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Effect of Chlorfluazuron on the Mitotic Plant Cell Division and Germination of Onion (*Allium cepa* L.) and Faba Bean (*Vicia faba* L.)

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Abstract. I.K.I is a trade name for the chitin synthesis inhibitor insecticide (chlorfluazuron). The insecticide I.K.I. is tested for its cytological effects on onion root tip cells and its effect on germination of onion and *faba bean* seeds. The results obtained indicated the ability of the insecticide to cause different mitotic alternations ranging from reductions in mitotic activity to the production of a large number of mitotic abnormalities. Additionally, the decrease rate of germination for both onion and *faba bean* seeds was associated with lower mitotic index especially when higher concentrations of insecticide were used.

Introduction

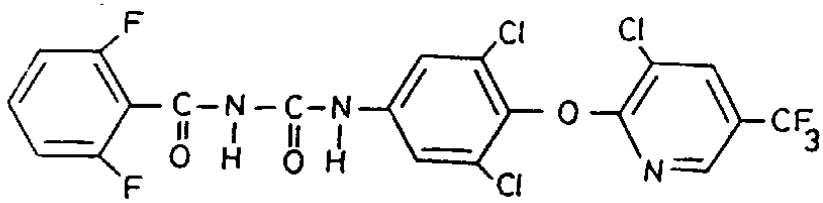
Considerable research has been directed towards defining the effect of pesticides on seed germination, seedling growth and chromosomal behaviour. There have been a number of researchers who concluded that some insecticides are of no serious genetic risk [1], while others insisted that the potential for damage is significant and suggested further testing [2-4]. The objective of the present study is to determine the potential of Benzoylaryl urea insecticide (Chitin synthesis inhibitor), chlorfluazuron known as I.K.I. on the mitotic process in treated onion root tips and germination of treated onion and *faba bean* seeds.

Materials and Methods

Bulbs of the common onion (*Allium cepa*, 2n=16) were used for studying the cytogenetical effects of chlorfluazuron. The bulbs were grown in tap water. Roots,

while intact, were treated with different concentrations of aqueous solution of I.K.I ranging from 0.00 ppm. to 50 ppm. for 4,24,48 and 72 hours. Roots of the control treatment were simultaneously treated with tap water. After each interval, the roots were cut and immediately fixed in acetic-alcohol (1:3) (v/v) for 24 hours. Cytogenetical investigations were carried out from permanant slides prepared by Feulgen squash technique [5]. Mitotic index was calculated for each treatment as the number of dividing cells/100 cells. Abnormalities were counted in the different mitotic stages. The effect of I.K.I on the seed germination rate of onion and *faba bean* seeds was carried out according to Habib *et al.* [6].

Chemical structure of I.K.I



N (2,6 diflorobenzoyl) N′ [2,6,2′-trichloro-4′ (trifloromethyl) diphenyl ether] urea.

Results and Discussion

Mitotic abnormalities induced by chlorfluazuron

Different types of mitotic abnormalities involving all mitotic stages were induced after treatment with I.K.I. The frequencies of these abnormalities varied with applied concentrations (Tables 1 and 2). In roots treated with high concentrations complete inhibition of cell division was noted. This inhibition may be due to the interference of the insecticide with the normal events of mitosis by reducing the number of cells entering it. Similar results were obtained with other pesticides [7;8].

Chromosomal aberations were the common type of abnormalities in prophase (Figs 1 and 2). In roots treated with high concentration (50 ppm/4hr), the abnormalities in metaphase stage were 100%. Chromosomal stickiness represents the most common type of metaphase disorder (Fig. 3), such stickiness was attributed to the process of depolymerization of DNA, which cause the chromosome surface to be sticky [9], as well as breakage and exchange of the basic folded fibre-unit of chromatids [10]. These results were in agreement with those obtained in earlier studies [11-13]. In addition, c-metaphase in which the chromosomes are scattered all over the cell represents a second type of metaphase abnormalities (Fig. 4).

Table 1. Number of total cells examined, total mitosis, different mitotic phases, mean of mitotic index and mean percentage of abnormal mitosis after treating *A. cepa* root with different concentrations of I.K.I for different periods.

Time of treatment hrs.	Conc. in p.p.m.	Total cells examined	Total mitoses	Prophase			Metaphase			Ana. & Telophase			Mitotic index seen	Mean. Aba. mitoses
				Nor.	Abn.	Total no.	Nor.	Abn.	Total no.	Nor.	Abn.	Total no.		
4	50.00	8593	5	0	0	0	0	2	2	0	3	3	0.06	0.06
	37.50	5848	16	7	0	7	2	3	5	1	3	4	0.27	0.01
	25.00	8058	83	12	0	12	23	11	34	25	12	37	1.03	0.28
	12.50	6404	70	3	2	5	27	3	30	25	10	35	1.09	0.23
	10.00	7126	126	45	1	46	27	15	42	20	18	38	1.77	0.34
	7.50	5069	119	49	1	50	18	20	38	11	20	31	2.35	0.81
	0.00	6533	143	36	0	36	44	0	44	63	0	63	2.19	0.00
24	12.50	6792	73	24	0	24	21	5	26	22	1	23	1.07	0.09
	10.00	7933	93	12	1	13	34	4	38	37	5	42	1.17	0.13
	7.50	6832	49	3	4	7	13	6	19	15	8	23	0.72	0.26
	5.00	7263	156	38	4	42	47	10	57	47	10	57	2.15	0.33
	0.00	8835	226	41	0	41	80	0	80	104	1	105	2.56	0.01
48	7.50	7854	63	7	1	8	11	11	22	23	10	33	0.80	0.28
	5.00	6506	150	39	2	41	40	7	47	48	14	62	2.31	0.35
	2.50	11075	223	23	8	31	67	18	85	92	15	107	2.01	0.37
	1.25	6948	187	41	2	43	53	8	61	79	4	83	2.69	0.20
	0.00	6978	182	67	0	67	45	1	46	69	0	69	2.61	0.01
72	7.50	7271	194	35	7	42	51	14	65	63	24	87	2.67	0.62
	5.00	6681	227	32	10	42	53	13	66	99	20	119	3.40	0.64
	2.50	8223	187	30	2	32	48	7	55	89	11	100	2.27	0.24
	1.25	6614	139	17	3	20	38	11	49	62	8	70	2.10	0.33
	0.00	8570	259	98	2	100	73	0	73	84	2	96	3.02	0.05

Table 2. Number of different types of metaphase and ana-telophase abnormalities after treating *A. cepa* root tips with different concentrations I.K.I for different periods

Time of Treatment hrs.	Conc. in p.p.m.	Metaphase abnormalities								Ana. & Telophase abnormalities								
		C.M. (4n)	C.M. (2n)	Star	Stick.	Mon. congr.	C.M. cond.	Abn.	Total	Chr. bridge	Tree chr.	Dist.	Diag.	Star	Multi-polar	Lagg. chr.	Abn.	Total
		no.	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.
4	50.00	–	–	–	2	–	–	–	2	–	–	3	–	–	–	–	–	3
	37.50	2	–	–	–	–	–	1	3	1	–	–	–	–	–	–	–	3
	25.00	–	–	1	9	1	–	–	11	–	1	–	–	–	–	–	11	12
	12.50	–	–	–	3	–	–	–	3	2	1	1	–	–	–	–	5	10
	10.00	8	–	2	2	3	–	–	15	2	1	1	3	3	1	–	4	18
	7.50	8	7	1	1	3	–	–	20	2	–	–	2	5	–	–	1	20
	0.00	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24	12.50	1	–	–	4	–	–	–	5	1	–	–	–	–	–	–	–	1
	10.00	1	–	–	–	3	–	–	4	–	1	1	–	–	1	–	2	5
	7.50	1	1	–	4	–	–	–	6	1	–	3	1	1	–	–	1	8
	5.00	4	1	–	1	4	–	–	10	2	1	3	–	1	1	1	–	10
	0.00	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1
48	7.50	1	–	–	5	3	2	–	11	–	2	2	–	2	–	–	–	10
	5.00	5	–	–	–	2	–	–	7	7	1	4	–	–	–	–	–	14
	2.50	5	–	–	4	5	3	1	18	4	–	8	–	2	–	–	–	15
	1.25	3	–	–	3	1	1	–	8	1	–	2	–	–	1	1	–	5
	0.00	1	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–
72	7.50	2	1	–	6	3	2	–	14	5	–	12	–	3	–	1	–	24
	5.00	5	–	–	2	5	–	1	13	7	2	1	–	–	–	2	2	20
	2.50	1	–	1	3	2	–	–	7	6	2	–	1	–	–	1	–	11
	1.25	2	–	1	1	4	2	1	11	3	1	1	–	–	1	1	–	8
	0.00	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	–	2

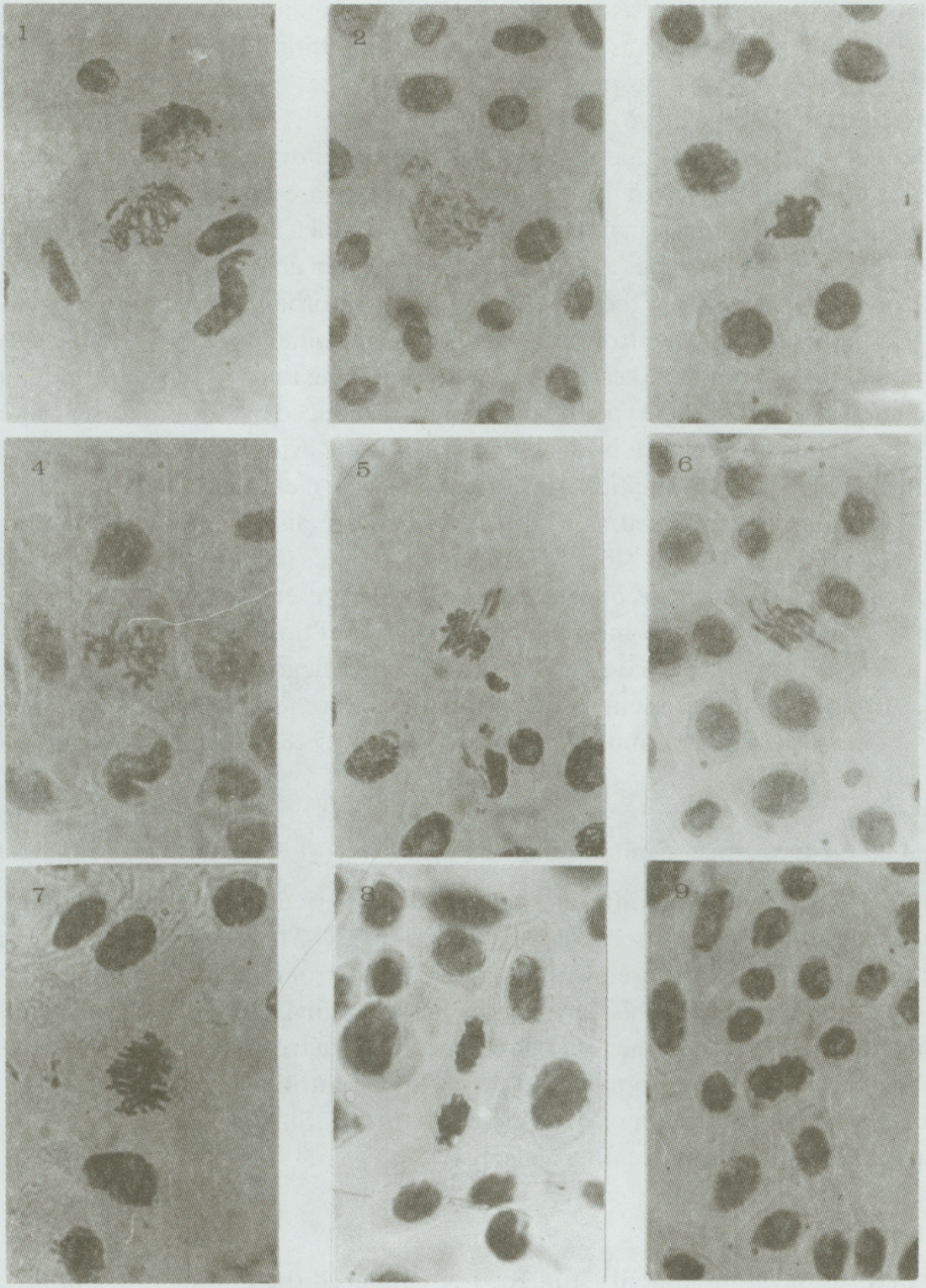


Plate (1). Effect of different I.K.I. concentration on the mitotic cell division of *A. Cepa* root tip cells

Fig. 1.	**2.5**	**p.p.m.**	**for 48 hrs**	**Figs 5 & 6.**	**2.5**	**p.p.m.**	**for 48 hrs**
Fig. 2.	**.**	**p.p.m.**	**for hrs**	**Fig. 7.**	**1.25**	**p.p.m.**	**for 72 hrs**
Fig. 3.	**1.25**	**p.p.m.**	**for 72 hrs**	**Fig. 8.**	**1.25**	**p.p.m.**	**for 48 hrs**
Fig. 4.	**5**	**p.p.m.**	**for 72 hrs**	**Fig. 9.**	**1.25**	**p.p.m.**	**for 72 hrs**

C-metaphase was produced as a result of inhibition of spindle fibre formation. Such an arrest may be one of the causes of mitotic index inhibition [14]. Non congression in which unoriented chromosome fails to reach the equatorial plate (Fig. 5), chromosomal break (Fig. 6) and metaphase tetrads (Fig. 7) are also among the metaphase abnormalities that appeared as a result of different I.K.I concentration and during various phases of the treatments. In anaphase and telophase stages different types of induced abnormalities were observed which include: (i) sticky anaphase and telophase chromosome (Figs 8 & 9), (ii) disturbed anaphase where the chromosomes spread irregularly in the cell due to spindle disturbance (Fig. 10), (iii) c-anaphase (Fig. 11) where the centromere of each chromosome failed to reach the specific cell pole, and (iv) polyploid cell as a result of doubling the chromosome number for the c-anaphase cell. Other types of abnormalities induced by the insecticide were the chromatid and chromosome bridges (Figs 12-14), the bridge may be due to the general stickiness of the chromatids and chromosomes at metaphase stage and in certain cases also may be produced as a result of breakage and reunion [13], multipolar anaphase (Fig. 15), lagging (Fig. 16), chromatid breaks (Figs 17 & 18), chromosome fragments & micronuclei at different stages in the present study indicate that I.K.I has the ability to cause chromosome breakage in root tips of *A. cepa,* star anaphase (Fig. 19), abnormal anaphase (Figs 20 & 21), and diagonal telophase (Fig. 22) as well. Among the interphase nuclei abnormalities were micronucleus (Fig. 23), vacuolated nucleus (Fig. 24), multinucleate cells (Figs 25 & 26), and restitution polyploid nucleus (Fig. 27).

Effect on the frequency of mitotic stages

The frequency of the different mitotic stages varied according to the duration and concentration of the insecticide (Table 1 & Figs 28-31). The frequency of prophase and ana-telophase differed without a clear trend from that of the control in the 4 and 24 hours treatments while significantly reduced in 48 and 72 hours treatments. Metaphase frequency, on the other hand, increased in treated roots during all treatments. These changes in frequency of the different mitotic stages indicate that I.K.I affects the relative duration of each stage differently. Similar results were obtained using other pesticides for treating cells [8;13;15].

Effect on mitotic index

In most of the treatments studied, I.K.I reduced the mitotic index in *A. cepa* roots. However, there was a slight increase in the index in roots treated with lower concentrations for the 4,48 and 72 hours durations as compared to the control (Fig. 32). These were in harmony with those reported by other researchers using other pesticides [8;13;16;17]. The reduction in the mitotic activity could be partially due to

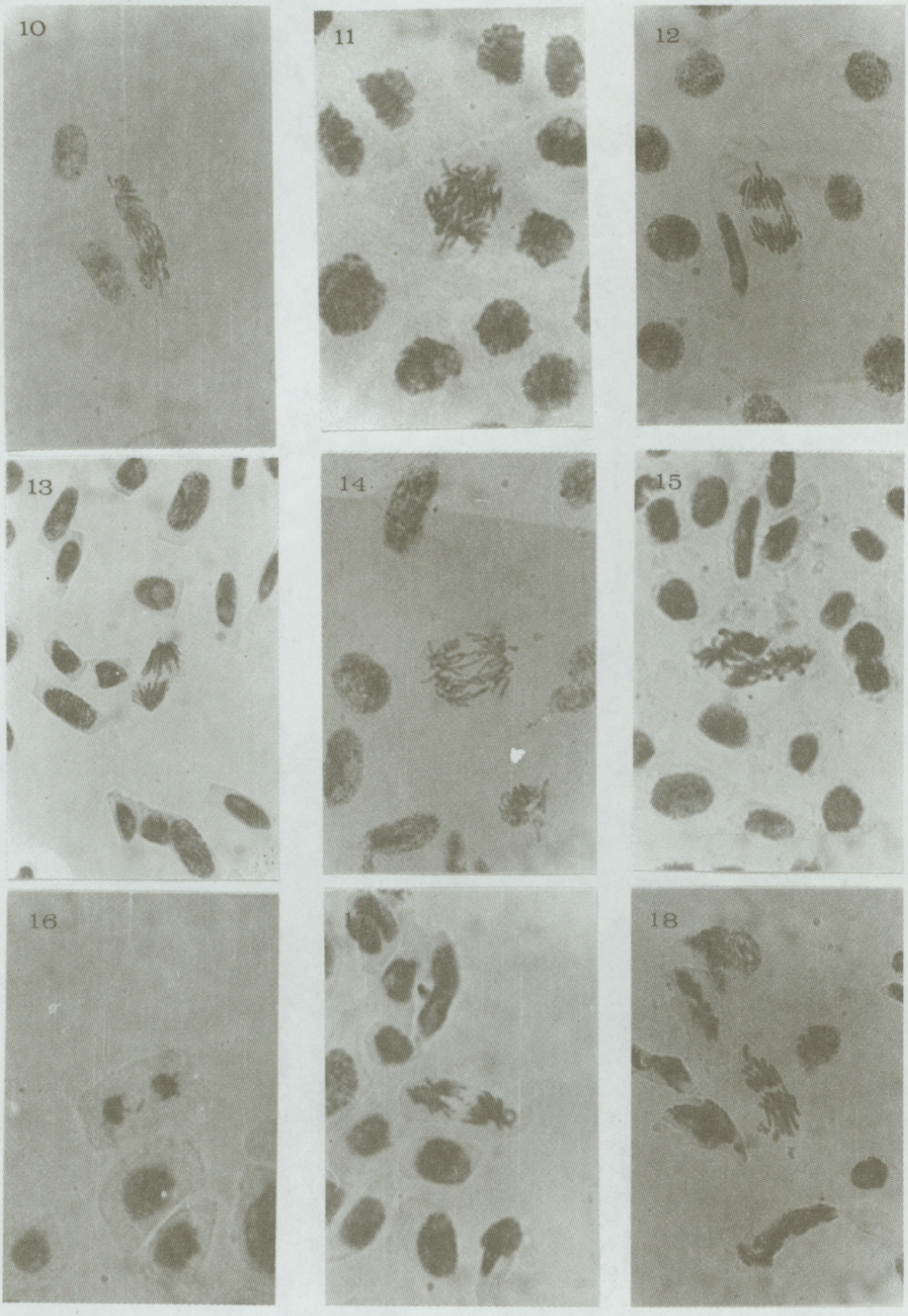


Plate (2). Effect of different I.K.I. concentration on the mitotic cell division of *A. cepa* root tip cells

Fig. 10.	**2.5**	**p.p.m. for 48 hrs**	**Figs 14.**	**1.25**	**p.p.m. for 48 hrs**
Fig. 11.	**5**	**p.p.m. for 72 hrs**	**Figs 15,16 & 17.**	**1.25**	**p.p.m. for 72 hrs**
Fig. 12 & 13.	**2.5**	**p.p.m. for 48 hrs**	**Fig. 18.**	**2.5**	**p.p.m. for 48 hrs**

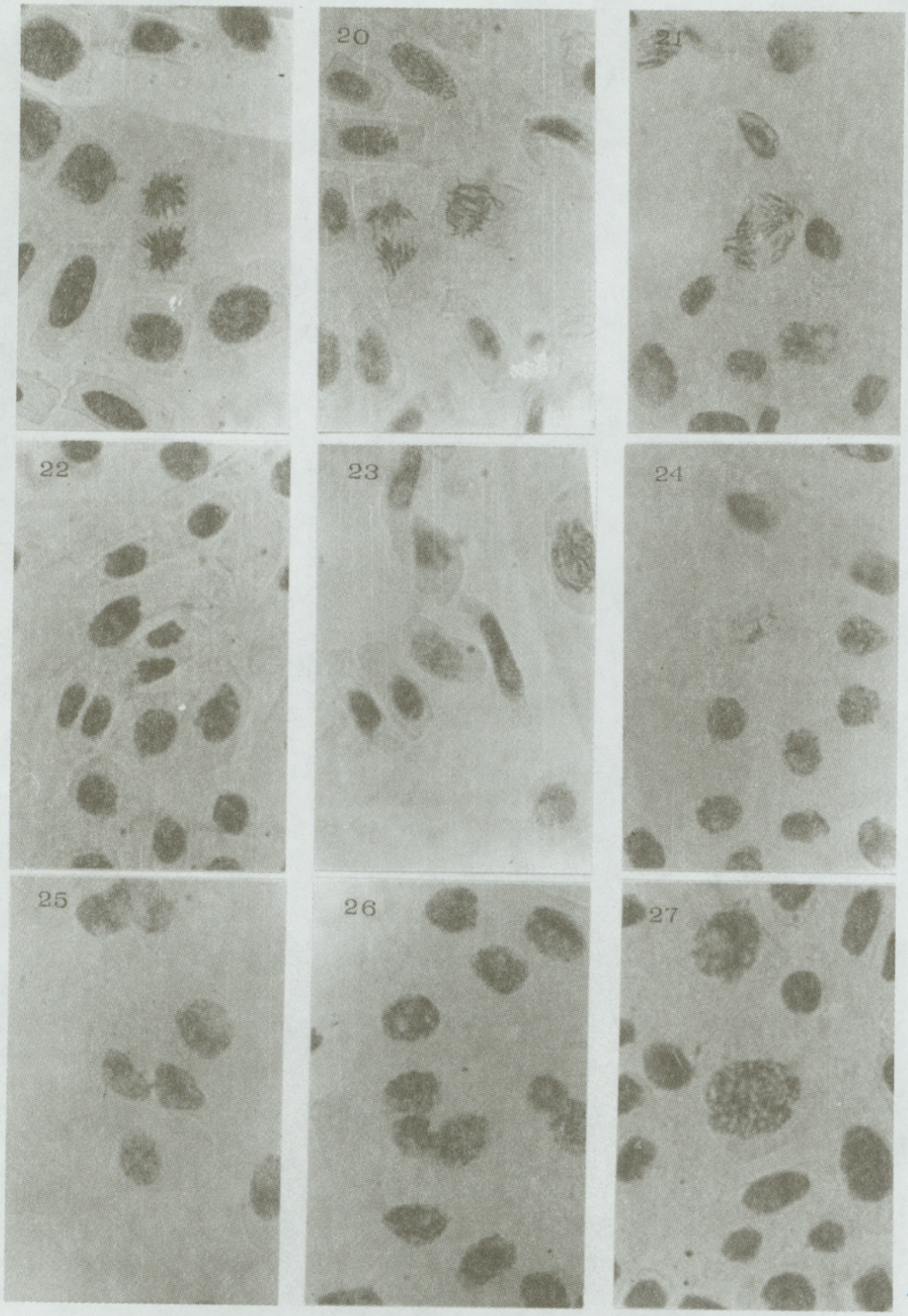


Plate (3). Effect of different I.K.I. concentration on the other mitotic cell divsion of *A. cepa* root tip cell

Figs 19 & 20.	**1.25 p.p.m. for 48 hrs**	**Fig. 25.**	**2.5 p.p.m. for 48 hrs**
Fig. 21.	**2.5 p.p.m. for 48 hrs**	**Fig. 26.**	**1.25 p.p.m. for 24 hrs**
Fig. 22.	**1.25 p.p.m. for 72 hrs**	**Fig. 27.**	**1.25 p.p.m. for 72 hrs**
Figs 23 & 24.	**1.25 p.p.m. for 48 hrs**		

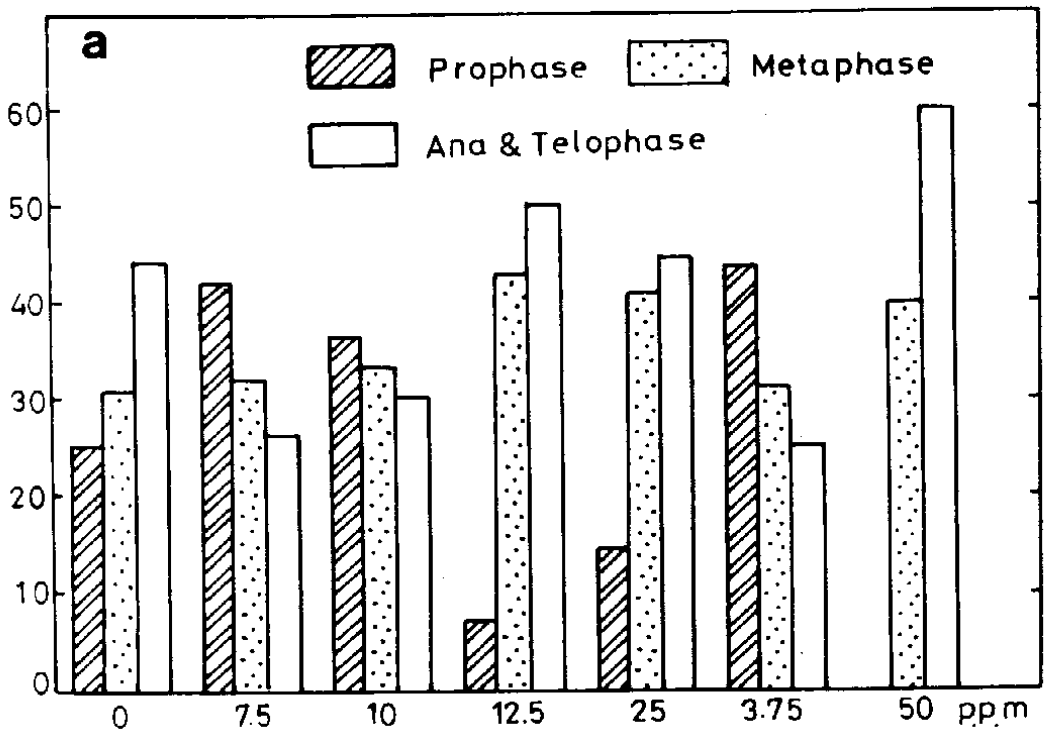


Fig. 28. Frequencies of mitotic phase after treating *A. cepa* root tips with different concentrations of chlorfluazuron (IKI) for 4 hrs.

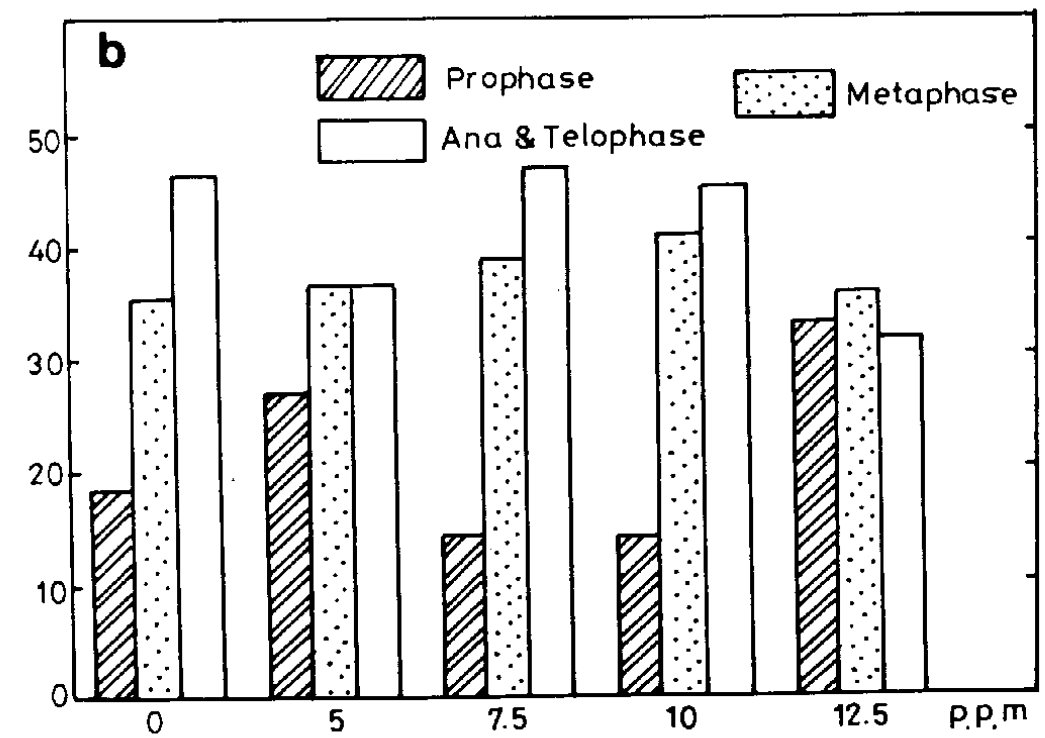


Fig. 29. Frequencies of mitotic phases after treating *A. cepa* root tips with different concentrations of chlorfluazuron (IKI) for 24 hrs.

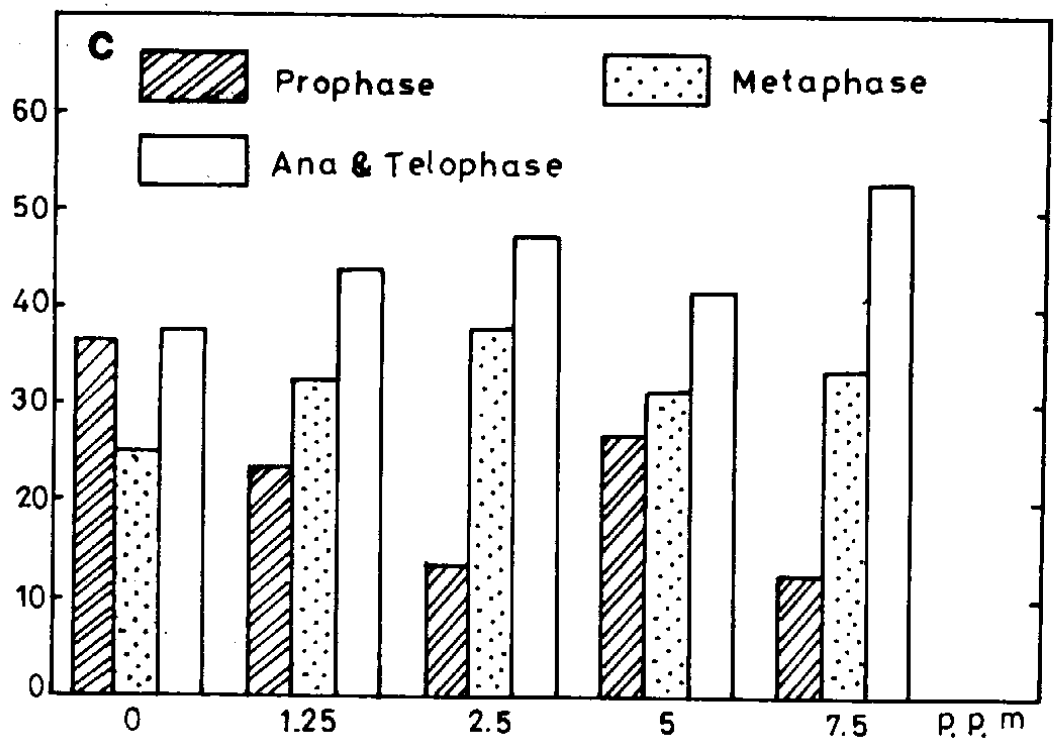


Fig. 30. Frequencies of mitotic phases after treating *A. cepa* root tips with different concentrations of chlorfluzuron (IKI) for 48 hrs.

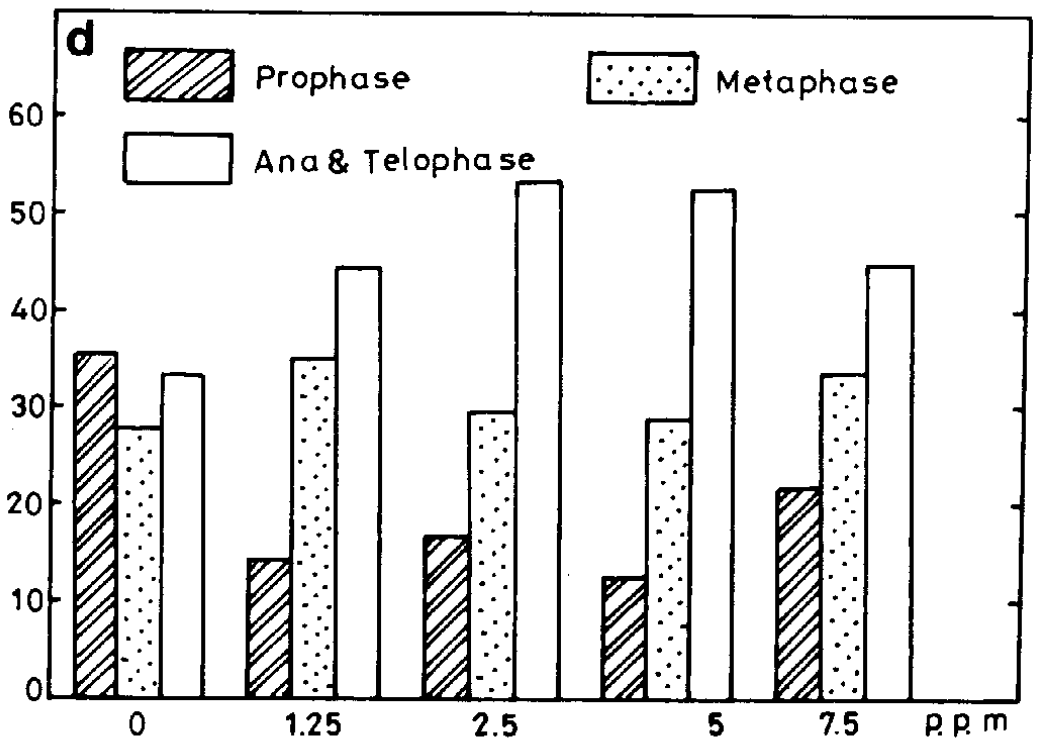


Fig. 31. Frequencies of mitotic phase after treating *A. cepa* root tips with different concentrations of chlorfluzuron (IKI) for 72 hrs.

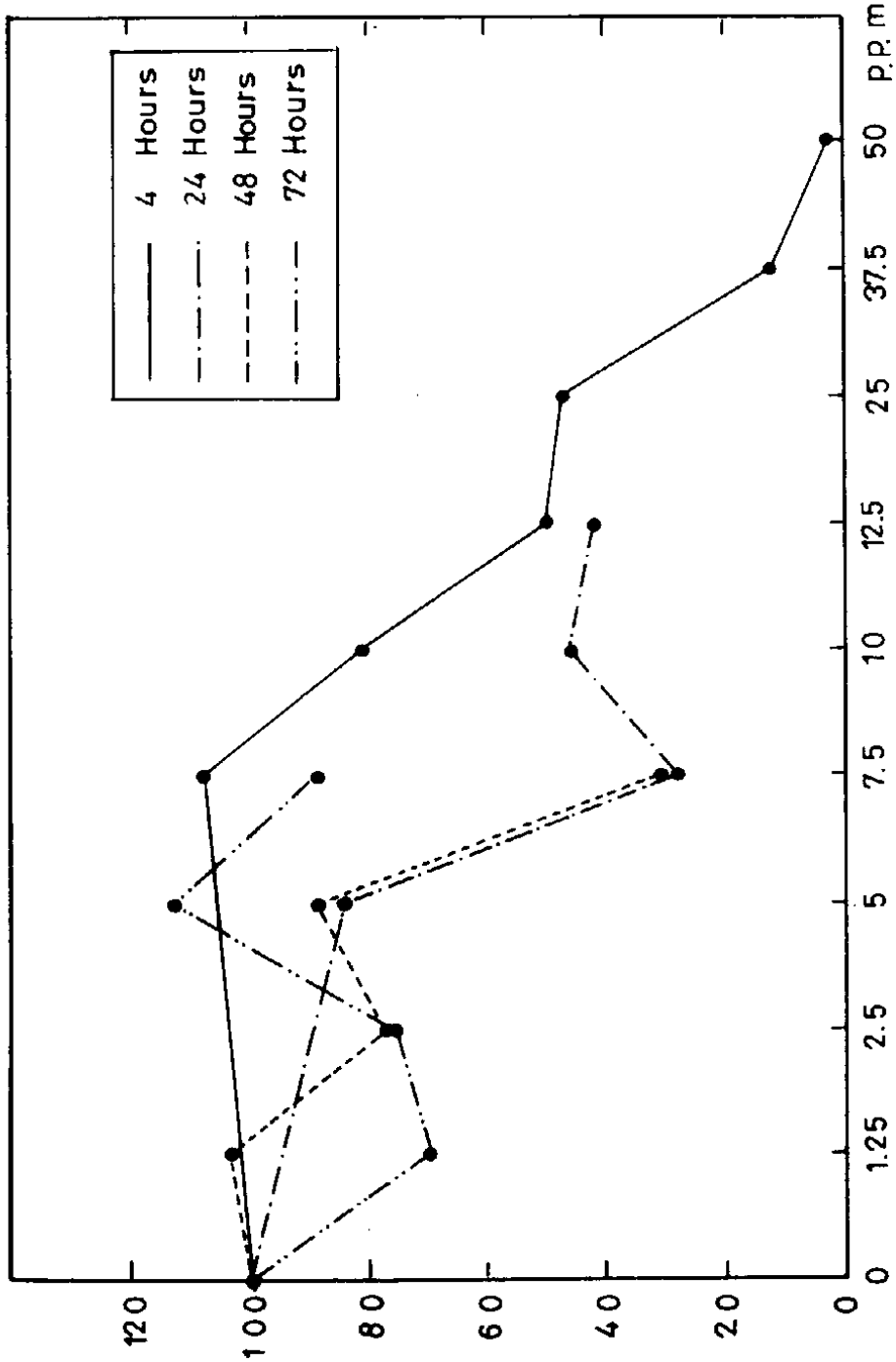


Fig. 32. Mitotic index difference after treating *A. cepa* root tip cells with different concentrations of chlorfluazuron (IKI) for different periods

inhibition of DNA synthesis [18]. In support to this conclusion Beu *et al.* [19] showed that exposing the root tips of *V. faba* to high concentrations of the herbicide "paraquate" led to inhibition of DNA synthesis.

Effect on the germination rate of *A. cepa* and *V. faba seeds*

Data presented in Table 3 and Figs 33-38, show the effects of insecticide concentrations as well as duration on the germination rate of *A. cepa* and *V. faba*. The relation between the concentration of I.K.I and the germination percentage is shown in Fig. 39 where the reduction in germination rate in case of *A. cepa* is much more as compared to that of *V. faba*. The same results were in agreement with those obtained by Habib *et al.* [6] using the fungicide (bupirimate). They attributed their findings to the difference in the effective dose needed for onion and *faba bean*. Similar results were obtained by using ammonium salt of 2,4 D for pea seeds [20] and Lindane insecticide on clover and beam seedlings [21].

In conclusion, it was found out that there were consistent reductions in both germination rate and mitotic index as a result of using the insecticide I.K.I especially for longer durations and at high concentrations.

Table 3. The percentage of germination (after one week) of *A. cepa* and *V. faba* seeds treated with different concentrations of I.K.I. for 4,24 and 48 hours

Con. (p.p.m)	*A. cepa*			*V. faba*		
	4 hrs	24 hrs	48 hrs	4 hrs	24 hrs	48 hrs
50	38.00	32.00	12.00	98.00	94.00	86.00
37.50	46.00	42.00	32.00	100.00	100.00	96.00
25.00	52.00	32.00	36.00	96.00	98.00	100.00
12.50	48.00	38.00	46.00	96.00	100.00	100.00
10.00	48.00	54.00	48.00	96.00	100.00	100.00
7.50	42.00	42.00	42.00	100.00	100.00	96.00
5.00	84.00	62.00	46.00	96.00	100.00	100.00
2.50	90.00	62.00	42.00	100.00	100.00	100.00
1.25	74.00	58.00	54.00	100.00	100.00	100.00
0.00	100.00	100.00	100.00	100.00	100.00	100.00

Fig. 33. Effect of I.K.I on the germination of *A. cepa* for 4 hrs treatments. The concentration used were:0,5,10,37.5 and 50 p.p.m. respectively.

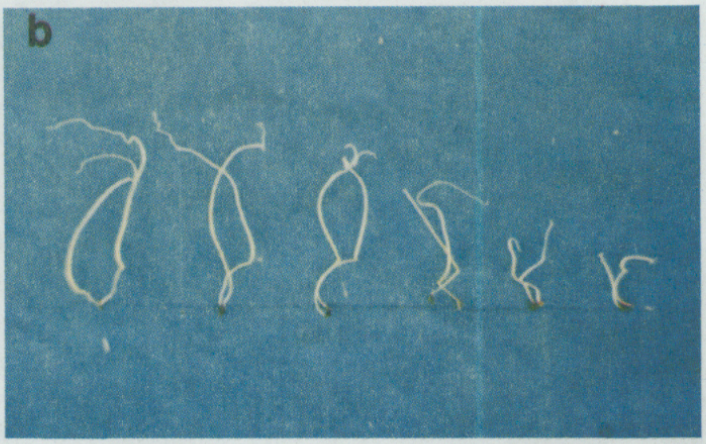


Fig. 34. Effect of I.K.I on the germination of *A. cepa* for 24 hrs treatments. The concentration used were:0,5,10,37.5 and 50 p.p.m. respectively.

Fig. 35. Effect of I.K.I on the germination of *A. cepa* for 48 hrs treatments. The concentration used were:0,5,10,37.5 and 50 p.p.m. respectively.

Fig. 36. Effect of I.K.I on the germination of *V. faba.* for 4 hrs treatments. The concentration used were:0,10,25,37.5 and 50 p.p.m. respectively.

Fig. 37. Effect of I.K.I on the germination of *V. faba.* for 24 hrs treatments. The concentration used were:0,5,10,25, 37.5 and 50 p.p.m. respectively.

Fig. 38. Effect of I.K.I on the germination of *V. faba.* for 48 hrs treatments. The concentration used were:0,5,10,25, 37.5 and 50 p.p.m. respectively.

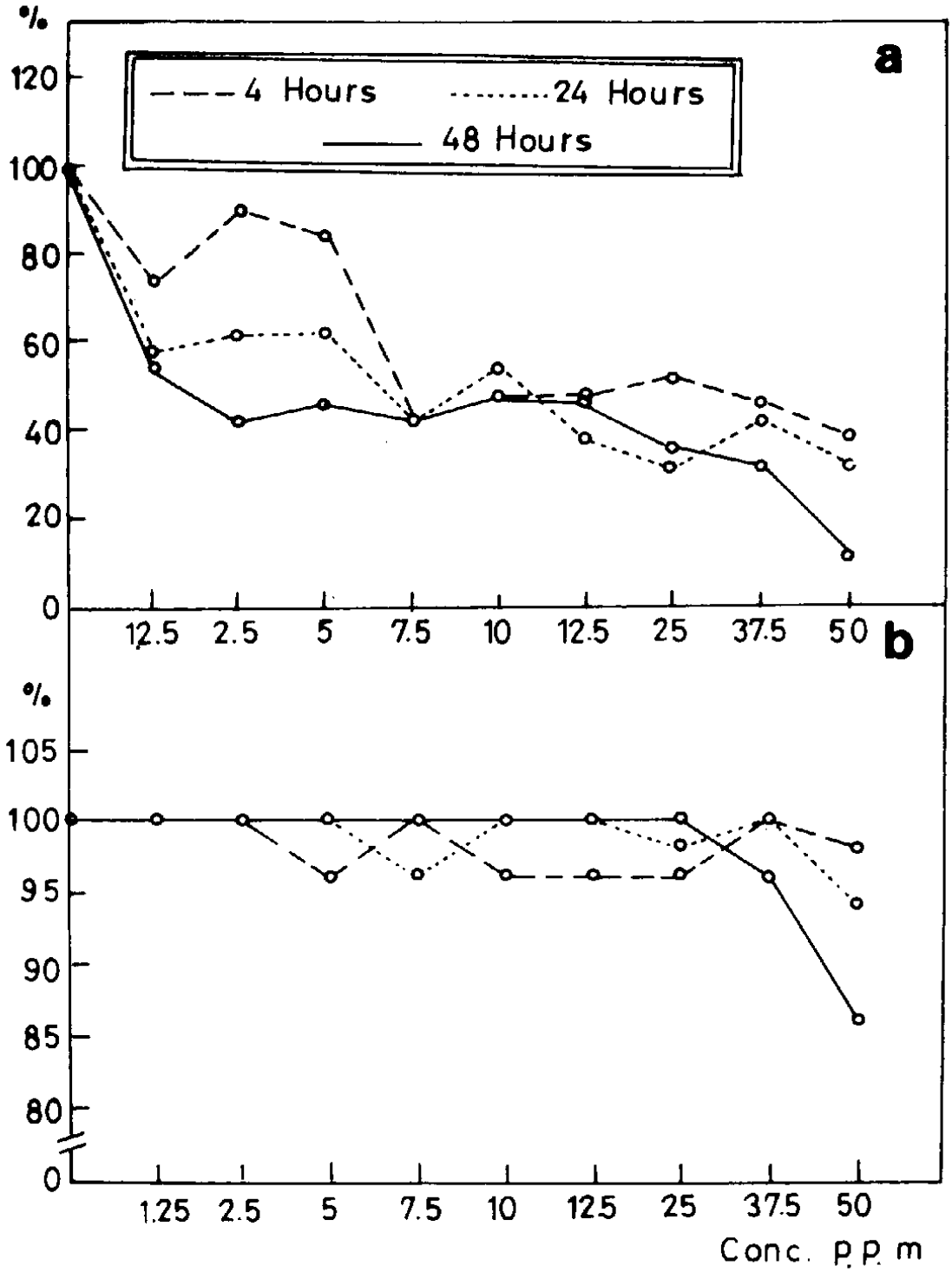


Fig. 39. Effect of chlorfluazuron (IKI) on the germination of: a) *A. cepa* and b) *V. faba* seeds for 4,24 and 48 hours.

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تأثير المبيد الحشري «كلورفلوازورون» (I.K.I) على معدّل الانقسام الخلوي غير المباشر في النبات وعلى معدّل الإنبات في نباتي البصل والفول

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ملخص البحث. اختبر تأثير المبيد الحشري «ذات التأثير المثبط لتكوين الكيتين الحشري والمعروف بمادة كلورفلوازورون (I. K. I)» على الانقسام الخلوي غير المباشر (الميتوزي) لخلايا القمم النامية في جذور نبات البصل، بالإضافة إلى تأثيره على معدّل إنبات بذور نباتي البصل والفول.

دلّت النتائج على أنّ للمبيد المستخدم المقدرة على إحداث بعض التغيّرات في الانقسام الخلوي غير المباشر تتراوح ما بين تثبيط معدّل هذا الانقسام الخلوي وإنتاج بعض الشذوذ في مراحله المختلفة بالإضافة إلى وجود علاقة بين نقص معدّل إنبات بذور نباتي البصل والفول وانخفاض معدّل الانقسام الخلوي غير المباشر (الميتوزي) خاصةً عند استخدام التركيزات المرتفعة من المبيد المستخدم.