Chromotoxic effects of Cumicidin and Curacron insecticides on Vicia faba L.

A.M. AL- Shehri and H.M. AL-Wadi

Department of Biological sciences, College of Science
P.O.Box 9019, King Khalid University
Abha, Saudi Arabia

Abstract :

Treatment of *vicia faba* L.with different concentrations of cumicidin and curacrone for 3,6,12 and 24 h, showed chromotoxic effects on mitotic and meiotic cells. Both compounds reduced the mitotic activity and resulted in accumulation of metaphase cells, where other phases are reduced with few exceptions. Effects also include inhibition of cell division and induction of chromosomal abnormalities. The percentage of mitotic abnormalities increased as the concentration of the insecticide increased and when the time of treatment is prolonged. Types of abnormalities as c-metaphase, stickiness, disturbed, bridges and Lagging were observed.

Meiotic analysis of sprayed plants showed that all treatments increase the percentage of abnormal pollen mother cells and pollen sterility. This relationship follows the same trend as for mitotic results. Few types of meiotic abnormalities were observed such as Lagging, disturbed, and sticky cells.

In general it could be concluded that inspite of mutagenic effects of both insecticides cumicidin was found more effective on mitosis and induce abnormalities than curacrone. Such genotoxic effects warrants frequent use of these insecticides to protect plant. These results Lead to the importance of cytogenetic studies such chemical products before applications.

Introduction:

Numerous insecticides extensively used in modern agriculture practice for disease control. Such control to the pathogenic organisms infecting crop and horticulture plants is an accepted despite of the secondary consequences of most of these insecticides Wuu & Grant 1966, 1969 Rao *et al.,* 1987 and Pandy *et al.,* (1994).

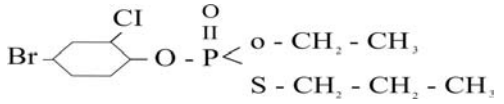
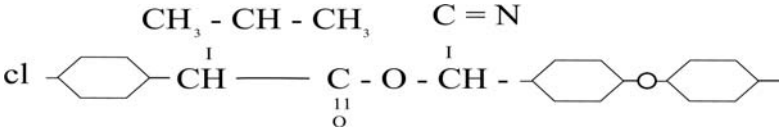
Previous studies investigate the harmful effects of insecticides and possibility to change heredity material of tested plants, with the tendency to

pass to the next generations. However, the potentialities of insecticides as mutagenic agents to the non-target organisms are worthy of extended study (Amer & Farah, 1983). In this contest, it seems highly reasonable to study the consequence effects of these two well known insecticides on mitosis and meiosis of *Vicia faba* L.

Materials and methods:

Root meristems (1 - 2 cm Length) of common broad beans (*Vicia faba* L., 2n = 12) were exposed to different concentrations (0.1, 0.5, 1and 2 (percent) of Cumicidin 80 % and Curacron 70% insecticides, structural and chemical formula listed below) for four time intervals (3, 6, 12 and 24 h). The range of concentrations used was below the recommended dose used for crop protection. After treatments root tips were excised, fixed in 3: 1 ethanol - acetic acid solution and stained by feulgen reagent (Badr. 1988) Control experiment was carried out for each concentration and time of treatment in order to compare cytological parameters studied. For each treatment four slides were prepared and examined for mitotic analysis.

For meiotic study, plants (7-10 plants for each treatment) were sprayed twice at flowering stage using the same concentrations of preurous mitotic treatments. Flower buds were gathered 36 hours after the last spray, fixed in fixing solution (same as mitosis) for 24 hours and examined using 2% aceto-carmine stain.(Haroun and Ali 1993)

Name	Structural and Chemical Formula
Curacrone 70	 **0-4 bromo -2- chlorophenyl. O-ethyl -S- propyl- phosphorothioate.**
Cumicidin 80	 **Cyano (3-phenoxypheny1) methyl 4-chloro alpha (1- methyl ethyl) benzeneacetate.**

Results and Discussion:

1 - *Mitotic studies.*

A - Cumicidin

Mitosis found normal in the root, cells of the untreated plant (control) with little reduction in mitotic index as the time of treatment prolonged. Only few numbers of aberrated cells were observed in control cells recording 1.4 % of abnormal metaphase at 24h treatment.

Mitotic analysis showed gradual decrease in the mitotic index either by increasing, the concentrations of Cumicidin or the time of treatment prolonged (Fig 1), similar to the same trend reported by Pandy *et al.,* 1994, and Adam *et al.,* 1990. Compare the mutagenic effects of this insecticide, within the applied concentrations , our results showed that mitotic index values were slightly lower than that of the control values at lower doses (0.1and 0.5%) treatments. whereas MI values of the other two concentration (1 and 2 %) , were found sharply decreased. This reduction, decrease as the time of treatment increase recording value of 2.8 MI at 2% concentration and 24h. treatment, (Table 1). The increases in the concentration and time significantly increase mitotic inhibition and ensures the harmful effect of Cumicidin on the mitotic division (Fig 1)

In contrast the percentage of stage index at metaphase increased which indicate the accumulation of cells at this stage (Badr 1988) which consequently affect the following stage index (anaphase and telophase). Within the applied concentrations and the time of treatment, the percentage of stage abnormalities showed positive relationship. It increase as the concentration increases and or the time of treatment is prolonged as previously reported by Golam & Alam (1986) Rao *et al* (1987) ,Grover & Malhi (1988) and Badr (1988)

Detail analyses of mitotic abnormalities recorded various types of aberrations. The highest values were recorded at the higher concentration and longer time of exposure (42.5 % for 2% concentration and 24h. treatment Table 2 and Fig 2). Types of c - metaphase and multipolar cells recorded the higher percent of abnormalities. It increased gradually as the time prolonged at all concentrations applied. In contrast sticky types were found lower post 3 and 6 h. time and increased significantly post 12 and 24-h of exposure whears, lagging types exposure did not show any definite relation with neither concentration nor time of exposure . Other types of

abnormalities such as disturbed, breaks only increased significantly post 24-h, exposure .

B - Curacron:

Mitotic index, stage index and abnormalities of this insecticide are listed in table 3. At time of 3 and 6 h treatment, mitotic index found not differ greatly from control value especially at lower doses of 0.1 and 0.5 concentrations. As seen in Fig.3 it seems that mitotic index values decrease as the concentrations increase and the time of treatment prolonged (pandy *et al* 1994 Adam *et al* 1990 and Golam & Alam 1986, Grover & Malhi 1988 Haroun & Ali 1993). Compare this effect with that of Cumicidin found that curacron is less lethal on mitotic index than cumicidin, (the lowest values of MI recorded were 2.8 and 3.2 for cumicidin and curacron respectively).

Regarding stage index, the data listed in Table 3 showed with few exceptions , that the values of prophase, index anaphase and telophase I Index were decreased that of the in contrast to that of the values metaphase which increased compared with to the control.
The abnormality results showed that it follow the same trend as that of Cumicidin, where its percentage increases as the time of treatments is prolonged (Table 3).

Types and percentage of abnormalities caused by Curacron are listed in Table 4. C-metaphase type is more frequent than c- anaphase.

It recorded a high percentage compared to the other types in general. All types of aberration, showed a positive relationship to the concentration of insecticide and to the time of treatment (Fig.4). Nevertheless sticky and multipola cell types were recorded somehow in high concentrations. Lagging and bridges with some other types of aberrations as micronuclei, disturbed and fragments were also recorded.

2 - Meiotic studies

Our results showed that treatment of flowering plants with both insecticides (Cumicidin and Curacran) had induced abnormal pollen mother cells (Table 5). The percentage of meiotic abnormalities in observed PMC`s (pollen mother cells) found to be increased as the concentration of both insecticides and increased as compared to the control, (Fig.5) record values

29 and 21.8 percentage for Cumicidin and Curacron at 2% concentration respectively. Applications of these insecticides show somehow higher percentage of abnormalities compared with the other types of insecticide investigated by Amer & Farah (1987).

Induction of abnormal PMC's by insecticides treatments was previously recorded by many authors (Amer & Farah 1980, 1983, 1987, Golame & Alam (1986), and Reddy & Annadurai (1992), confirming the mutagenic effects of insecticdes on meiotic chromosomes. No doubt these effects accumulate in the gametic cells and concequently affect pollen viability where cells are poorly functioning and can not withstand in competition with normal ones (Haroun & Ali, 1993). Positive relationship was recorded between percentage of pollen viability and percentage of meiotic abnormality (Table 5). Types and percentage of meiotic abnormalites were also recorded. Lagging, sticky, disturbed and micronuclei types of abnormalities were recorded at different concentrations and increased as the concentration in both insecticides increased.

In general, it could be concluded that the tested insecticides were found have lethal effect on mitotic and meiotic cell division. They induce darresting the rate of dividing cells and induce cytological disturbance during mitosis and meiosis recording different types of abnormalities. A. linear relationship was recorded between percentage of concentration and percentage of mitotic and meiotic abnormalities showed negative relationship. The same results were also recorded between rate of cell division and the applied concentrations and or time of exposure to presently tested insecticides. Data analysis confirm the genotoxic effects of these tested pesticides similar to the toxic pesticides and chemicals which are previously tested by Adam *et al* (1990), Bellani *et al* (1991), kumar & Sinha (1991), and Ahmad & Yasmin (1992) and Pandey *et al* (1994).

Results in this study pointed out to the chromotoxic effects of both insecticides. Together with previous works the results warrants indiscriminate spraying plant with pesticides and the wide use of other chemical products. It also point to the importance of mutagenic testing of the applied chemicals and pesticides before use.

(Table 1)

Effects of different concentrations of Cumicidin insecticidc on mitotic index (MI), stage index and abnormalities (%abn.) of each stage in root tips of *Vicia faba L.*

Treat. %	MI ± S.E.	Prophase.		Metaphase.		Anaphase.		Telophase	
		index	%abn.	index	%abn.	index	%abn.	index	%abn.
3h. Cont.	11.88±0.21	44.2	0.7	27.1	0.9	17.5	0.9	11.2	0.7
0.1	11.10 ± 041	49.2	2.5	26.2	4.2	17.1	4.7	7.5	3.7
0.5	10.23 ± 0.7	47.5	2.7	26.9	10.7	16.5	11.2	9.1	10.1
1	7.64 ± 0.52	40.7	5.7	33.8	25.1	15.7	16.5	9.8	14.5
2	5.12 ± 0.52	40.7	5.7	33.2	25.1	16.3	19.3	9.8	17.5
6h. Cont.	11.4 ± 0.37	43.1	0.9	28.2	1.2	16.4	0.7	12.3	1.0
0.1	10.9± 0.56	42.2	3.1	32.2	4.2	15.4	5.1	10.2	4.1
0.5	9.9 ± 0.11	41.7	4.9	30.3	11.1	14.9	10.9	13.1	4.1
1	7.8 ± 0.82	40.9	6.2	31.1	19.6	14.2	17.2	13.8	15.1
2	5.7 ± 1.3	38.3	7.1	34.7	26.5	15.5	22.1	11.5	18.4
12h. Cont.	10.6 ± 0.72	41.5	1.0	27.5	1.2	18.5	0.9	12.5	0.9
0.1	9.7 ± 0.61	41.1	4.2	33.7	4.9	12.1	4.3	13.1	4.3
0.5	9.5 ± 0.7	40.2	5.0	33.9	13.2	11.8	11.7	14.1	11.7
1	7.1 ± 0.66	38.7	6.4	35.1	27.5	13.1	17.2	13.1	17.2
2	6.3 ± 0.37	35.7	7.5	38.7	35.1	12.8	20.1	12.8	20.1
24h. Cont.	9.3 ± 0.61	40.1	1.3	25.7	1.4	20.1	1.2	14.1	1.0
0.1	8.7 ± 0.24	35.2	4.4	28.7	5.2	17.5	7.3	18.8	4.9
0.5	7.5 ± 0.31	32.1	6.1	33.4	15.7	17.1	15.3	17.4	13.7
1	4.1 ± 0.81	30.5	7.2	37.2	25.1	15.3	22.1	17.0	14.2
2	2.8 ± 0.46	29.7	7.9	39.7	35.4	10.2	29.9	20.4	22.5

(Table: 2)

Types and percentage of abnormalities recorded in *Vicia faba* L. root tips treated with different concentrations of Cumicidin as compare to the control.

Treatment %	Total % abn.	Types and percentage of abnormalities						
		CM.	CA	St	Bridge	Lagg	nultip	Others
3h Cont.	2.7	0.0	0.0	0.3	0.4	0.0	2	0.0
0.1	4.1	0.0	0.0	1.1	0.9	0.0	2.1	0.0
0.5	13.5	2.1	0.0	2.3	2.9	1.7	2.7	1.8
1	19.1	5.0	0.0	3.1	3.0	1.8	4.8	1.4
2	25.2	6.0	1.1	3.9	4.1	2.2	5.7	2.2
6h Cont.	2.6	0.0	0.0	0.0	0.5	0.0	2.1	0.0
0.1	5.6	0.0	0.0	1.3	0.9	0.7	2.3	0.4
0.5	17.2	3.3	0.0	3.1	2.3	2.3	4.7	1.5
1	25.3	7.2	1.8	3.1	2.7	3.1	5.3	2.1
2	33.3	11.0	2.2	3.3	3.4	3.8	6.9	2.7
12h. Cont.	2.8	0.0	0.0	2.0	0.5	0.0	0.3	0.0
0.1	7.1	2.4	0.0	1.7	0.7	0.0	2.3	0.0
0.5	22.9	5.6	2.3	2.1	1.8	3.1	6.0	2.0
1	27.7	7.7	2.7	2.7	2.3	3.0	7.1	2.2
2	35.1	9.9	4.5	3.4	2.9	3.4	7.7	3.3
24h. Cont.	2.2	0.0	0.0	2.0	0.2	0.0	0.0	0.0
0.1	14.7	2.6	1.1	3.1	2.4	0.0	3.7	1.8
0.5	27.6	6.9	3.0	3.3	2.8	2.2	6.4	3.0
1	31.5	8.2	3.4	3.4	2.9	3.0	7.1	3.5
2	42.5	10.7	7.1	4.2	3.7	3.3	8.1	5.4

CM :C - meataphase
CA :C - anaphase
St : stickness

(Table: 3)
Effects of different concentrations of Curacron insecticide on mitotic index (MI), stage index and abnormalities (abn.) of each stage in root tips of *Vicia faba* L.

Treat. %	MI ± S.E.	Prophase.		Metaphase.		Anaphase.		Telophase	
		%	abn.	%	abn.	%	abn.	%	abn.
3 Cont.	11.88 ±0.12	44.2	1.1	27.1	1.5	17.5	0.9	11.2	0.7
0.1	12.02 ± 0.6	44.8	2.1	25.2	3.7	18.9	3.2	13.1	2.1
0.5	11.70 ± 0.3	43.1	2.3	25.0	7.9	19.2	9.2	12.7	7.4
1	10.50 ± 0.5	46.5	4.2	23.7	10.3	18.1	12.4	11.7	11.7
2	7.81 ± 0.43	50.1	5.1	22.1	19.1	17.6	15.6	16.2	15.1
6 h. Cont.	11.40 ±0.36	43.1	0.9	28.2	1.2	16.3	0.7	12.4	1.0
0.1	11.71 ± 0.7	47.2	3.7	30.7	4.0	8.4	4.3	13.7	2.5
0.5	10.50 ±0.22	47.0	3.9	30.5	8.7	9.6	7.9	12.9	8.5
1	8.11 ±0.37	44.1	5.1	33.2	15.1	12.6	13.7	10.1	13.7
2	7.99 ±0.71	41.2	6.2	33.9	23.2	14.7	19.2	10.2	15.2
12h. Cont.	10.6 ±0.26	41.5	1.0	27.5	1.2	18.5	1.0	12.5	0.9
0.1	9.9 ± 0.4	35.9	2.9	29.2	3.1	18.1	5.2	16.8	3.7
0.5	10.1 ± 0.33	37.2	4.7	29.7	10.2	17.4	10.7	15.7	10.5
1	7.2 ±0.62	37.7	5.3	30.1	23.1	16.7	18.3	15.5	15.4
2	5.0 ±0.51	39.2	6.9	33.7	31.2	16.3	22.6	10.8	21.1
24h. Cont.	9.3 ±0.72	40.1	1.3	25.7	1.21	20.1	1.2	14.1	1.0
0.1	6.5 ± 1.1	34.2	5.0	27.7	4.2	20.2	7.0	17.9	4.5
0.5	7.1 ±0.11	37.5	5.7	31.5	15.1	17.6	11.7	13.4	11.7
1	5.0 ±0.31	32.1	6.1	32.1	22.4	17.9	19.2	17.9	17.2
2	3.2 ± 0.2	30.7	6.9	34.2	34.1	15.9	25.1	19.2	22.6

(Table : 4)
Types and percentage of abnormalities induced by Curacron insecticide in *Vicia faba* root tips

Treatments %	Types and percentage of abnormalities							Total. %
	CM	CA	Stick	brid.	Lagg	mnlltipol	others	
3h. Cont.	-	-	-	0.7	-	2.0	-	2.7
0.1	-	-	-	0.9	-	2.2	-	3.1
0.5	1.9	-	1.5	1.2	-	2.5	-	7.1
1	1.9	-	1.7	2.5	1.5	3.7	1.5	12.8
2	3.1	-	3.4	2.7	2.0	4.6	1.8	17.6
6 h. Cont.	-	-	-	0.5	-	2.1	-	2.6
0.1	-	-	1.3	0.7	0.5	2.3	-	4.8
0.5	3.1	-	2.9	1.5	-	3.5	1.0	12.0
1	3.7	1.7	3.2	1.9	3.0	4.5	1.7	19.7
2	5.4	3.3	3.3	2.5	3.5	4.1	3.2	25.3
12 h. Cont.	-	-	-	0.5	-	2.3	-	2.8
0.1	3.1	-	1.0	1.1	-	2.7	-	7.9
0.5	3.5	-	2.6	1.4	1.0	3.2	1.2	12.9
1	5.2	3.3	3.1	1.7	2.5	3.5	2.1	21.4
2	7.5	3.4	3.2	2.5	2.9	5.1	2.7	27.3
24 h. Cont.	-	-	0.8	0.0	-	2.0	-	2.8
0.1	2.3	1.4	1.5	2.1	-	3.0	-	10.3
0.5	4.5	2.7	2.7	2.5	2.9	5.1	2.6	23.0
1	7.6	2.1	3.0	2.7	2.9	5.8	3.1	27.2
2	9.2	3.0	3.7	3.4	3.0	8.6	3.9	34.8

(Table : 5)

Percentage, types of abnormalites, and pollen fertility induced by different treatments of Cumicidin and curacron insectside .

Treatment	PMC's observed	% of abn. PMC's	Percentage and types of abnormalites				% of Pollen fertility
			Lagg	Stick	disturb	Micro	
Control	62	1.6		0.5	0.6		98.7
Cumicidin 0.1	80	13.7	0.5	5.9	4.2	3.1	79.1
0.5	65	17	1.9	6.1	5.0	4.0	75.5
1	72	23.6	2.7	7.5	8.9	4.5	61.7
2	69	29	3.6	8.4	12.4	4.6	57.3
Curacron 0.1	71	11.3	1.0	4.0	3.1	3.2	82.3
0.5	87	14.9	1.9	4.5	4.7	3.8	76.1
1	98	19.4	2.4	7.2	5.8	4.0	65.2
2	64	21.8	2.7	7.7	7.2	4.2	61.9

PMC's (Pollen Mother Cells) .

abn. abnormal

References

1. Adam, Z.M., F.A. and El Sheikh, I.A. (1990): Alternations in nucleic acid, protein content and mitotic division of *V. faba* root tips as afected by Malathion and Tamron insecticides. Cytologia 55:349-355.
2. Ahmad, S. and Yasmin, R. (1992): Effects of methyl parathion and Tri-miltox on the mitosis of *Allium Cepa.* Cytologia, 57155-160.
3. Amer, S. and Farah, O.R. (1980): Cytological effects of pesticides X. Meiotic effects " Cytologia " 45: 241: 245.
4. Amer, S. and Farah, O.R. (1983): Cytological effects of pesticides XIII. Meiotic effects of insecticides " Dursban ". Cytologia 48: 557-563.
5. Amer, S. and Farah, O. (1987): Cytological effects of pesticides VIII. Meiotic effects of the insecticides Methamidophos Cytologia 52: 303 - 307.
6. Badr. A. (1988) : Cytogenetic activities of some fungicides. Cytologia 53 : 633 - 640.
7. Bellani , L . M., Rinallo, C. and Bennici, A. (1991): Cytomorphological alterations in *Allium* roots induced by surfactants. Enviromental and Expermintal Botany 31 (2): 179- 185.
8. Golam, K. and Alam, S. (1986): Cytological effects of insecticides (Carbicron - 100 EC and Vapona - 50) on Barley (*Hordeum vulgare L.).* Cytologia 51: 885-892.
9. Grover, I.S. and Malhi, P.K. (1988): Genotoxic effects of some organophosphorus Pesticides III. in Vivo chromosomal aberration bioassay in root meristems of *Allium* and *Hordeum.*
10. Haroun, S.A. and Ali, A. (1993): Cytological studies on Gamma irradiated wheat (Triticum aestivum L.).J. Agric. Res. Tanta Univ., 19 (3): 618 - 623.
11. Kumar, U. and Sinha, S.S.N. (1991): Genotoxic effects of two pesticides (Rogor and Bavishn) and antibiotic (streptomycin) in meiotic cells of qrasspea *(Lathyrus sativus L.)*. Cytologia 56 : 209-214.
12. Pandy, R.K., Shukla, R. and Datta, S. (1994): Chromotoxic effects of one fungicide (Dithane M-45) and Two insecticides (Aldrex-30 and Metacid-50). Cytologia 59: 419 - 422.
13. Rao, B.V., Sharma, C. R. and Rao, B.G.S. (1987): Cytological effects of organophosphorus insecticides on *Allium cepa* Root meristems.
14. Reddy, V.R.K. and Annadurai, M. (1992): Cytological effects of differentt muttagens in lentil (*Lens Culinoris* Medik). Cytologia 57: 213 - 216.
15. Wuu, K.D. and Gront, W.F. (1966) : Morphological and somatic chromosomal aberrations induced by pesticides in barley (*Hordeum vulgare*). Can. J. Genet. Cytol. 8: 481 - 501.

تأثير مبيد السيموسيدين والكلوراكرون على كروموسومات نبات الفول البلدي

عبدالرحمن بن محي الشهري، حسين ما نع الوادعي
قسم علوم الحياة – كلية العلوم - جامعة الملك خالد
أبها – ص.ب ٩٠١٩ – المملكة العربية السعودية

الملخص :

أظهرت المعالجة بالتركيزات المختلفة لكل من المبيد الحشري سيموسيدين ٨٠ ، كوركرون ٧٠ لمدة ٣، ٦، ١٢، ٢٤ ساعة تأثيراً ضاراً على كروموسومات الانقسام الميتوزي والميوزي . ووجد أن كلا االمركبين يساعد على خفض النشاط الانقسامي وتراكم الانقسام في مراحل معينة مما يؤثر على معدل النشاط ، كما أظهرت المعالجات تأثير ضار على الكروموسومات وأحدثت شذوذا في الكروموسومات . كما أظهرت النتائج أن النسبة المئوية لشذوذ الانقسام تزداد مع زيادة التركيزات لكل من المركبين وكذلك مع زيادة فترة التفاعل ظهور بعض الشذوذ مثل الاستوائي الكولشيسيني والقنطرة الكروموسومية وكذلك الكروموسوم المفقود .

بالنسبة للإنقسام الميوزي وجد أن المعاملة بالمبيدات تؤثر على نسبة الشذوذ في أمهات حبوب اللقاح وزيادة عقم حبوب اللقاح . كما كانت زيادة العقم والتشوهات تزداد مع زيادة التركيز وفترة التفاعل كما في الإنقسام الميتوزي . وأيضاً ظهرت بعض الشذوذ في هذا النوع من الانقسام ، عموماً فإن دراسة التركيز أثبت وجود تأثير ضار على الإنقسام الميتوزي والميوزي ومن حيث المقارنة كان تأثير مركب السيموسيدين أكثر فاعلية من المركب الثاني كوراكرون . وأن مثل هذه التفاعلات في المبيدات الحشرية لحماية النبات من الحشرات لها تأثير ضار على النبات نفسه حيث يؤثر على الانقسام وبالتالي نمو النبات . ومن هذا يجب دراسة التأثيرات الجانبية لمثل هذه المبيدات قبل استخدامها في مكافحة الحشرات وغيرها .