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Cvtolgenetic Effects of the Insecticide (Dralgo) and Herbicide (Fusilade) on the Cellular Characters of *Viciafaba* L.

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Vicia faba L. plants were used to test the combined genotoxic effects of Fusilade (Herbicide) and Drago (Insecticide) and their interaction. Also, germination vigour, vegetative growth and biochemical parameters were used to test the combined physiological effects of two pesticides alone and in combination.To study the cytotoxic effects, the meristem cells in root tips of *plant* were treated with nine different concentrations (1, 5, 10, 50, 100, 500, 1000, 5000, 10000 ppm) at four different time (3, 6, 12, 24 hrs.). The concentrations of the two pesticides were tested on growth of seeds of *Viciafaba* L. compared with control where the mean root length of seeds was measured at different times between 24, 48 and 72hrs. Furthermore, after 72h, root degeneration occurred turning the root tips into black colour, which wasn't measurable. lethal concentration LC_{50}% was decreased with the increase in time which caused an increase in percentage of mortality using Probit tables .All the above treatments of two pesticides alone and in combination have been shown to decrease the mitotic index . In the combined pesticides treatments, the decrease in (MI) wasn't like in the individual pesticide treatments which was less when compared with

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pesticides alone either herbicide or insecticide at the same concentration and duration. Increase the percentage of mutation frequency in each pesticide alone and the combined (Fus.-Dra.) after 6hrs of treatment whereas at the combined the relation was inversed when compared with control, indicating an antagonistic effect. Also, all treatments used have caused different kinds of mitotic abnormalities and chromosomal aberrations, which were generally as follow: C-metaphase, chromosome disturbance, stickiness, breaks and fragments, laggard and bridges, multipolar and ring chromosome, micronuclei and binuclear cells. In addition the pesticides have been shown to decrease the protein and DNA and RNA contents below the control level.

Key Words: Cellular characters, cytogenetics, herbicide, insecticide, *Viciafaba,*

Introduction

The widespread use of pesticides in modern agriculture to control the diseases in crop plants has resulted in the initiation of studies on the mutagenic effects of these chemicals The risk a pesticide presents depends on the type of pesticide, how toxic it is, where and how it is used, how much is used, how often it is used, how long it remains in the environment, if it concentrates through the food chain and how it impacts the habitat and the environment in general (Crosby, 1982). Different pesticides, insecticides and fungicides are being extensively used in modern agriculture (Pandey *et al.,* 1994). El-Zoka *et al.* (2000 a) studied the mutagenic potentialities of the two organophosphorus insecticides, Curacron and Hostathion, on mitosis in root-tips of *Allium cepa* as well as on meiosis in flower buds of *Viciafaba* plants. They also studied the effects of the two insecticides on the seed storage protein banding patterns of M_2 *Viciafaba* plants. The obtained data showed that a significant decrease in the mitotic index was pronounced at all concentrations and time of treatments even after recovery for 24 hours. Highly significant values of total mitotic abnormalities were induced as the concentration and time of treatment increased. The induced abnormalities included stickiness, bridges, disturbed configurations and laggards. Dixit, (2001) explained that the five organophosphorus pesticides (Ekalux, Metasystox, Monocrotophos, Rogor and Methylparathion) induced the

chromotoxic and antimitotic effects on mitotic chromosomes of rye *(Secale cereal).* El-Ghamery *et al.* (2000) conducted an investigation to determine the effect of the herbicide Goal "Oxyfluorfen" on cell division and nucleic acids content in root tips of *Allium cepa* and *Viciafaba* L. They used four different concentrations (25%, 50%, 75% and 100%) and four treatment times at 4h, Sh, 12h and 24h. They found that the herbicide was an antimitotic agent; all treatment exerted a mitodepressive action and resulted in the alteration of the mitotic phases. This effect increased when both concentration and treatment time increased. Also, the herbicide Goal resulted in reduction in the amounts of DNA and RNA.

Ateeq *et al.* (2002) indicated that the meristematic mitotic cells of *Allium cepa* is an efficient cytogenetic material for chromosome aberration assay on environmental pollutants. They studied the genotoxicity of Pentachlorophenol (PCP), 2,4-Dichlorophenxyacetic acid (2,4-D) and 2-Chloro-2,6-diethyl-N- (Butoxymethyl) acetanilide (Butachlor), 50% effective concentration (EC_{50}), c-mitosis, stickiness, chromosome breaks and mitotic index (MI). The toxic effects of pesticides accumulated in soil or water can be measured on intact plants grown in the natural communities or in field, on intact plants cultivated in the greenhouse or growth chambers, or on plant cell cultures *in vitro* (Frans *et al.,* 1988). Over the last decades, environmental contamination with heavy metals constitutes a major component of the environmental pollutants accumulating in the biosphere (Sengupta and Ghosh, 1993).

Chauhan and Gupta (2005) indicated that substituted urea herbicides-Isoproturon (ISO) or Diuron (DIU) and a synthetic pyrethroid insecticide-Deltamethrin (DEL) induced combined cytogenetic and ultrastructural effects on the root meristem cells of *Allium cepa.* Simultaneous or successive use of herbicides and insecticides is a common agricultural practice, which due to synergistic interactions can cause serious ecotoxicological problems. Therefore, toxicity evaluation of the herbicide/insecticide interaction is very essential.

In this work we used *Viciafaba* L. plants as a model system to study the combined cytotoxic effects of Fusalide (Herbicide) and Drago (Insecticide), and their interaction on *Viciafaba* L.

Material and Methods

(I) Materials

The pesticides used were use in this study:

a) Drago (Dimethoate)

Dimethoate is an insecticide used to kill mites and insects systemically and on contact. It is moderately toxic by ingestion, inhalation and dermal absorption.

Chemical structure: *0,*0-dimethyl S-methylcarbamoylmethyl phosphorodithioate

Molecular formula: CsH12N03PS2

b) Fusilade (Fuazifop-p- butyl)

It is selective postemergence phenoxy herbicide used for control of most annual and perennial grass weeds in cotton, soybeans, stone fruits.

Chemical structure: Butyl 2-(5-trifluoromethyl-2-pyrdyloxy) phenoxy) propionate.

c) The plant used in this study is *Viciafaba* L.; healthy seeds were supported by (ASTRA AGRICULTURAL COMPANY; Spain).

(II) Methods

Determination of lethal concentration at 50%

Nine different concentrations were calculated to determine the lethal concentration at 50% for each pesticide, these concentrations were I, 5, 10, 50, 100, 500, 1000, 5000 and 10000 ppm. The nine different concentrations were examined on seed germination of *Vicia faba.* Length of roots was measured and comparative with root of control with three replicates.

Cytological preparations

The cytological preparations were carried out to study single effect of lethal concentration at 50% , quarter and half of pesticides on behavior of *Vicia faba* and essay knowledge range effect the different concentrations of pesticides on Mitotic index and abnormality as described by (Badr and Ibrahim, 1987) using root tips of *Viciafaba* L. These processes were carried out in compliance with type of pesticide (Herbicide – Insecticide) and concentration of pesticide compliance with existence control treatment in all treatments with concentration zero ppm. In alternatively combined treatments, the primary roots were exposed to 6+6 and 24+24 hours to each concentration of Fusilade, followed by each concentration of Drago [Fus.-Dra.] or to Drago following by Fusilade [Dra.-Fus.], and they were exposed

for 3, 6, 12 and 24 hours (Sharma and Grover, 1970) to determine whether the combined effects of the two compounds were synergistic or antagonistic.

Physiological Preparations

Healthy seeds of *Viciafaba* were selected for the uniformity of size and color and then washed and soaked in distilled water for 24hrs, then 10 seeds were germinated for 24, 48h at 22-25°C.in the botanical green house of King Saud University for 3 weeks. A leaf area meter (Model Li-CoR 3000) was used to measure leaf area. Shoot and length, and root fresh weight were measured.

Mixture of Drag and Fusilade

Possible interactions between pesticides were estimated using Abott's formula (Gisi, 1996). We only used this model to analyze the inhibition of growth caused by mixtures of Drago and Fusilade. In this widely used model, the expected inhibition of mixture, expressed as percent Coxp=A+B-(AB/l00), in which A and B were the inhibitions given by single pesticides. The ratio of inhibition (Rl) was then calculated as follows for each pesticide concentration:

Rl = Observed inhibition/Coxp Synergism or antagonism was evaluated by comparing Rl with 1. A Rl value > 1 indicated synergism between the two pesticides; Rl = 1 simple additivity; and Rl < 1, antagonism between the chemicals.

Metabolic features

Protein extraction for electrophoresis as described by Wang *et al.* (2006), and DNA and RNA were extracted and estimating as described by Corniquel and Mercier (1994).

Results

Cytological Results:

Effect of pesticides used on root length of *Vicia faba* L we used measurement root length of bean plants to determine the lethal concentrations of the pesticides (LC_{50}%). All the treatments showed significant reduction in the growth rate of root tips. Comparing the treatments to the control, all the treatments show gradual decrease in root length as we increase the concentration and duration of treatment. Figures (1, 2, 3, 4) show all the *Viciafaba* L. root tips with Drago, Fusalide and mix of them both together as (Dra.- Fus.) and (Fus.-Dra.).

Fig (1): Effect of Drago on root length of *Vicia taba* L. (cm) at 24,48 and 72 hrs. and different concentration.

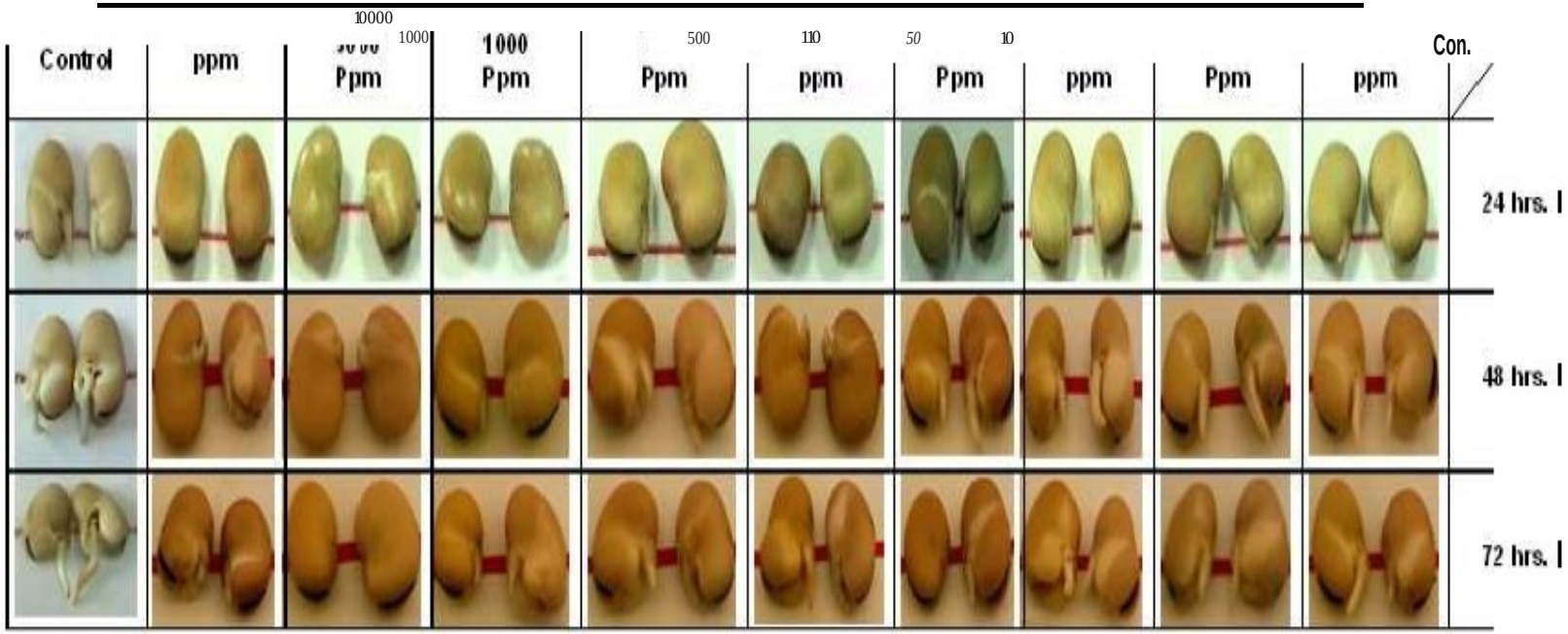


Fig12): Effect of Fusilade on root length of *Vicia faba* L. (cm) at 24,48 and 72 hrs. and different concentration.

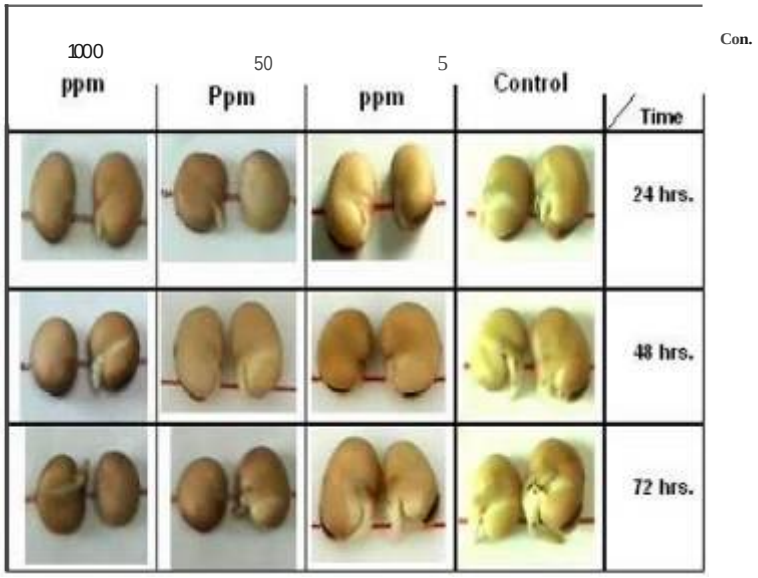
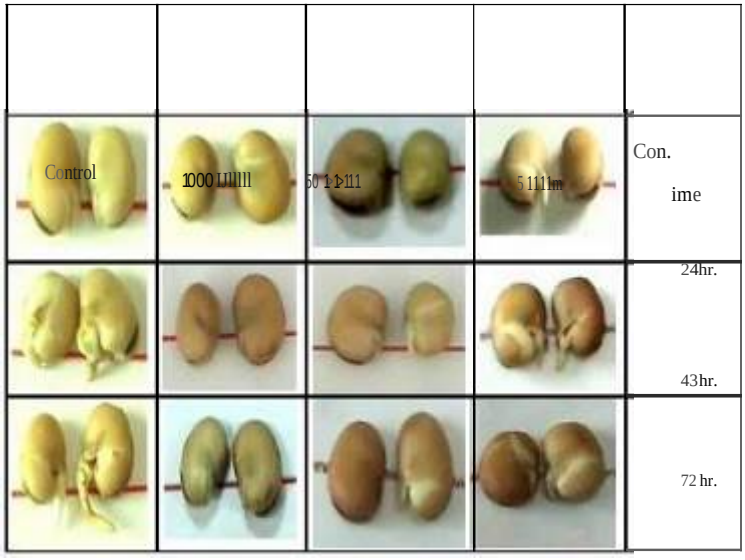


Fig (3): Effect of combined 11esticid es [Orn..Fus.) on root length of *Vicia* fab.1 L.(cm) at 24, 48 and 72 hrs.and different concentration.



Fig(4): Effect ofreve1se combined 11esticid es [Fus..Ora.] on root length of *Vicia* falx1L. (cm) at 24, 48 anti 72 lus.and different conccntr,1tion.

Determination of the lethal concentration at 50% of the two pesticides used:

Statistical analysis is completed by using regression analysis and the help of probit tables to get concurrent concentrations with the percentage of mortality at 50% after 24, 48, 72hrs.of 1reatm.ent with two pesticides.

The results of the determination of lethal concentration at 500/o (LC_{50}%) for the two pesticides used. Also, the Fig. (5 - 8) show the linear relationship between the percentage of mortality and log different concentrations. Figure (6) shows the projections determing the different lethal concentration at 50% at 24, 48 and 72hrs which was 707.95, 158.49 and 79.43 ppm, respectively, where: lethal concentration LC_{50}% was decreased with increue in time which caused increase in percentage of mortality from 41.52% at 24hrs, 46.65% at 48hrs to 49.600/o at 72hrs which at concentration 50 ppm of Drago. In figure: (7) the lethal concentration (LCso%) for combined pesticides (Fus.-Dra.) at the mean of different time intervals (24, 48 and 72hrs) was 354.81ppm where the percentage of mortality increased with the increase in concentration which was 18.75%, 49.53% and 58.96% at 5, 50 and 1000 ppm, respectively. Figure (8) shows the lethal concentration (LCso%) for combined pesticides (Dra. – Fus.) at the mean of different time intervals (24, 48 and 72hrs) was 630.96 ppm where the percentage of mortality increased with the increase in concentration which was 38.47%, 43.03% and 63.91% at 5, 50 and 1000 ppm, respectively.

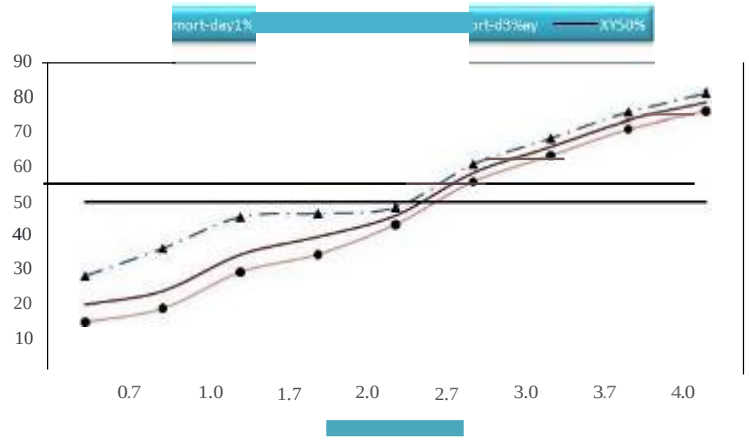


Fig. (5):Lethal concentration at (LCso%} of Fusilade of *Vicia faba* L. root length and percentage of mortality for different times and concentrations.

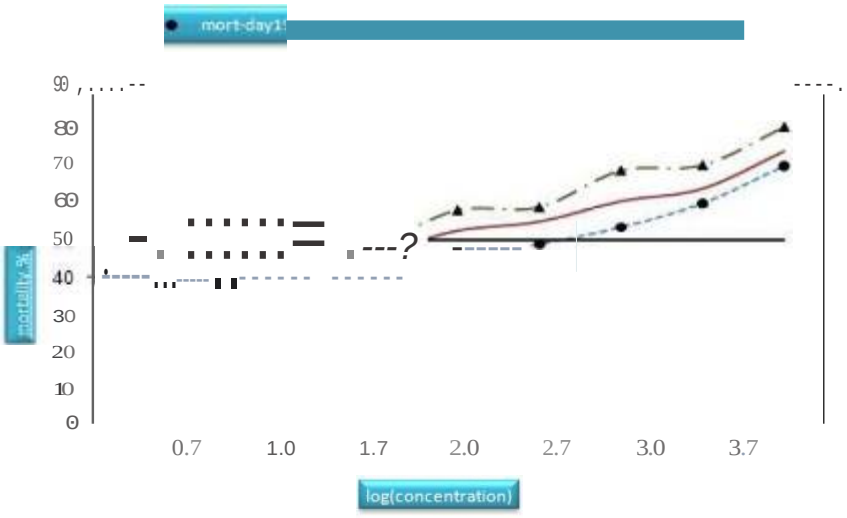


Fig (6): Lethal concentration at (LCs0%) of Drago *of Vi ciafaba L.* root length and percentage of mortality for different times and concentrations.

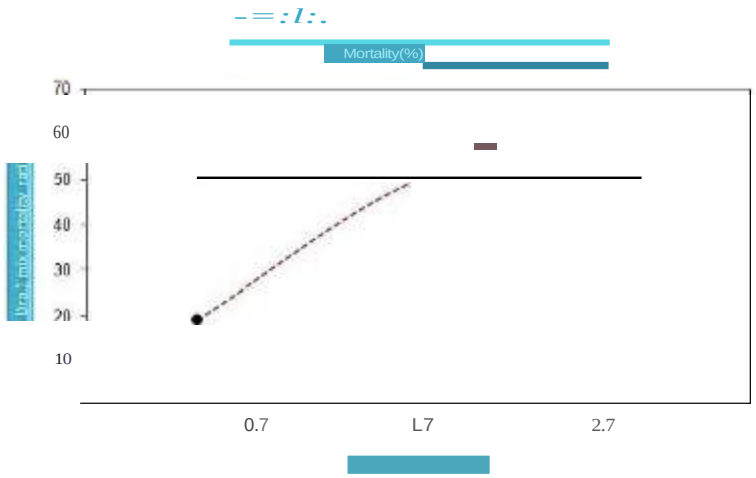
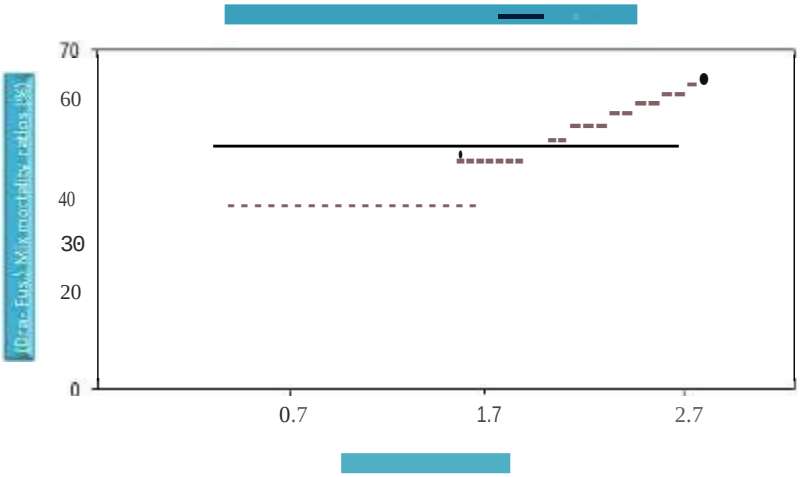


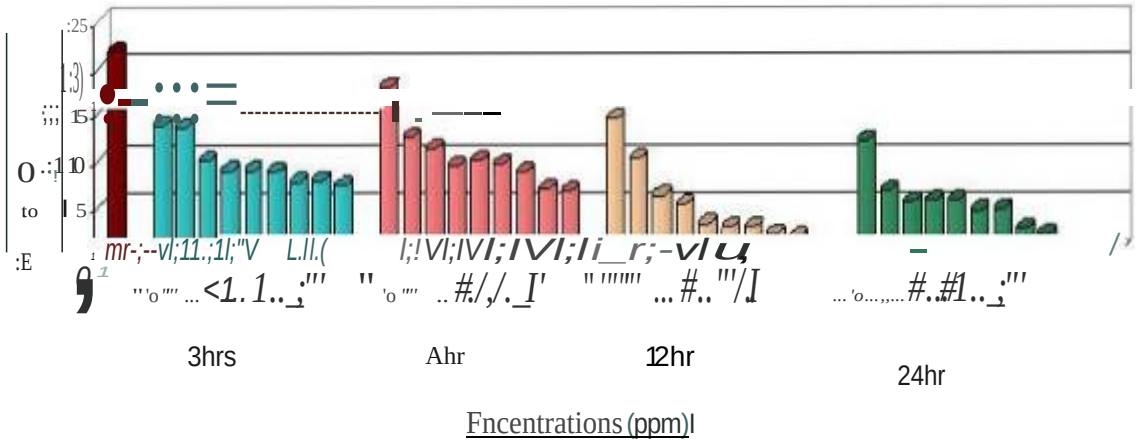
Fig (7): Lethal concentration at (Leso %) of (Fus.- Dra.) of *Vicia faba* L. root length and percentage of mortality for different times and concentrations.



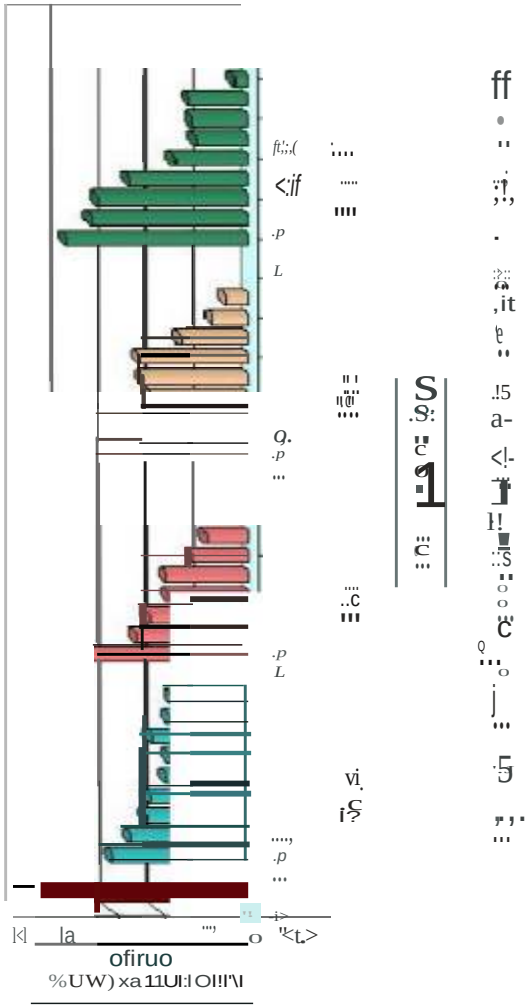
l.f1a (l): Lethal concentration at (LC,o%) of (Dra. - Fus) of *Vicia faba* L. root l.ength and percentage of mortality for diferent times .

Cytologiaal effects of patiddes used on mitom of .,*Viciafaba* L

The mitotic index in root meristems of *Vicia/aba* treated in different concentrations of Drago, Fusilade, and different combinations with Drago and Fusalide are given in Figures (9 - 12). The data showed that all treatments for the two pesticides at different time and concentrations led to a significant depression of the mitotic ind.ex as compared with the control. Using the herbicide Fusilade (Fluazifop-p-butyl), the mitotic index was gradually decreased with increasing the concentration of herbicide at 3, 6, 12, 24hrs. where; (Ml) decreased from 13.2% at lppm to 6.8% at 10000 ppm after 3hrs treatment, from 17.5% at lppm to 6.3% at 10000 ppm after 6hrs trea1ment, from 14.1% at lppm tol.2% at 10000 ppm after 12hrs treatment, and from 11.6% at lppm to 1.6% at 10000 ppm after 24hrs treatment which compared with the control which mitotic index was 21.2%. The mitodepression has reached its maximum action at the high concentration 10000 ppm of the herbicide at all the period of the treatment which was 1.2% and 1.6% after 12, 24hrs, respectively Figure 8).



Figw'e (9): Effect of Fusi.lade on Mitotic Index (Ml) illtile root tip of *Yiciafaba* L (2n • 12)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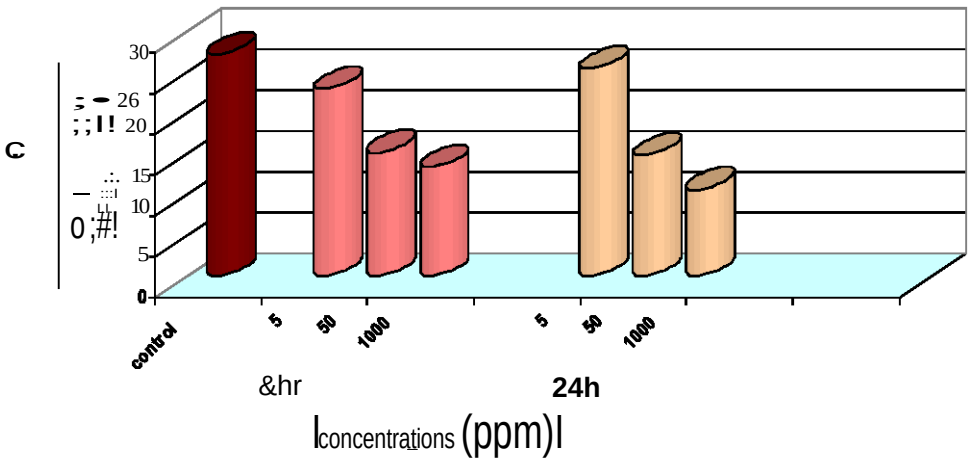


Figure (11): Effect of the combined pesticides (Fus.- Dra.) on Mitotic Index (MI) in the root tip of *Viciafaba* L. (2n = 12).

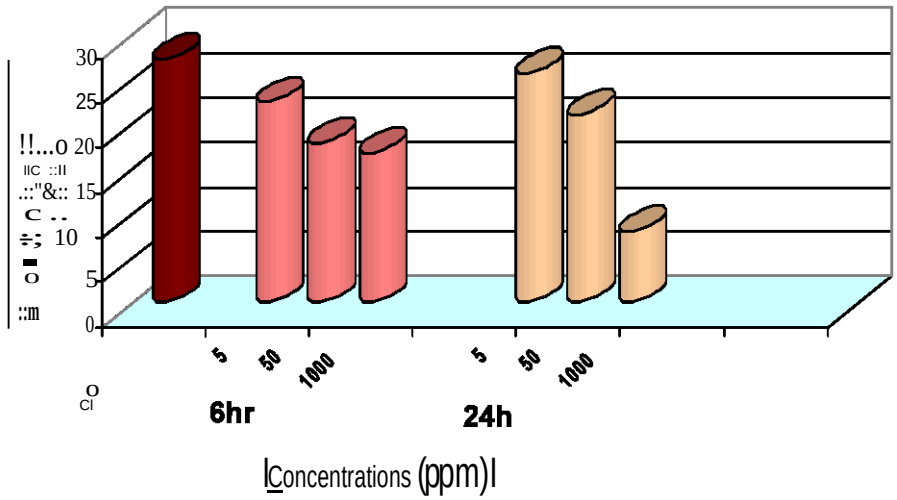


Figure (12): Effect of the reverse combined pesticides (Dra.- Fus.) on Mitotic Index (Ml) in the root tip of *Viciafaba* L. (2n = 12).

Also, when we used Insecticide Drago (Dimethoate), we get the same results in decreasing the mitotic index with increasing of the concentrations and duration of the treatment where; (MI) decreased from 14.6% at lppm to 8.3% at 10000 ppm after 3hrs treatment, 17.5% at lppm to 1.7% at 10000 ppm after 6hrs treatment, from 16.1% at 1ppm to 2.4% at 10000 ppm after 12hrs treatment, and 19.3% at lppm to 1.4% at 10000 ppm after 24hrs treatment. Therefore the maximum values of MI was 19.3% at lppm after 24hrs treatment which decreased to 1.4% at 10000 ppm at the same treatment as compared with the control value which was 21.2% (Figure 9).

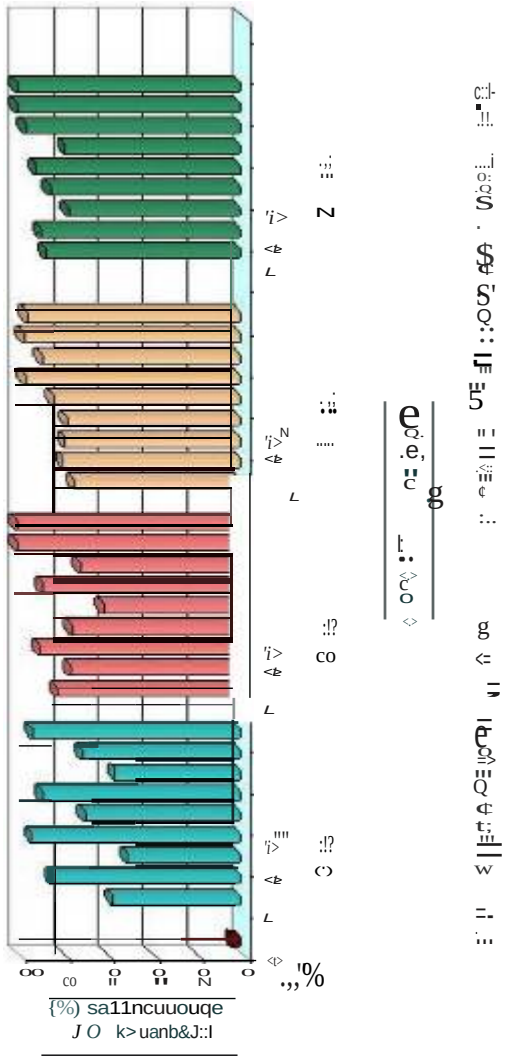
The data showed that the effect of combined of pesticides on mitotic index where; the mitotic index indicated a proportionate decrease with increasing the concentrations of the combined pesticides applied. Mitotic Index in using the combination of [Dra.- Fus.] decreased from 22.8% at 5ppm to 13.3% at 1000 ppm after 6hrs treatment, and from 25.3% at 5ppm to 10.5% at 1000 ppm after 24hrs treatment as compared with the control value which was 27.1% (Figure 12).

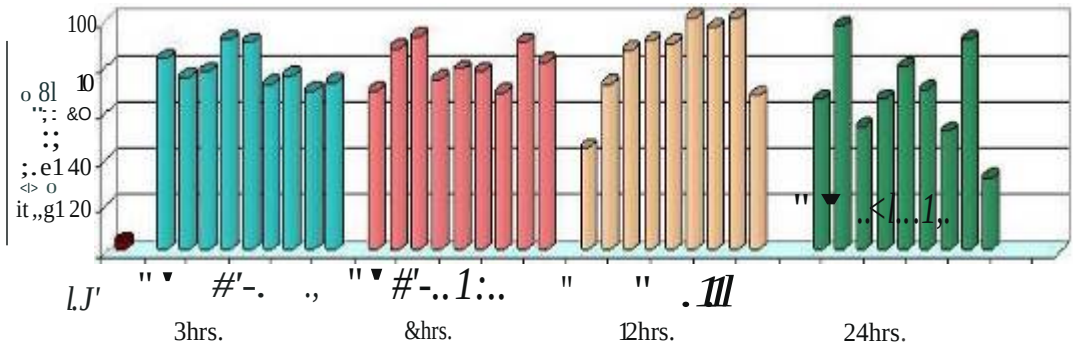
Furthermore, Mitotic Index of the reverse combination [Fus.- Dra.] decreased from 22.3% at 5 ppm to 16.6% at 1000 ppm after 6hrs treatment, and from 25.5% at 5 ppm to 7.8% at 1000 ppm after 24hrs treatment (Figure 11). In the combined of pesticides, the decrease in mitotic index wasn't like at the individual of pesticides which was less when compared with pesticide alone either herbicide or insecticide at the same concentrations and duration. Effect of pesticides on the Frequency of abnormalities in root tip of *Viciafaba* L.

The data showed that the frequency of abnormality was direct proportionately increased as the concentration and duration of treatment increased. Treated root meristems of *Viciafaba* L. by Insecticide (Drago) with different concentrations and time induced increase the frequency of abnormalities where; the maximum value was 100% at 5000 ppm, 10000 ppm after 12hrs, 24hrs, respectively, and the minimum value was 50.4% at 10 ppm after 3hrs treatment (Figure 13).

As presented in Figure (14), the frequency of abnormalities increase with increasing concentrations and exposure time when compared to means

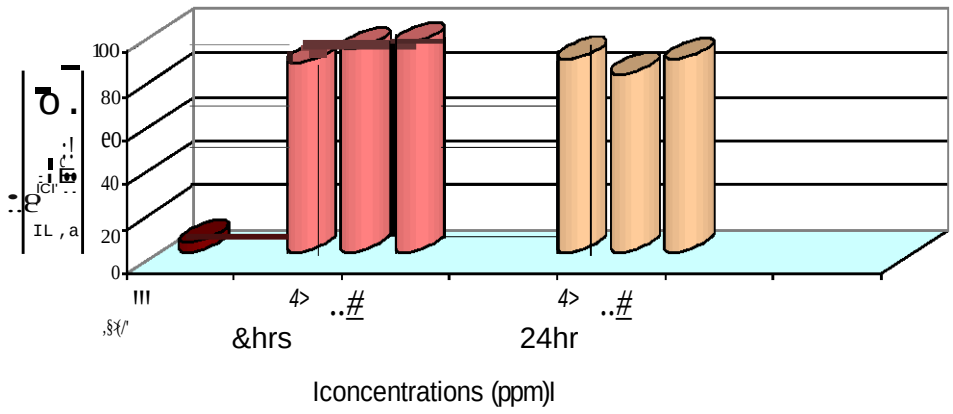
of control value, Herbicide (Fusilade) induced increase the frequency of abnonnalities on root tips : from 44% at 1ppm after 12hrs treatment to 100% at 5000 ppm in the same treatment Whereas, when root tips treated with the combined pesticides, the frequency of abnormalities was approximately decrease with die conesponding increase in concentration and duration of treatment (Figures 15, 16).





concentrations (ppm)

Fig (14): Ettect of Fnslade (F1tmzifo11-11-1Jutyl) on Fre11uency of olmonnalities in root ti11of *Vicia t1ba* L. (2n=12).



J'ig (15): .htlect otthe combined pesticides {.l'us.-Ura.) on .l'tequency ot abnormalities in the root tip of *Viciafaba* L. (2n = 12).

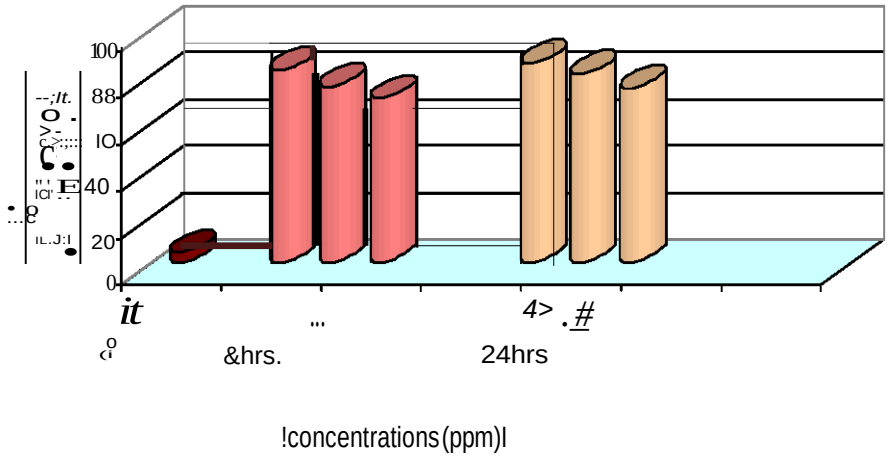


Fig (16): Effect of the reverse combined pesticides (Dra.-Fus.) on Frequency of abnormalities in the root tip of *Viciafaba* L. (2n = 12).

Statistical analysis of comparing the Mitotic index (MI)

The significance of data was determined by statistical analysis presented in Tables (1 - 4) and showed that the decline in mitotic abnormalities was highly significant ($p < 0.01$) at 5 ppm of Insecticide (Drago) and significant ($p < 0.05$) at 50 ppm and 1000 ppm, respectively, after 6hrs of treatment. On the contrary, the decline was significant at 5 ppm and 50 ppm, respectively, and high significant at 1000 ppm after 24hrs of treatment as compared with the untreated control. Data showed treating the primary roots of *Vicia faba* with Herbicide (Fusilade) caused high significant reduction in mitotic abnormalities at 5 ppm and significant reduction at 50 ppm and 1000 ppm, respectively, after 6hrs while it caused a various reduction which was high significant at 5 ppm, a significant at 50 ppm and a non-significant at 1000 ppm after 24hrs of treatment when compared with the control.

In combined effect of pesticides (Fus.- Dra.), the reduction was a significant at all treatments except the lowest one (5 ppm) after 24hrs of treatment as compared with the untreated control. Similarly, when the reverse combined effect of pesticides (Dra.- Fus.) was applied, the results reveal a significant reduction at all treatments except at 1000 ppm after 6hrs of treatment when compared with the control.

Table(l): Combined cytotoxic effect of Drago followed by Fusilade in root meristems of *Viciafaha* L. plants.

Cytological effects	Pesticides treatments				
	(Draao) Slnlle effect (A)	(Futilade) Smale effect (B)	Additin effect (A+B)	Celllbined treatment (AB)	Combined effect (AB/A+B)
Mitotic Index (Ml)	10.1	7.5	16	16.95	0.96
Pen:entaae or Mitotic Freq•eney	82.32	73.5	lSS.82	78.13	0.S0
Percentage or Chromosomal Aberration	3099	30.65	6164	26.45	0.43

Table(2): Combined cytotoxic effect of Fusilade followed by Drago in root meristems of *Viciafaha* L. plants.

Cytological effecU	Pesticides treatments				
	(Draao) Sinpeffect (A)	(Futilade) Siqle effect (B)	Additive effect (A+B)	Celllbined treatment (AB)	Cembined effect (AB/A+B)
Mitotic Index (Ml)	10.1	7.5	16	15.99	0.91
Percmtq;e of Mitotic F•eney	82.32	735	lSS.82	87.37	0.56
Percent.ge or Chromosomal Aberration	30.99	30.65	6164	29.67	0.48

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	looo	1000	13!	42	II)	41	.ii	21	23	20	IG	W	W	rn	mr	Im	;04	133 I (.G3	10.2 I 3.11	.OlI'
	5	1000	22!	41	31	II	N	53	ii	44	n	ms	11.!f	rn	41.00	IW	f.23	22.3 t W	75.3t lGI	.0!2
m.-RJ1.	50	1000	113	35	25	55	41	41	33	<ii)	IG	4i!O	311	UI	312i	I.II	3.71	1111m	4.3 I 0.62	
	1000	1000	iGG	3G	21	5i	41	42	Jl	33	25	mo	I.is	rn	$125	3.22	GI	IG.G 1m		
conl\11		IOI!	21G	30	3	IOI	5	25	3	G0	I	GS.00	WI	11.0I	!.00	1.G3	0.12	2i.lt li04		

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t

Tal:le (4):Numbers of lltalcells ell3minedand total mitoses, total and amocrllll rritaic phases, meil'l and rritOlic index (MO andmean 'iofabnonral rritoses allE!'tE!lting *Vtialaba* L. roottiwlhdifllrentconcentrationsoffusilade, Drago, (Fus, l:r..)il'ld (i:ta-Fus.) ater 24hrs oftreatllllnt.('):signitcant;it5'4 (+):signitcant;it1'i

l.11K-ol tu.i 11nl (11>1	C..._ l\'fl	1..; «lJ CU:llai.	l''-'I ml	r'O'''' 11•1		\(,fJ.Lll!lo IN•I		:U.1- I I0I		ldoplJ.. f\01		\kl:I III	m Ill	I[III	••• IAl<•I	l\l»I	f. l.\\tl	\!JWC IIIIII \hJll.tst	\lLJul\j.tf .'lo• .WJi̇ilM	Ht IAltl) r'''j'I
	5	1007	Gl	Tl	l3l	35	35	T0	l0	T	lG	IS.SO	13.I!	G.5$	15.00	IUI	G.Ti	U t W	fffi't.11	.061'
	io																	l.lt l.oi -	'£n.	.oiii-
fJtll3d!		1600	51	18	G	11	10	8	T	II	II	11.00	rn	2.08	8.50	l.11	1.1$		1.1$	
	I000	I000	13	31	11	I	2	l	I	3	l	I0.15	1$.SI	T.TG	W	7.GI	3.11	-rrtnr	SI.I d.II	.010
	5	1000	IGT	30	20	33	II	31	13	12	G3	II.Tl	20.ll	10.11	31.!5	2IJO	IQ.IS	11T 110.11	13.l t IQ.IS	.031'
01i9)	SO	001	118	.til	11	13	13	10	17	l3	3G	mo	3.41	rn	11.15	T.li	3.33	12.T t 4.11	IS.It 3.33	.03$'
	1000	1000	GO	15	II	25	is	5	▮	15	IS	IS.00	3.lt	108	14.l0	3.St	rn	G.o .m	3U t rn	.003
		103!	n0	113	2	H	2	10	▮	5		mo	41.Gl	23.80	rn	1.1i	rn	2 12123.85	3.G t o.ll	
onlrol	s	1000	155	GI	1$	IG	II	GO	53	•S	Tl	ms	IW	l.0T	5rn	17.\li	I.SI	2S.1 1 8.0T	IS.It I.SI	.012 .013'
				20	13		21			lO		13.50	W	3.28	15.15		3.13			
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fll.[111.	,-	1003	2II	S!	II	ST	T		f-48	u	80	mo	13.li	G.31	ffff	It.»	I.ii	i5.3 t t.31	1.m	.OSI
tra. f\JI.		'l0''io	IIT	3S	25	I	32	!!	33	:1	22	IW	15	3.15	23.l0	151	W	ll.7 11.75	!0.2t W	.040'
	I000	1000	105	22	IG	38	35	21	18	I	22	lW	W	3.!i	22.ts	3,$;;	1.21	10.S t !.5	IG.Gt UT	.ow
o:nrol		10 1!	lG	30	3	I0I	5	2S	i	10	I	G!.00	3U7	17.04	fo'0	W	'.fl2	27.lt 11.0I	cl t o.32	

Determination the percentage and types of chromosomal abnormalities for four mitotic stages:

The percentages of types of chromosomal aberrations were given in Tables (5-8) and represented in Figures (17-25). Both the compounds induced high percentage of aberrations at four times exposure. The kinds of aberrations were similar except ring chromosome which showed at 1 ppm after 6hrs with insecticide (Drago) and 5 ppm after 24hrs with (Dra.- Fus.), and binucleate cells which didn't showed with herbicide (Fusilade).

In treated root tips, irregular prophase was observed in almost all treatments of the herbicide (Fusilade) with a relatively high percentages which reached a maximum value of 76.7% at 1 ppm after 3hrs of treatment. Also, chromosomal stickiness was observed in almost all treatments and reached a maximum value of 66.6% at 5000 ppm after 6hrs of treatment. Metaphase had the highest percentage of abnormalities than ana-telophases after different treatments with the herbicide except 10000 ppm after 24hrs. C-metaphase was the most common type of metaphase abnormalities approximately observed in all treatments in treated root tips which reached a maximum value of 86.3% at 10000 ppm after 6hrs of treatment. Also, the herbicide induced sticky metaphase nearly at all treatments which reached a maximum value of 83.3% at 100 ppm after 12hrs of treatment. The disturbance metaphase was observed in treated root tips with the herbicide but it was less than c-metaphase and sticky metaphase which reached a maximum value of 52.9% at 1 ppm after 3hrs of treatment and decreased by increasing the applied concentration and duration time. The percentage of type of chromosomal abnormalities in anaphase was low compared with metaphase and prophase. In anaphase abnormalities, sticky anaphase was the most common of anaphase abnormalities in this study which reached a maximum value of 66.6% at 100 ppm after 3hrs of treatment. Multipolar was observed in anaphase which increased from 12.5% at 500 ppm after 3hrs to 66.6% at 5000 ppm after 24hrs of treatment. Lagging chromosome was observed which reached a maximum value of 38.8% at 50 ppm after 6hrs of treatment. The percentage of the bridge, diagonal and breaks were the lowest in anaphase.

In telophase abnormalities, the percentage of sticky telophase was increased by increasing the applied concentration and duration time which increased from 3.5% at 1 ppm after 3hrs to 71.4% at 5000 ppm after 24hrs

of treatment. Micronucleus was observed which reached a maximum value of 80% at 5000 ppm after 12hrs of treatment (fable 5).

The data in Table (6) showed that the insecticide (Drago) induced high percentage of type of chromosomal abnormalities in mitotic phases where; it caused high percentage of disturbance chromosome in prophase which increased from 13.8% at 10 ppm after 3hrs to 70% at 5000 ppm after 6hrs of treatment. Stickiness also observed in prophase at all treatments and duration times which increased from 13.8% at 10 ppm after 3hrs to 75% at 10000 ppm after 12hrs of treatment. Also, c-metaphase was the highest value of metaphase in treated root tips with the insecticide (Drago) which showed in all treatments and times, it reached a maximum value of 96.5% at 50 ppm after 24hrs of treatment. Sticky metaphase noted on metaphase but with low percentage which reached a maximum value 54.3% at 500 ppm after 3hrs and 50% at 10000 ppm after 24hrs of treatment.

The disturbance metaphase was observed in treated root tips with the insecticide which the lowest value when was compared with c-metaphase and stickiness in metaphase, it reached a minimum value of 2.5% at *5* ppm after 6hrs of treatment. In anaphase, stickiness was the most common type of anaphase abnormalities on treated root tips with the insecticide which increased from 5.8% at 10 ppm after 3hrs to 66.6% at 10000 ppm after l2hrs of treatment. Multipolar anaphase was observed which was between 9.5% at 50 ppm after 12hrs and 60% at 500 ppm after 24hrs of treatment. Bridges and diagonal anaphase were observed but were had less percentage than stickiness or multipolar. Bridges were between 2.8% at 1 ppm after 12hrs and 50% at 5000 ppm after 24hrs of treatment. Diagonal anaphase was between 2.5% at 1ppm after 3hrs and 25% at 10000 ppm after 24hrs of treatment. The percentage of lagging chromosomes was between 5% at 5000 ppm after 3hrs and 42.8% at 100 ppm after 6hrs of treatment. Breaks at anaphase were observed but had the lowest percentage which reached a minimum value of 4.7% at 50 ppm after 12hrs of treatment.

Intelophase, stickiness was showed in all treatments and duration times which increased from 18.1% at 1 ppm after 3hrs to 85.7% at 10000 ppm after 12hrs of treatment. Cells abnormalities with micronucleus observed approximately in all treatments in treated root tips which reached a maximum value of 86.6% at 1000 ppm after 24hrs of treatment. The percentage of binucleus was low in telophase. Itjust observed at 5000 ppm

after 3hrs which reached 20% and at 5, 10 ppm after 24hrs of treatment which reached 16.6%, 17.6% respectively.

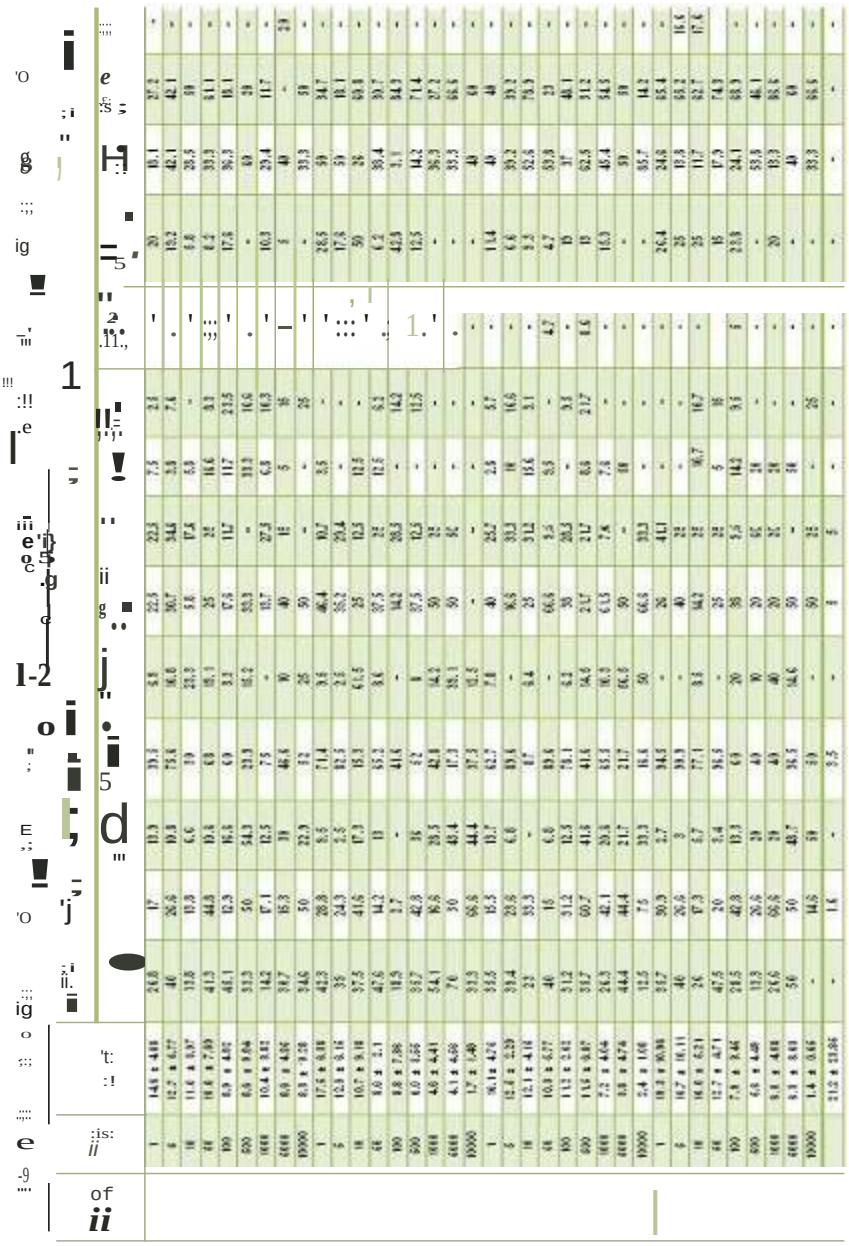
The percentage of type of chromosomal abnormalities in the combined effect of pesticides was illustrated in Table (7). The combined effect (Fus.-Dra.) increased the percentage of abnormalities in dividing cells which increased the disturbance prophase from 59.5% at 5 ppm to 71.4% at 1000 ppm after 6hrs of treatment while it was decreased from 25.4% at 5 ppm to 18.1% at 1000 ppm after 24hrs of treatment. Stickiness was observed which was increased from 20.2% at 5 ppm after 6hrs to 54.5% at 1000 ppm after 24hrs of treatment. The percentage of stickiness in metaphase was increased from 23.7% at 5 ppm to 55.3% at 1000 ppm after 6hrs of treatment. C-metaphase was showed which reached a maximum value of 72.9% at 50 ppm after 6hrs of treatment. The percentage of disturbance of metaphase was low which reached a minimum value of 6.2% at 50 ppm after 6hrs of treatment. In anaphase, the percentage of stickiness was decreased from 40% at 5 ppm after 6hrs to 19% at 1000 ppm after 24hrs of treatment. Multipolar anaphase was showed which reached a maximum value of 37.5% at 1000 ppm after 6hrs of treatment. The percentage of diagonal anaphase was increased from 5% at 5 ppm after 6hrs to 23.8% at 1000 ppm after 24hrs of treatment while the percentage of lagging chromosome was decreased from 20% at 5 ppm to 8.3% at 1000 ppm after 6hrs and from 27.7% at 5 ppm to 14.2% after 24hrs of treatment. In telophase, the percentage of stickiness was between 11.9% and 60%. The percentage of micronucleus reached a maximum value of 82.1% at 5 ppm after 24hrs of treatment. binucleus just showed at 5ppm after 6hrs and 24hr, respectively.

The percentage of type of chromosomal abnormalities in the combined effect of pesticides was illustrated in Table (8). The reverse combination (Dra. - Fus.) increased the percentage of abnormalities in dividing cells which increased the disturbance prophase from 20.4% at 5 ppm to 38.8% at 1000 after 6hrs while it was decreased after 24hrs of treatment from 43.7% at 5 ppm to 20% at 1000 ppm. On contrast the percentage of stickiness in prophase decreased after 6hrs of treatment from 55.1% at 5 ppm to 27.7% at 1000 ppm while it was increased after 24hrs of treatment from 26.5% at 5 ppm to 45% at 1000 ppm. In metaphase, c-metaphase was less than sticky metaphase which increased from 18.3% at 5ppm to 41.8 at 1000 ppm after 6hrs of treatment while it was decreased from 21.7% at 5 ppm to 14.8% at 1000 ppm after 24hrs of treatment. Disturbance was showed but the

percentage was the lowest which reached a minimum value of 7.2% at 1000 ppm after 6hrs of treatment. In anaphase, sticky anaphase was observed where the percentage increased from 33.8% at *5* ppm to 47.6% at 1000 ppm after 6hrs and from 25% at *5* ppm to 36.3% at 1000 ppm after 24hrs of treatment while multipolar anaphase decreased from 30.5% at *5* ppm to *9.5%* at 1000 ppm after 6hrs and from 21.6% at *5* ppm to 18.1% at 1000 ppm after 24hrs of treatment. The percentage of bridges and breaks were the lowest which reached a minimum value of 1.6% at 5, 50 ppm after 24hrs, respectively. Lagging chromosomal also showed which reached a maximum value of 18.1% at 1000 ppm after 24hrs of treatment. In telophase, the percentage of micronucleus was the highest value which reached a maximum value of 68.2% at *5* ppm after 24hrs of treatment. Sticky telophase also observed which between 17% and 30.3%. Binucleus was noted which just at *5* ppm (2.2%) after 6hrs of treatment.

Table (5): Frequencies of different types of prophase, metaphase, anaphase and telophase abnormalities after treating *Vicia faba* L. root tips with different concentrations of Fusilade for different period (hrs).

Time of treatment	Conc. ppm	MI% ± SE	% of prophase abnormalities		% of Metaphase abnormalities			% of Anaphase abnormalities						% of Telophase abnormalities		
			[illegible]	Stickiness	Sticky Metaphase	C-Metaphase	Disturbance	Sticky Anaphase	Multipolar	Bridge	[illegible]	[illegible]	[illegible]	Sticky Telophase	Micro-nucleus	bi-nucleus
3 hrs.	1	16.2 ± 6.59	76.7	7.1	6.8	32.3	53.9	40	-	6.6	[illegible]	[illegible]	13.3	3.5	50	-
	5	10.1 ± 6.10	53.6	19.8	-	70.2	21.6	33.3	27.7	-	[illegible]	[illegible]	22.2	16.7	28.3	-
	10	9.6 ± 6.27	66.6	17.5	24.1	44.8	16.3	50	21.4	-	[illegible]	[illegible]	14.2	5	7.6	-
	50	6.4 ± 4.79	66.6	23.3	7.1	65.7	3.5	60	20	-	[illegible]	[illegible]	-	12.5	68.7	-
	100	6.4 ± 6.28	44.7	33.4	13.4	44.4	33.3	66.6	-	-	[illegible]	[illegible]	-	21	42.8	-
	500	6.8 ± 6.66	32.5	42.5	19	38	19	37.5	12.5	12.5	[illegible]	[illegible]	12.5	31	21.4	-
	1000	7.1 ± 2.70	58.1	36.3	28.8	28.5	14.2	33.3	16.6	8.3	[illegible]	[illegible]	8.3	21.7	69.3	-
	5000	7.8 ± 4.28	52.17	17.3	7.4	74	-	26.6	-	-	[illegible]	[illegible]	20	12.5	50	-
	10000	6.8 ± 4.02	74.1	11.1	14.2	35.7	21.4	25	25	-	[illegible]	[illegible]	12.5	42.1	19.5	-
6 hrs.	1	17.8 ± 7.70	24.3	14.6	11.9	44.7	22.3	16.7	16.7	7.8	[illegible]	[illegible]	16.7	16.7	65.5	-
	5	12.8 ± 10.07	37.1	62.5	18.6	34.8	46.5	33.3	16.6	-	[illegible]	[illegible]	16.6	33.3	44.4	-
	10	19.7 ± 18.69	64.5	12.9	40.6	42.1	17.1	33.3	33.3	-	[illegible]	[illegible]	-	27.3	54.5	-
	50	8.0 ± 2.99	34.6	11.5	10.2	75.3	-	27.7	16.6	-	[illegible]	[illegible]	38.8	35.2	41.1	-
	100	8.4 ± 7.94	37.7	44.4	20.8	20.8	46.8	16.7	-	8.3	[illegible]	[illegible]	31.2	31	30	-
	500	9.1 ± 9.29	56.5	8.6	29	50	20	33.3	-	-	[illegible]	[illegible]	33.3	10.3	79.3	-
	1000	6.3 ± 9.99	56.2	4.1	15.7	21	47.3	16.6	33.3	16.6	[illegible]	[illegible]	-	5	54.5	-
	5000	4.4 ± 1.0	26	66.6	19	28.5	28.5	33.3	33.3	20	[illegible]	[illegible]	-	71.4	21.4	-
	10000	6.0 ± 4.98	25	50	13.6	86.3	-	25	25	25	[illegible]	[illegible]	-	11.1	55.5	-
12hrs.	1	14.1 ± 26.46	23.1	19.2	-	25	75	33.3	33.3	-	[illegible]	[illegible]	-	11.1	66.6	-
	5	8.7 ± 6.28	35	22.6	35	45	5	25	-	10	[illegible]	[illegible]	20	23.6	47	-
	10	8.7 ± 3.48	26	63.6	28.5	66.6	-	60	10	-	[illegible]	[illegible]	-	66.6	13.3	-
	50	4.8 ± 2.22	37.5	37.5	23.8	70.5	-	33.3	22.2	-	[illegible]	[illegible]	11.1	33.3	60	-
	100	5.7 ± 3.67	58.8	23.6	83.3	-	16.6	-	-	-	[illegible]	[illegible]	-	33.3	66.6	-
	500	3.8 ± 3.77	50	50	17.6	70.5	11.7	-	-	-	[illegible]	[illegible]	-	66.6	33.3	-
	1000	2.8 ± 4.08	50	50	6.5	56.5	22.2	-	-	-	[illegible]	[illegible]	-	51	-	-
	5000	1.6 ± 1.28	66.6	33.3	71.4	-	28.5	-	-	-	[illegible]	[illegible]	-	21	88	-
	10000	1.2 ± 1.08	50	-	50	50	-	-	-	[illegible]	[illegible]	[illegible]	-	-	66.6	-
24hrs.	1	11.6 ± 1.00	34.4	13.7	17.6	26.4	17.6	16.5	37	[illegible]	[illegible]	[illegible]	3.7	10.7	64.2	-
	5	6.2 ± 6.69	27.2	54.5	14.2	42.8	42.8	10	50	[illegible]	[illegible]	[illegible]	10	33.3	66.6	-
	10	6.1 ± 2.86	12.5	12.5	-	61.5	7.6	31.2	12.5	[illegible]	[illegible]	[illegible]	-	16.6	16.6	-
	50	6.2 ± 2.08	11.1	22.2	16.6	41.6	25	12.5	37.5	[illegible]	[illegible]	[illegible]	-	14.2	64.2	-
	100	6.2 ± 6.07	15.7	31.5	28.5	53.5	17.8	-	-	[illegible]	[illegible]	[illegible]	-	21	50	-
	500	4.2 ± 2.18	12.5	50	28.5	42.8	-	42.8	14.2	[illegible]	[illegible]	[illegible]	-	21	41.6	
	1000	4.8 ± 7.76	38.2	11.7	25	-	25	-	50	[illegible]	[illegible]	[illegible]	-	33.3	33.3	-
	5000	3.1 ± 1.02	33.3	33.3	25	37.5	25	33.3	66.6	[illegible]	[illegible]	[illegible]	-	71.4	28.5	-
	10000	1.6 ± 1.78	-	50	-	-	-	11.1	11.1	[illegible]	[illegible]	[illegible]	-	-	-	-
Control		21.2 ± 23.84	-	3.6	-	3.5	-	3	3	[illegible]	[illegible]	[illegible]	-	-	-	-



Tcble(7): Frequencies of different types of proph.ase, metaphase, anaphase andtelophate abnormalties after treating *!Acia faba* L root tips wffh different concentrations of (Dra.·Fus) for different period (his).

Tlmt or t.„t11ent	Qlno, ▮▮▮▮	1.11tC6	f p1ii°$liCfiii\i11tt–rr.1ttip1111ui\1e					%if:;;-",\p:i.rudniimillt.					Tii°C1111&u.'b11trm&11tt			
			r.q1r.11121\ 1.	atcl!tieet	Uc Mttiph'°'	CfMt'flillc.t	Cl!tltb11nc.e	ctdly Jt!,ph'1W	"11PCl11r	81dOt	C1 11cn111 ,n,,ph,Ht	Ell"t.'1'1t Am1ph.w	11100ho Chromomt'	etc ldoph11w	r.tcn:i. llllOltll'	" 1111010f1
	S	12.3 1 5.17	20.4	55.1	40.8	18.3	30.9	33.8	305		10.1		11.8	29.5	40.9	2.2
	1000															
6hrs.	S0	118 t 4.41	40	31.4	<I(	16.3	18.1	45.8	8.3		10.4	4.1	83	30	35	
		IG.G t 4.88	38.8	27.7	30.11	41.8	72	476	9.5		7.1		11.9	30.3	45.4	
	▮	11.1 1 1.07	41.7	26.5	54.3	21.7	13	25	216	1.6	20	5	15	24.7	68.2	
241Ys.	10	10.3 ' .11	31.9	29.7	2	39.2	12.5	15	25	10	16.6	1.6	13.3	17	5M	
	1000	7.1 ∎ .11	20	45	592	148	148	363	181				18.1	25	65	
ntiil	1	11.11 17.0I	2.2	11	1	2			4		8				1Aj	:_

Table (8): Frequencies of different types of prophase, metaph. e. anaphase and telophase abnormalfies after beating *Vciaf•b•* L root ips wffh different concentrations of (Fus.·Dra) for different period (hr).

llhH t f h•1tne11t	(>>M, ▮▮▮▮▮	l.lltCE	tpropb'1c.tllbllltllmll H		r t.1el'lpll11e 11bntrm1111 tt.			tf.An:ipll11c.11b11t nn1111tet.						tUtlcph11u1mcrm\llhe		
			q.lr.ill :nt. ▮▮▮	ttcllllt t.t.	Ctc MttlPMW	0.1tttipllitc.t	Cltt11b11(1&	Hd!y C.Mpllaw	l.t.11tpcl11r	a1t111•	Ct,\f,ICllll 11111111aw	a kut ;l.Mf!IMW	lllf,IQllll t11m;ccimu	He· ltltflll W	l.1C- llllGlflllt	nllGt!l
6hrs.	S	22.8 t 13.55	59.5	20.2	23.7	16.9	50.8	40			5	10	20	14.9	64.1	4.4
	S0	15.1 t UT	40.6	46.8	18.7	72.9	62	483	193		12.9		96	15	72:5	
	1000	IU t C.U	71.4	23.8	55.S	42.5		332	375	4.1	12.5		82	60	2	
241Ys.	▮	11.3 1UI	25.4	50.8	24.5	59.6		25S	25S		9.2		27.7	11.9	82.1	11
	10	1 7 ı3.71	37.1	34.2	31.7	39	73	38S	227	6.8	4.5	6.8	13.6	55.5	25.$	
	1000	16.$ ı3.37	18.1	54.5	28.9	31.5	315	19	23.8	4.7	23.8		14.2	41.6	5	
con1r1		21.1t 17.04	2.2	11	H	23			4		8				1.6	

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