 7.30	15.18 ± ± 4.79	40.56 ± ± 2.87	34.75 ± ± 10.10	30.86 ± ± 13.05	59.44 ± ± 2.87
Dieldrin	5	2819	628	22.28	44.11	1.47	45.58	53.88	0.54	54.42
	50	2618	568	21.70	27.65	18.07	45.72	39.60	14.68	54.28
	500	2827	467	16.52	19.08	20.18	39.26	15.87	44.87	60.74
	1000	2416	202	8.36	8.81	23.21	32.02	12.40	55.58	67.98
		2670.0 ± ± 97.49	466.2 ± ± 94.14	17.21 ± ± 2.22	24.91 ± ± 7.46	15.98 ± ± 4.87	40.64 ± ± 3.24	30.43 ± ± 9.87	28.92 ± ± 12.82	59.35 ± ± 3.24
Aldrin	5	2408	532	22.09	43.40	1.08	44.48	53.54	1.98	55.52
	50	2517	539	21.41	27.79	16.19	43.98	37.24	18.78	56.02
	500	2412	338	14.01	12.84	23.98	36.82	11.85	51.29	63.14
	1000	2921	210	7.19	7.25	23.67	29.92	9.97	60.11	70.08
		2564 ± ± 121.48	404.75 ± ± 79.90	16.17 ± ± 3.52	22.82 ± ± 8.11	16.23 ± ± 3.11	38.80 ± ± 4.44	28.15 ± ± 10.50	33.04 ± ± 13.64	61.19 ± ± 3.43
Mean		2597.23 ± ± 50.18	449.86 ± ± 43.27	52.17 ± ± 1.56	25.61 ± ± 4.08	15.79 ± ± 2.76	40.00 ± ± 11.09	31.11 ± ± 5.22	30.94 ± ± 6.90	59.99 ± ± 1.69
CD at										
5 %		130.18	88.96	3.22	8.40	5.68	22.81	10.73	14.19	3.46
1 %		139.46	120.25	4.35	11.34	7.67	30.83	14.51	19.17	4.68

Results. The cytotoxic effects on root tips cells of *Vicia faba* have been presented in Table 1. The perusal of the table indicates that there was an exponential relationship between the percentage of aberrations and concentrations of pesticides. The species showed significant decrease in mitotic index as the dose increased. The metaphase abnormalities gradually increased by the increase in concentration of pesticides in the *Vicia faba* i.e. 50–1000 ppm doses of endosulfan, dieldrin and aldrin. Different concentrations of chemicals used also showed significant variation in mitotic index. The aqueous solution of pesticides enhanced the mitotic index than treated values, when a recovery period of 2 hrs was given to the treated materials of all used concentrations. Different concentrations of pesticides induced various types of chromosomal aberrations at metaphase and anaphase. The aberrations with their mean percent values have been given in Table 2. Almost all treatments revealed significant effect on the frequency of mitotic phases. The stickiness of chromosomes was induced by only higher concentrations of pesticides and the mean value of stickiness was 5.20 %, 5.13 % and 6.02 % in 500 ppm, which increased to 8.17 %, 6.27 % and 8.06 % in 1000 ppm of endosulfan, dieldrin and aldrin, respectively. The 5, 50 ppm treatments were unable to induce stickiness of chromosomes.

At metaphase, chromatid separation, disturbed metaphase, stickiness of chromosomes, precocious movement, C-mitosis were observed (Figure). All chromosomal abnormalities like chromatid separation, disturbed metaphase, precocious move-

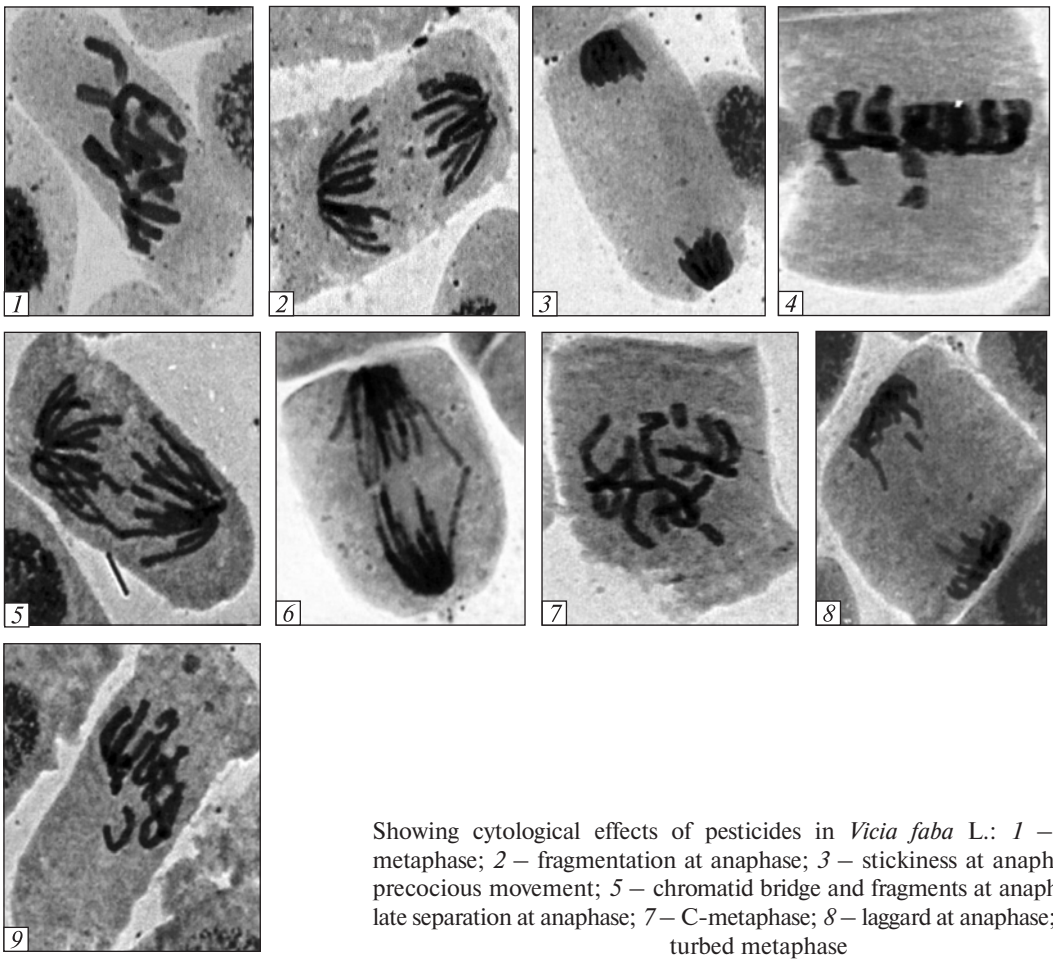
Table 2

Chromosomal aberrations (%) in mitotic cells of root tips of *Vicia faba* L.

Treatments	Concentration (ppm)	Distubed metaphase	Precocious movement	C-mitosis	Laggards	Stickiness at anaphase	Fragmentation at anaphase	Chromatid bridge	Late separation at anaphase
	Control	–	–	–	–	–	–	–	–
Endosulfan	5	–	–	1.05	1.78	–	–	–	–
	50	4.30	5.37	8.29	6.02	2.06	3.29	2.12	2.87
	500	3.87	6.48	9.07	18.17	5.20	11.37	6.02	7.46
	1000	3.95	7.17	11.19	24.68	8.17	13.42	8.17	2.66
		4.04 ± ± 0.13	6.34 ± ± 0.52	7.4 ± ± 2.20	12.66 ± ± 5.30	5.14 ± ± 1.76	9.36 ± ± 3.09	5.43 ± ± 1.77	4.33 ± ± 1.56
Dieldrin	5	–	1.47	–	0.54	–	–	–	–
	50	3.85	5.09	9.13	6.79	2.05	3.08	1.02	1.74
	500	3.42	7.29	9.47	17.97	5.13	9.95	4.93	6.89
	1000	3.87	7.33	12.01	24.19	6.27	12.20	6.14	6.78
		3.71 ± ± 0.14	5.29 ± ± 1.37	10.20 ± ± 1.11	12.37 ± ± 5.41	4.48 ± ± 1.26	8.41 ± ± 2.74	4.03 ± ± 1.54	5.14 ± ± 1.69
Aldrin	5	–	1.08	–	1.98	–	–	–	–
	50	5.39	3.31	7.70	8.14	2.11	3.28	2.16	3.09
	500	4.91	6.95	12.17	20.37	6.02	11.10	5.78	7.97
	1000	4.57	7.04	12.06	24.73	8.06	12.07	7.43	7.82
		4.96 ± ± 0.23	4.59 ± ± 1.46	10.64 ± ± 1.47	13.80 ± ± 5.28	5.39 ± ± 1.74	8.81 ± ± 2.78	5.12 ± ± 1.55	6.29 ± ± 1.60
Mean		4.23 ± ± 0.22	5.32 ± ± 0.71	9.41 ± ± 1.05	12.94 ± ± 1.65	5.00 ± ± 0.81	8.86 ± ± 1.44	4.86 ± ± 0.84	5.26 ± ± 0.86
CD at									
5 %		0.45	1.45	2.16	3.39	1.66	2.97	1.72	1.77
1 %		0.60	1.97	2.91	4.59	2.25	4.02	2.33	2.39

ment, stickiness of chromosomes and C-mitosis at metaphase were induced only by higher concentrations (Table 2). The mean values of laggards at anaphase were 6.02 %, 6.79 % and 8.14 % in 50 ppm, which increased to 24.68 %, 24.19 % and 24.73 % in 1000 ppm of endosulfan, dieldrin and aldrin, respectively. The 5 ppm concentration was not able to induce laggards. The other chromosomal abnormalities like stickiness, fragmentation of chromosomes and late separation at anaphase were induced only by 500 ppm and 1000 ppm (Table 2). Mitotic index values were low in high concentrations of endosulfan, dieldrin and aldrin and also varied in various concentrations of these pesticides (Table 1).

Discussion. Pesticides applied to either soil or crop plants are subjected to volatilization, leaching, and induce chromosomal aberrations, chemical modification and microbial degradation, which have higher risk of bringing about environmental pollution. The application of pesticides to the root meristems of *Vicia faba* L. have revealed decrease in mitotic index in increasing concentrations of endosulfan, dieldrin and aldrin. Inhibition of mitosis was also reported by [4, 7, 11, 15, 27, 34, 35] by the treatment of substituted urea compound, phenols, trifluraline, nitraline, carbamates, terbutryn, atrazine and gasegard in *Hordeum* and *Tradescantia*, *Nigella, Allium cepa, Vicia faba* and in barley, respectively. Significant reduction in mitotic index (MI), noted in the present study may be due to the mitodepressive action of the chemicals indicating thereby the pesticides used interfere in the normal cell cycle resulting in decrease in number of dividing cells. Similar results had also been reported by [11, 19, 24, 28, 33, 36] on various crop plants. The inhibition of certain cell cycle specific proteins remains as a possible pesticide target site which inhibit DNA polymerase as well as other enzymes resulting in antimitotic effect [22].



Showing cytological effects of pesticides in *Vicia faba* L.: *1* – normal metaphase; *2* – fragmentation at anaphase; *3* – stickiness at anaphase; *4* – precocious movement; *5* – chromatid bridge and fragments at anaphase; *6* – late separation at anaphase; *7* – C-metaphase; *8* – laggard at anaphase; *9* – disturbed metaphase

The treatments of pesticides showed significant effect on the percentage of the metaphase stages, the mean values were 22.31 %, 23.21 % and 23.67 % in 1000 ppm of endosulfan, dieldrin and aldrin, respectively. As far as anaphase percent was concerned the mean values were 57.10 %, 55.58 % and 60.11 % in 1000 ppm [5, 6] also observed a similar trend by the treatment of trichlorphon and rotenone in other crops. Various types of chromosomal abnormalities like fragments, disturbed metaphase, C-mitosis, laggards, chromatid bridge, stickiness, precocious movement and late separation were observed, the percentage of abnormalities were gradually increased in higher concentrations of pesticides. These results are in agreement with *Allium cepa* root tips with different pesticides, such as sencorer [21, 28], cyanazine [30], gasegard [28], carbetamex & paradone plus [9]. The treatments of various pesticides such as gespex [10] in *Vicia faba*, gasegard and igran [35] in barley have shown similar effect as in the present study. Cytotoxic effects of the pesticides used in the present case were in conformity with that of [1, 13] with the treatment of toxic pesticides and chemicals.

Chemical abnormalities such as C-mitosis, lagging chromosome (s) and precocious movement were the result of disturbance in the spindle fiber formation caused by the pesticides [10, 21] attributable to lipophylic chain of spindle proteins, which causes the banding of polypeptides [18]. Stickiness is induced either by the effect of pesticides on chromosomal protein attributed to the improper folding of chromosome fibers which render the chromatid connected by means of subchromatid bridges [12, 26] or may be due to the action of pesticides on the polymerization process, resulting in the fragmentation of chromosomes and bridges at anaphase stage forms sticky chro-

mosomes [18]. Rank et al. [32] have reported that the chromosome bridges and fragments lead to structural changes in chromosomes of crop plants and in other organisms in the environment. Hence the higher concentrations of endosulfan, dieldrin and aldrin may become motostatic, chromotoxic and clastogenic in crop plants, therefore, its higher concentration is not suggestive.

From the foregoing discussion, it is obvious that pesticides present in the environment can be absorbed by the plants, which may adversely affect the genetic systems, causing damage to chromosomes in crop plants and other organisms. Regular application of pesticides in agricultural practices is a potential threat to genetic constitution of crop plants and animals. Therefore, judicial application of chemical pesticides is essential. Indiscriminate use of pesticides should be discouraged as far as practicable.

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Р.М. Панди

ЦИТОТОКСИЧЕСКОЕ ДЕЙСТВИЕ ПЕСТИЦИДОВ НА СОМАТИЧЕСКИЕ КЛЕТКИ *VICIA FABA* L.

Изучено влияние пестицидов (Endosulfan, Dieldrin, Aldrin) на деление клеток и морфологию хромосом *Vicia faba* L. Результаты свидетельствуют, что пестициды в высоких концентрациях подавляют митозы, а в низких являются митопромоторами, и индуцируют разнообразные хромосомные аномалии такие как слипание хромосом, фрагменты, разделение хроматид, нарушения метафаз, С-митозы, отставания, преждевременное движение и позднее разделение в тех случаях, где доминировали отставшие хромосомы. Для всех пестицидов, использованных в настоящей работе, концентрации свыше 500 ppm проявляли значительный токсический эффект. В остальных случаях митотический индекс хоть и улучшался, но был меньшим, чем в контрольных экспериментах.

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ЦИТОТОКСИЧНА ДІЯ ПЕСТИЦИДІВ НА СОМАТИХНІ КЛІТИНИ *VICIA FABA* L.

Вивчали вплив пестицидів (Endosulfan, Dieldrin, Aldrin) на ділення клітин та морфологію хромосом *Vicia faba* L. Результати свідчать, пестициди у високих концентраціях пригнічують мітози, а в низьких є мітопромоторами та індукують різні хромосомні аномалії, такі як злипання хромосом, фрагменти, розділення хроматид, порушення метафаз, С-мітози, відставання, передчасний рух та пізнє розділення в тих випадках, коли домінують відсталі хромосоми. Для всіх пестицидів, використаних в даній роботі, концентрації понад 500 ppmпроявляли значний токсичний ефект. В інших випадках мітотичний індекс хоч і покращувався, але був меншим, ніж в контрольних експериментах.

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