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A COMPARATIVE STUDY ON THE EFFECT OF THE GROWTH RETARDANT CYCOCEL ON MITOSIS OF Pisum sativum L. and Vigna unguiculata L.

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ABSTRACT

The effect of the growth retardant "Cycocel" has been studied on root-mitosis of Pisum sativum and Vigna unguiculata. The root-tips were treated with 25, 50, 100, 250, 500, 1000, and 2000 ppm of the growth retardant for 24 hours.

The growth retardant "Cycocel" showed an obvious effect on the mitotic activity and caused a reduction in the mitotic index values of both plants. The frequencies of different mitotic stages in the treated roots in both plants were changed than those in untreated roots due to the accumulation of the metaphase stage.

Cycocel treatments induced several types of mitotic abnormalities. The total percentage of abnormalities was found to be time and concentration dependent. The percentage of abnormalities in Pisum was higher than that in Vigna. C-metaphase was the most frequent abno-

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rmal type in Pisum but chromosome stickiness was the main dominant type of abnormalities in Vigna root-tip cells. Other types of abnormalities were recoreded in Pisum, e.g. stickiness, bridges, lagging chromosomes, chromosome fragments, C-anaphase and disturbed anaphase. Also, very few interphase cells with micronucleus were observed. In Vigna, the other induced types of abnormalities were C-metaphase, lagging chromosomes, unequal chromosomes distribution and disturbed anaphase. Also, irregular prophase, vacuolation and chromosome fragments were observed in very low percentages.

INTRODUCTION

The growth retarding chemical (cycocel) 2-chloroethyl trimethyl ammonium chloride was found to be have a retarding effects on the growth and metabolism of a number of plants (Thomas, 1964; El-Sharaawi, 1976; Wareing and Phillips, 1976 and Farghal, 1990). Similar to other growth retarding chemicals e.g. alar, Amo-1618 and phosphon, the most obvious effect of cycocel is shortening of plant heigh or inhibiting shoot growth (Sachs, 1961 and Cathey, 1964; El-Khodary, 1972 ; Wareing and Phillips, 1976). Moreover, Sachs and Kofranek (1963) reported that cycocel and other growth retardants (Amo-1618 and Phosphon) have a direct inhibiting effects on nuclear cell division in the subapical meristems of Chrysanthemum. Later on, Kabarity and El-Khodary (1974 a; b) studied the effect of different concentrations of cycocel on V. faba root-tips. They concluded that the cycocel caused a reduction in the mitotic index and induced several types of chromosomal abnormalities.

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The cytological effects of cycocel on the pea and cowpea plants have not been investigated. The purpose of the present study, is to investigate the behaviour of the root-mitosis in both plants following treatment with different concentrations of cycocel.

MATERIALS AND METHODS

Seeds of two plants (Pisum sativum L., "var. vectory freezer", and Vigna unguiculata L. Walp) and the growth retardant "cycocel" were used in the present study. The growth retardant was dissolved in distilled water and the applied concentrations were, 25, 50, 100, 250, 500, 1000 and 2000 ppm.

Seeds were immersed in water for 24 hours, then germinated on moistened filter paper in large petri dishes. Young seedlings with root 3 cm in length were transferred to other dishes containing the used concentrations of cycocel for 24 hours. After the treatment, seedlings were washed throughly in water and root-tips were cut and immediately fixed in freshly prepared glacial acetic acid and absolute ethyl alcohol (1:3 v/v) overnight. Another group of young seedlings was treated with distilled water as control. Germination of the seeds and treatment of the young seedlings were carried out at room temperature (23-25 °C).

For each concentration, examinations were made on 5 roots in permanent root-tip squash preparations stained by the Feulgen technique. Mitotic index was estimated as the number of dividing cells to all counted cells. Chromosomal abnormalities percentage and types were determined in the different mitotic phases and in interphase.

RESULTS AND DISCUSSION

Results given in Tables 1 and 2 show that all treatments of the used growth retardant "cycocel" caused a reduction in the mitotic index values in root-tips of Pisum sativum and Vigna unguiculata. Such reduction was increased with increased cycocel concentration. This was clearly noticed when roots treated with the highest cycocel concentration of 2000 ppm. In both plants, pea and cowpea, MI reduction was about 50% or less of the respective control values at concentrations more than 50 ppm. Minimum MI values of 1.12% and 0.88% were recorded in pea and cowpea, respectively, at the 2000 ppm compared to the control values of 6.61% and 5.78%, respectively. A marked depression in the mitotic index was previously obtained following treatment of Vicia faba root-tips with different concentrations of CCC ranging from 800 to 12000 ppm and applied for 4 & 24 hours (Kabarity and El-Khodary, 1974a). Also, These authors noticed that the treatment for 12 hrs showed a similar trend in all the applied concentrations except the lowest two (800 & 1200 ppm CCC). The inhibitory effect of cycocel and some other growth retardants

on the subapical cell division in Chrysanthemun had been observed by Sachs and Kofranek (1963). Our results also agree with that obtained by El-Sharaawi (1976) on wheat plants. He observed a reduction in stem length and suggested that cycocel decreased or retarded cell division in subapical meristems. The reduction in the mitotic indices of pea and cowpea was also observed after treatment with the growth retardant B-9 (El-Ghamery and Abd Al-Moaty, 1991). Similarly, El-Khodary (1980) reported that high dosage of B-995 caused a reduction in the mitotic index of Allium cepa root-tips. She suggested that the decrease in the value of the indices could be mainly attributed to a corresponding decrease in the prophase. Similarly our results could be attributed to the accumulation or arrest of cell division at the metaphase since high percentage of abnormal cells was recorded at this stage (Tables 3 & 4). Also, the reduction of division rate in this study, could be produced either by prolonging the time of mitotic cycle as a whole, or through the permanent inhibition of mitosis of some cells. This is in accordance with the results recorded by El-Sadek and Ashour (1983).

Results in Table 1 also reveal that the percentages of the different mitotic stages in pea root-tip cells as compared to the controls show no appreciable changes at the lower concentrations. Increasing the concentrations directly decreased prophase frequency and increased metaphase and

ana-telophase frequencies. A positive correlation was observed between the percentage of metaphase and the applied concentrations of the cycocel. On the other hand, the percentages of ana-telophase fluctuated with the concentrations. At 2000 ppm, prophase frequency reached a minimum value of 40.60% compared to control value of 66.20%. On contrast the metaphase and ana-telophase frequencies reached a miximum values of 31.20% and 18.20%, respectively (see Table 1).

The data in Table 2 also show a decrease in the percentage of prophase and ana-telophase in cowpea root-tip cells with all treatments of the cycocel. No correlation was observed between decreasing the percentage of prophase and ana-telophase and increasing the applied concentration of cycocel. A minimum percentage of prophase and ana-telophase frequencies of 43.65% and 18.50% was found in the concentration of 250 ppm of cycocel. The concentration of 500 ppm of cycocel showed the highest frequency of metaphase (38.20%) compared to a control value of 24.14%. Similar results obtained after the treating Vicia faba roots with different concentrations of cycocel (Kabarity and El-Khodary, 1974a), treating Allium cepa roots with B-995 (El-Khodary, 1980) and treating pea and cowpea with B-9 (El-Ghamery and Abd Al-Moaty, 1991).

The percentage of total chromosomal abnormalities recorded in treated root-tips of pea and cowpea plants

increased as the concentration increased with one exception the highest concentration (2000 ppm) (Tables 3 & 4). At all concentrations, the percentage of total abnormalities in pea root-tips was higher than that in cowpea. The minimum values of total chromosomal abnormalities in both plants, pea and cowpea, were 32.83% and 13.7% respectively, and were recorded after the treatment with 25 ppm of cycocel. As the concentration increased to 1000 ppm, the total percentage of abnormalities reached a value of 71.18% and 47.91% in pea and cowpea, respectively. In this respect, the effect of cycocel on both plants simulates its effect on Vicia faba (Kabarity and El-Khodary, 1974a & b) and the effect of B-995 on Allium cepa roots-tips (El-Khodary 1980) and B-9 on pea and cowpea plants (El-Ghamery and Abd Al-Moaty, 1991).

Cycocel induced different types of abnormalities at all stages of division. The most conspicuous was at metaphase and anaphase stages. The percentage of abnormalities at metaphase increased with increasing growth retardant concentration, and this was higher than that at ana-telophases, at all applied concentrations. The percentage of each type of abnormalities differed in relaion to the applied concentration of cycocel in both plants. In cowpea, few interphase cells with vacuolated nucleus and irregular prophase configurations were observed with the treatment of 1000 and 2000 ppm of cycocel. In pea, micronucleated cells were induced at interphase following treatment with cycocel concentrations higher than 500 ppm

(Fig. 6). C-metaphase (Fig. 2) was the most frequent type of abnormalities in pea, but chromosome stickiness (Fig. 7) was the most common type in cowpea. Induction of chromosome fragmentation was recorded with concentrations less than 100 ppm in cowpea, on the contrary with the concentrations more than 500 ppm in pea. The frequency of chromosome stickiness (Fig. 7), C-metaphase (Fig. 8) and lagging (Fig. 9) in cowpea root-tips were higher than those in pea root-tips (Figs 1,2 & 3), in contrast to the chromosome disturbance including multipolar anaphase and disturbed anaphase (Figs 4 & 10). Other types of abnormalities were also observed in pea with all applied concentrations. These are chromosome bridges (Fig. 5) and C-anaphase.

One of the major type of abnormalities was chromosomal stickiness. Such stickiness was mostly evident at prophase and metaphase in both pea and cowpea plants. Stickiness frequency increased with increasing concentration. Darlington and Mc Leich (1951) and La Cour and Rutishausen (1954) have suggested that chromosome stickiness could be due to a polymerisation of DNA which made the chromosome surface to be sticky. The occurrence of chromosome stickiness was previously reported after treating V. faba roots with a relatively high concentrations (8000 ppm) of cycocel (Kabarity and El-Khodary, 1974a) and treating Allium cepa with a high dose (5000 ppm) of B-995 (El-Khodary, 1980).

These authors concluded that high doses of the growth retardants increase the viscosity of the protoplasm. In this study, the induction of this anomaly that was recorded at all applied concentrations could be due to the effect of cycocel on DNA protein cross-links (El-Sadek and Ashour, 1983; Ashour, 1988).

C-metaphase configuration was another frequent abnormality at metaphase. This type was approximately similar to that induced by colchicine treatment (Levan, 1938 & 1956). A partial effect of the growth retardant on spindle fibers led to the formation of C-anaphase, disturbance of the spindle fibers and the distribution of the chromosomes complement to more than two poles resulting in the formation of multipolar anaphase cells. El-Khodary (1972) concluded that cycocel disturbed the extra-chromosomal mechanism. This disturbance is restricted to the abnormal diffusion of the spindle organisms in many directions insteat two poles. Similar types were observed by Therman and Timonen (1950), Kabarity (1966), Kabarity & El-Khodary (1974a), El-Khodary (1980) & El-Ghamery & Abd Al-Moaty (1991).

Irregular prophase was recorded in few cells of cowpea plants and might be attributed to the irregular break of nuclear membrane. Similar type was observed by Kabarity (1966), Shehab (1979 & 1980) and El-Ghamery & Abd Al-Moaty (1991).

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Anaphase bridges induced in cowpea after cycocel treatments involved one or more chromosomes. Chromosomal bridges at anaphase may be due to the general stickiness of chromosomes. These results were similar to those obtained by Kabarity (1969), Gudkova et al., (1970), Kabarity & El-Khodary (1974a) and El-Khodary (1980). The presence of acentric fragments may point out that all bridges are not due to chromosome stickiness. Consequenlty some of the chromosomal bridges could be explained on the basis of chromosome breakage and reunion (Tomkins and Grant, 1972).

Lagging chromosomes and micronuclei were observed in some treatments of cycocel in both plants. Micronuclei observed in interphase cells may be attributed to lagging chromosomes or chromosome fragments. Both types (lagging chromosome and micronuclei) may be formed as a results of disturbances. The same results were obtained by Kabarity and El-Khodary (1974b).

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Table 1. Mitotic index and the percentage of the mitotic stages in Pisum sativum root-tips after treatment for 24 hours with 7 concentrations of the growth retardant "Cycocel".

Concen-tration (ppm)	count cells	Divid. cells	MI	Control %	Mitotic stages		
					Prophase %	Metaphase %	Ana-telo-phase %
Cont.	1800	119	6.61	100.00	66.50	21.15	12.35
25	4000	201	5.00	75.64	67.30	22.40	10.30
50	3666	123	3.35	50.68	67.70	22.90	9.40
100	4400	116	2.63	39.78	67.15	23.10	9.75
250	4400	130	2.50	37.82	64.10	25.60	10.30
500	5166	114	2.07	31.31	60.45	26.40	13.15
1000	5500	59	1.50	22.69	55.50	30.40	14.10
2000	3100	35	1.12	16.94	40.60	31.20	18.20

Table 2. Mitotic index and the percentage of the mitotic stages in V.unguiculata root-tips after treatment for 24 hours with 7 concentrations of the growth retardant "Cycocel".

Concen-tration (ppm)	count cells	Divid. cells	MI	Control %	Mitotic stages		
					Prophase %	Metaphase %	Ana-telo-phase %
Cont.	4000	250	5.75	100.00	55.19	24.14	23.77
25	3600	149	4.05	70.43	53.30	26.40	20.30
50	3666	95	2.59	45.04	50.25	27.45	22.20
100	4333	66	1.98	34.43	50.90	28.10	21.00
250	3730	67	1.79	31.13	43.65	35.85	18.50
500	4800	73	1.52	26.43	44.40	38.20	19.15
1000	4133	48	1.16	20.17	46.55	33.00	20.45
2000	4666	41	0.88	13.30	45.10	33.60	21.30

TABLE 3. Percentages of the different types of abnormalities in P. sativum roots, after root-treatments with the growth retardant "cycocel" for 24 hours.

Concen-tration (ppm)	Mitoses			Percentages of the different types of abnormalities relating to the number of abnormal mitoses.							
	divid. cells	abn. cells	% abn.	Stick	C-meta	Brid	Laq.	C-ana	Dist ana.	Frag.	Micr.
Cont.	119	-	-	-	-	-	-	-	-	-	-
25	201	66	32.83	4.32	20.00	1.64	0.50	5.62	0.74	-	-
50	123	40	32.50	4.32	17.00	1.63	0.55	7.00	1.10	-	-
100	116	48	41.37	7.30	19.50	3.40	-	9.40	1.70	-	-
250	130	59	45.60	10.00	20.46	3.30	0.76	9.30	0.76	1.00	-
500	114	57	50.00	12.00	18.46	6.42	0.87	7.30	2.33	1.75	0.87
1000	59	42	71.18	16.40	30.50	6.77	-	10.16	3.10	2.20	2.00
2000	35	24	70.50	15.50	24.60	11.60	-	11.60	2.90	2.30	2.00

TABLE 4. Percentages of the different types of abnormalities in V. unguiculata roots, after root-treatments with the growth retardant "cycocel" for 24 hours.

Concen-tration (ppm)	Mitoses			Percentages of the different types of abnormalities relating to the number of abnormal mitoses.							
	divid. cells	abn. cells	% abn.	Stick	C-meta	Brid	Laq.	C-ana	Dist ana.	Multi polar	Frag.
Cont.	250	-	-	-	-	-	-	-	-	-	-
25	149	20	13.70	-	-	11.60	-	0.68	0.68	-	0.45
50	95	26	27.36	-	-	20.56	1.05	3.25	0.35	1.25	1.41
100	66	21	31.82	-	-	20.59	2.50	4.54	1.00	2.00	1.00
250	67	23	34.32	-	-	26.62	2.50	2.00	0.50	2.50	-
500	73	30	41.90	-	-	34.84	2.21	2.73	-	0.90	-
1000	59	23	47.91	0.68	0.69	38.18	1.37	3.45	0.68	2.10	-
2000	35	15	36.58	1.62	0.81	25.00	1.62	5.72	-	0.81	-

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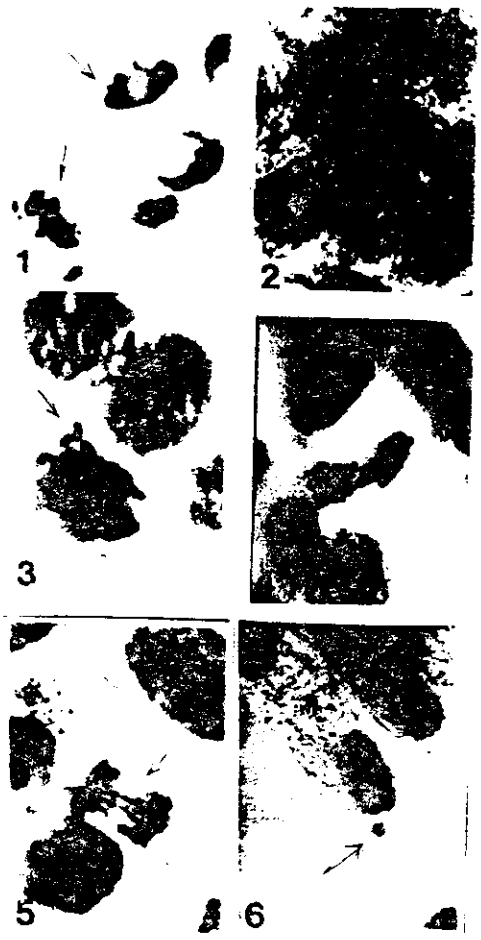

Figs 1-6. Different types of abnormalities induced in *Pisum sativum* root-tips cells after treatment with different concentrations of cycocel for 24 hours.
Fig. 1. Chromosome stickiness at prophase and metaphase.
Fig. 2. C-metaphase. Fig. 3. Chromosome lagging.
Fig. 4. Diaggonal anaphase. Fig. 5. Double chromosomal bridges.
Fig. 6. Interphase cell with micronucleus.

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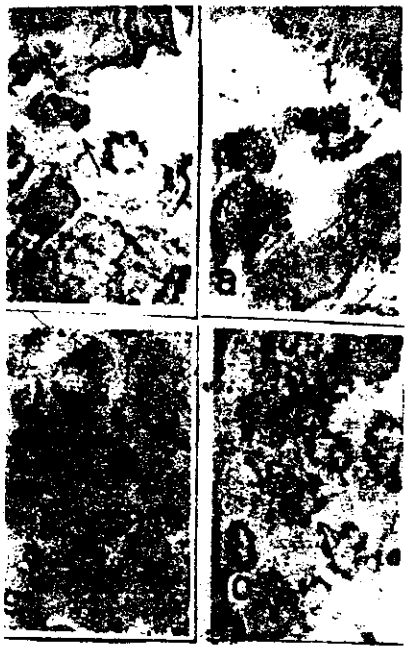

Figs 7-10. Different types of abnormalities induced in *Vigna unguiculata* root-tip cells after treatment with different concentrations of cycocel for 24 hours.
Fig. 7. Chromosome stickiness. Fig. 8. C-metaphase.
Fig. 9. Lagging chromosome. Fig. 10. disturbed anaphase.

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دراسة مقارنة لتأثير معوق النمو " سيكوسيل " على الانقسام الميتوزى فى نباتى البسلة واللوبيا

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تتناول هذه الدراسة مقارنة تأثير معوق النمو "سيكوسيل" على الانقسام الميتوزى فى خلايا القمة النامية لجذور نباتى البسلة واللوبيا قد غمرت البادرات الصغيرة فى تركيزات مختلفة من معوق النمو: ٢٥و ٥٠ و ١٠٠ و ٢٥٠ و ٥٠٠ و ١٠٠٠ و ٢٠٠٠ جزء فى المليون لمدة أربعة وعشرين ساعة. وقد أظهر معوق النمو "سيكوسيل" تأثيرا مثبطا على معامل الانقسام الميتوزى فى النباتين وان درجة التثبيط متوافقة مع التركيز. وكما كان معوق النمو أكثر تأثيرا على معدل الانقسام الميتوزى فى نبات البسلة عنه فى نبات اللوبيا.

أوضحت النتائج أن نسب خلايا الأطوار المختلفة للانقسام الميتوزى فى الجذور المعاملة تختلف عن نسبتها فى الجذور الغير معاملة فى نباتى البسلة واللوبيا وذلك لزيادة نسبة الخلايا فى الطور الاستوائى.

لحدث معوق النمو بعض صور الشذوذ الكروموسومى فى خلايا النباتين ،كانت النسبة الكلية للشذوذ الكروموسومى فى نبات البسلة أكبر منها فى نبات اللوبيا. ففى نبات البسلة كان الطور الاستوائى الكولشيسينى أكثر الأنواع ترددا ، أما الكروموسومات اللزجه فكانت أكثر الأنواع شيوعا فى نبات اللوبيا. أينما أدت المعاملة لنبات البسلة الى ظهور الكروموسومات اللزجه ، القناطر ، الكروموسوم التائة ، الطور الانفصالى الكولشيسينى ، الطور الانفصالى التشتتى والشظايا بالاضافه الى بعض الخلايا ذات الأنوية الصغيرة أما فى نبات اللوبيا فتم مشاهدة الطور الاستوائى الكولشيسينى ، الكروموسوم التائه ، خلل المغزل ، عدم انتظام الطور التمهيدى. خلايا بها نواة ذات فجوة ، الطور الانفصالى الكولشيسينى والشظايا.