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Genotoxicity of Two Organophosphate Insecticides Based on *Allium* Test

Adoracion T. Aranez and **Raquel O. Rubio**
Institute of Biology

ABSTRACT

The genotoxicity of two organophosphate insecticides, Folidol and Malathion, was each determined on root tip chromosomes of onion (Allium cepa *L.). Acetocarmine squash preparations of rootsgrown from seeds untreated (control) and treated with two concentrations of Folidol (0%, 0.5% and 0.75%) and three of Malathion (0.5%, 0.75%, and 1.0%) resulted in statistically insignificant differences in mitotic indices. The root cells grown from pesticide-treated seeds exhibited chromosomal abnormalities such as rings, laggards, bridges, disoriented and precocious chromosomes, as well as polyploidy. Frequency of chromosomal aberrations for seeds treated with 0%, 0.5%, and 0.75% Folidol were 2%, 10%, and 12%, respectively, while those treated with 0%, 0.5%, 0.75%, and 1.0% Malathion were 3.1%, 6.01%, 8.9%, and 8.3%, respectively.*

INTRODUCTION

Pesticides have long been regarded as valuable means of controlling pest infestation in field crops. They are usually synthetic compounds used by man to control pests such as insects, weeds, fungi, rodents, and other organisms. They should be toxic to the target organisms only. However, their absolute toxic specificity to target organisms has not been achieved and non-target organisms, including human beings, are usually at risk.

Among the widely used modern pesticides in the Philippines are the organophosphates Folidol and Malathion. These compounds are less persistent in nature than organochlorines (1). However, continuous and injudicious use of these pesticides may subject living forms to their chronic effects or to their acute effects in case large amounts are used. Organophosphates have alkylating properties, hence, can be correlated to mutagenicity (2).

The common onion (*Allium cepa* L.) has been found to be excellent for the assay of chromosomal aberrations due to its relatively large chromosomes and low chromosome number (3). In the past, the *Allium* test system lacked general acceptance because plant cells are characteristically different from the animal cells. However, recent studies have shown that for a specific chemical agent, similar genetic abnormalities are observed in plant and animal systems (3). Together with *Vicia faba, Allium cepa* L. they show very good correlation with the bacterial and mammalian systems (4) when used as bioassay test system for genotoxicity of pesticides.

This study investigates the effects of different concentrations of Folidol and Malathion on mitotic root tip chromosomes of *Allium cepa* L.

MATERIALS AND METHODS

Folidol

A total of 360 seeds of *A. cepa* was used in this study. They were divided into nine batches of 40 seeds each. Each batch was wrapped with a piece of gauze and soaked in water for six hrs at 30°C. Varying concentrations of Folidol (0,0-dimethyl-0-4-nitrophenyl phosphorothioate), a methyl-parathion containing pesticides, in distilled water were prepared (0.5% and 0.75%). The seeds were treated with 0% (control), 0.5% and 0.75% pesticide in distilled water for 4 hrs at 30°C. The treatment was done using water bath (Buch 461). The treated seeds were washed in running tap water for 2 hrs. Three replicates were made.

The treated seeds were placed in petri dishes with moist filter paper and then placed in a continuously lighted growth chamber maintained at 13 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity and 30 ± 1°C temperature.

Excision of root tips for the cytological studies was done when the root length was about 2–3 mm. The excised tips were immediately killed and fixed in freshly prepared Farmer's fluid (three parts of absolute ethyl alcohol and one part glacial

acetic acid) and placed in the refrigerator for about 24 hrs prior to squash preparation.

Acetocarmine squash technique was employed. The fixed root tip was treated with 1 N HCl, then stained with 1% acetocarmine. The specimen on a slide with acetocarmine was covered with a cover glass. The slide was heated gently over an alcohol lamp. After heating, the specimen was squashed carefully in order to separate and flatten the individual cells. Observations were made under the microscope. One hundred cells from each of ten good slides (30 slides for the three replicates) per treatment were scored for the mitotic index and chromosome morphology. The significance of variations in mitotic index of treated and untreated root tips were determined using analysis of variance (ANOVA).

Malathion

The above procedure was repeated using another organophosphate insecticide, Malathion (0,0-dimethyl S-[1,2-dicarbethoxyethyl] phosphorodithioate). An additional dose of 1% was included.

RESULTS

Cellular and Chromosomal Effects of Folidol

The mitotic indices of root meristems treated with 0%, 0.5%, and 0.75% pesticide were 7.0, 7.5, and 7.7, respectively. The values are not significantly different from each other by ANOVA test. The most number of dividing cells were in metaphase, followed by cells in prophase, telophase, and with cells in anaphase being the least both for the untreated and treated root tips of *A. cepa*. There was a slight decrease in the number of mitotic cells at Metaphase stage in the root tip meristem of treated seeds compared to control. However, more mitotic cells were at prophase and telophase in root tip meristems treated with pesticide than those in control specimens. Cells at anaphase were more or less similar in quantity for both treated and untreated root tip meristems (Table 1).

Chromosomal aberrations observed in cells treated with Folidol include ring chromosome (Fig. 1), laggard (Figs. 2–3), chromosome bridge (Figs. 4–5), disoriented chromosomes (Figs. 6–7), precocious chromosomes (Fig. 8), and polyploid cells (Figs. 9–10). Normal cells of *A. cepa* at metaphase are

in Figs. 11 and 12. Percentages of dividing cells with chromosomal aberrations were 2%, 10%, and 12% for seeds treated with 0%, 0.5%, and 0.7% Folidol, respectively (Table 2).

Cellular and Chromosomal Effects of Malathion

Mitotic index of root tip cells treated with 0%, 0.5%, 0.75%, and 1.0% Malathion were 8.7, 8.3, 8.2, and 8.4, respectively. As in Folidol, the differences in the mitotic indices of the different treatments observed were not statistically significant. The greatest number of dividing cells were at metaphase, followed by cells at prophase, then at telophase, with anaphase cells being the least number for both untreated and treated root tip cells. Also, there was a decrease in the metaphase index and an increase in prophase index of treated cells as compared with the control. There was a decrease in telophase index for mitotic cells from seeds treated with 0.75% Malathion, whereas a slight increase in the mitotic index was observed in cells treated with 1.0% Malathion (Table 3).

The types of chromosomal aberrations were similar to those observed in mitotic cells from roots treated with Folidol (Figs. 13–19). Oblique cell division (Fig. 20) was also observed. Percentage of dividing cells with chromosomal aberrations were 3.1%, 6.0%, 8.9%, and 8.3% for mitotic cells from seeds treated with 0%, 0.5%, 0.75%, and 1.0% Malathion, respectively (Table 4).

DISCUSSION OF RESULTS

The effects of varying concentrations of the two pesticides, Folidol and Malathion, on mitotic indices of *A. cepa* differed, however, the differences in the mitotic indices of treated and untreated *A cepa* were statistically insignificant. These results are in agreement with those of Amer and Farah (5). They did not observe any significant effect on the mitotic *Vicia faba* root tip cells which were treated with organophosphorus insecticide Dursban, although a high percentage of mitotic abnormalities were observed.

The chromosomal abnormalities observed in dividing cells may be due to the effects of the treatments on DNA or on the microtubules of the spindle fibers. Chromosome bridges, laggards, and ring chromosomes may be due to the effect of the agent on DNA. Organophasphates have alkylating properties (2). The N-7

of guanine is usually the site in DNA likely to be attacked by the alkylating agents (6,7). The release of 7-alkylguanine from alkylated DNA at neutral pH has been reported (8) and this will produce apurinic sites. Apurinic sites may result to splitting of the sugarphosphate chain and eventually the breaking of the chromosome (9, 10). The broken ends of chromosomes are sticky and two chromosomes with broken ends and with a centromere each may unite to form a dicentric chromosome. Anaphase bridges may reappear in succeeding cell generations because of breakage and reunion phenomenon. Ring chromosomes may be produced by the union of broken ends of segments of chromosomes with no centromere and hence cannot move to the poles.

Chromosome bridges may have resulted also from chromosomal stickiness and subsequent failure of anaphase separation (11). The stickiness of chromosomes could be attributed to intercalation of the pesticide with DNA leading to entanglement of chromatin threads (12, 13).

Polyploidy, precocious chromosomes, and disoriented chromosomes may be brought about by the action of the pesticides on the microtubules of the spindle fibers. Dysfunction of the spindle apparatus could explain the formation of polyploid cells (14). Treatment with pesticides could cause the failure of chromosomes to align at the equatorial plate because of dysfunction of the spindle and energy deficiency such that there is a delay in the division of the centromeric region (15). During cell division corresponding to anaphase, division of the centromere occurs with the separation of the daughter chromosomes and since there is lack of energy and definite poles, failure of assembly at definite poles by daughter chromosomes leads to the formation of polyploid cells (15). Lack of energy and definite poles may also be the reason why disoriented chromosomes were observed.

The presence of an insignificant number of chromosomal aberrations in the untreated *A. cepa* root tip cells could be due to spontaneous mutation.

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Table 1. Mitotic index and frequency of occurrence (%) of different mitotic phases in root tip cells of *A. cepa* untreated and treated with varying doses of Folidol (methyl parathion-containing pesticides)

Dose (%)	Cells scored (no.)	Dividing cells (no.)	Mitotic index* (MI)	Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
0	3000	208	7.0a	26.9	32.2	18.5	22.4
0.50	3000	225	7.5a	29.3	29.5	17.6	23.6
0.75	3000	230	7.7a	27.7	29.1	18.1	25.1

*not significant

$$Mitotic\ Index = \frac{number\ of\ dividing\ cells}{total\ number\ of\ cells} X\ 100$$

Table 2. Frequencies of chromosomal aberrations in root tip cells of *A. cepa* untreated and treated with varying doses of Folidol (methyl parathion-containing pesticide)

Dose (%)	Cells scored (no.)	Dividing cells (no.)	Cells w/aberrations (no.)	Aberrations (%)
0	3000	208	2	2.0
0.5	3000	225	22	10.0
0.75	3000	230	27	12.0

Table 3. Mitotic index and frequency of occurrence (%) of different mitotic phases in root tip cells of *A. cepa* untreated and treated with varying doses of Malathion (malathion-containing pesticides)

Dose (%)	Cells scored (no.)	Dividing cells (no.)	Mitotic index* (MI)	Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
0	3000	260	8.7a	28.9	31.5	19.6	26.2
0.50	3000	250	8.3a	26.4	26.8	20.8	26.0
0.75	3000	246	8.2a	33.7	24.8	18.7	20.3
1.0	3000	253	8.4a	30.0	22.9	20.6	27.3

*not significant

$$Mitotic\ Index = \frac{number\ of\ dividing\ cells}{total\ number\ of\ cells} X\ 100$$

Table 4. Frequencies of chromosomal aberrations in root tip cells of *A. cepa* untreated and treated with varying doses of Malathion (malathion-containing pesticide)

Dose (%)	Cells scored (no.)	Dividing cells (no.)	Cells w/ aberrations (no.)	Aberrations (%)
0	3000	260	8	3.1
0.5	3000	250	15	6.0
0.75	3000	246	22	8.9
1.0	3000	253	21	8.3

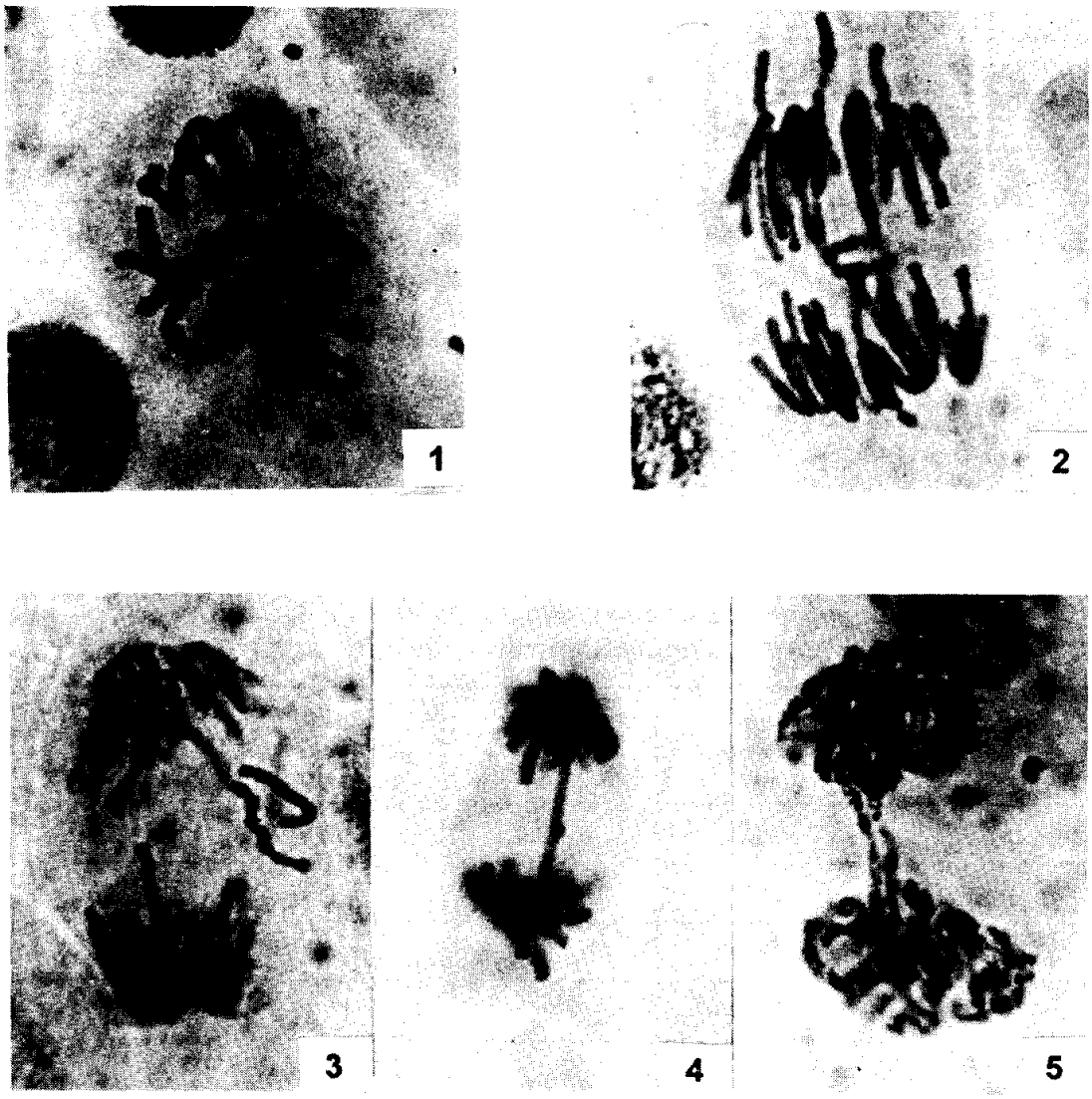

Plate I. Chromosomal aberrations due to effects of Folidol on DNA

1. Ring chromosome in A. cepa *root tip cell from seeds treated with 0.5% Folidol, X1389; 2. Laggard in the form of a ring in* A. cepa *root tip cell from seed treated with 0.5% Folidol, X1389; 3. Laggards in* A. cepa *root tip cell from seed treated with 0.75% Folidol, X1389; 4. Chromosome bridge in* A. cepa *root tip cell from seed treated with 0.75% Folidol, X1389; 5. Two chromosome bridges in* A. cepa *root tip cell from seed treated with 0.75% Folidol, X1389.*

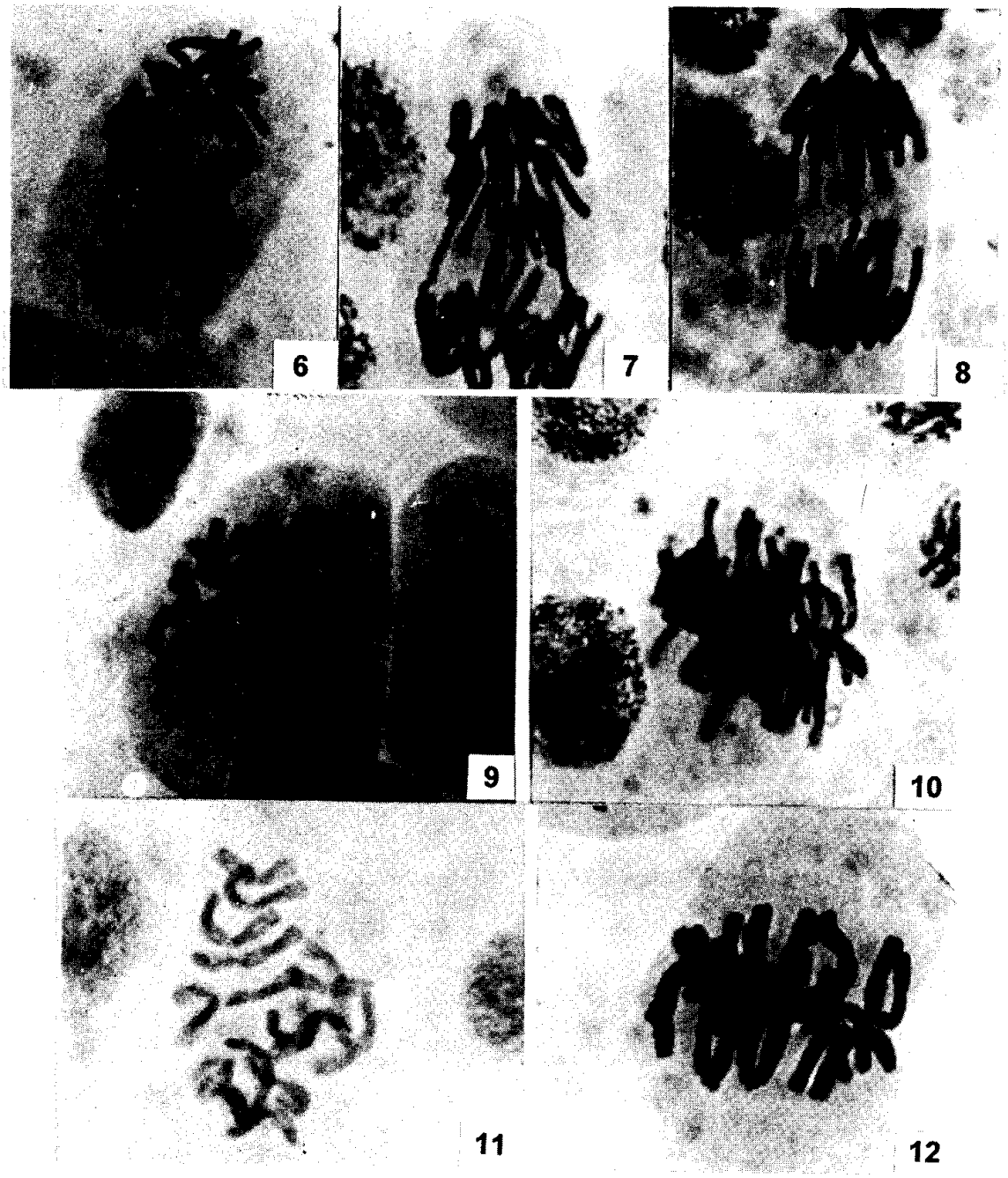

Plate II. Chromosomal aberrations due to effects of Folidol on spindle fibers

6. Disoriented chromosomes and chromosome bridge in A. cepa *root tip cell from seed treated with 0.5% Folidol, X1389; 7. Disoriented chromosomes and chromosome bridges in* A. cepa *root tip cell from seed treated with 0.5% Folidol, X1389; 8. Precocious chromosome in* A. cepa *root tip cell from seed treated with 0.75% Folidol, X1389; 9–10. Polyploid cells in* A. cepa *root tip cell from seed treated with 0.5% Folidol, X1389; 11–12.* A. cepa *root tip cell with normal chromosome number, X1389.*

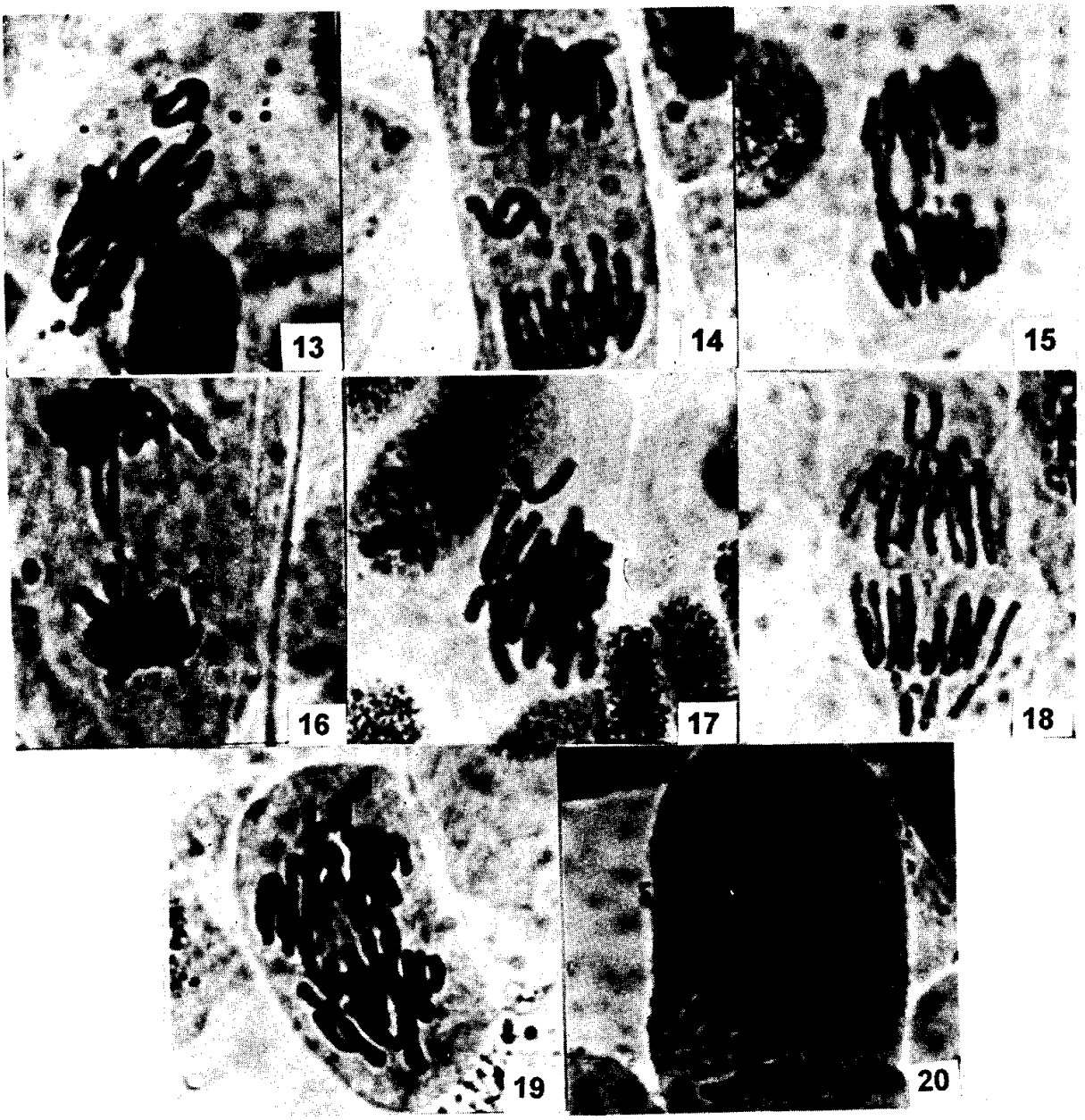

Plate III. Chromosomal aberrations due to effects of Malathion on DNA and spindle fibers

13. Ring chromosome in A. cepa *root tip cell from seed treated with 0.75% Malathion, X1389; 14. Laggards in* A. cepa *root tip cell from seed treated with 0.5% Malathion, X1389; 15. Chromosome bridges in* A. cepa *root tip cell from seed treated with 0.75% Malathion, X1389; 16. Chromosome bridges in* A. cepa *root tip cell from seed treated with 0.5% Malathion, X1389; 17. Precocious chromosomes in* A. cepa *root tip cell from seed treated with 0.5% Malathion, X1389; 18. Precocious chromosomes in* A. cepa *root tip cell from seed treated with 0.75% Malathion, X1389; 19. Disoriented chromosomes and chromosome bridges in* A. cepa *root tip cell from seed treated with 0.75% Malathion, X1389; 20. Oblique cell division in an onion root tip cell from seed treated with 1.0% Malathion, X1389.*