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CYTOTOXIC EFFECTS OF SOME SYNTHETIC AND BIO-FUNGICIDES ON *Vicia faba* L. PLANT

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تأثير السمية الخلوية لبعض المبيدات المخلقة والحيوية على نبات الفول البلدى

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ABSTRACT

The laboratory and field experiments were carried out to estimate the cytotoxic effects of two synthetic and two bio-fungicides on parents, F1 and F2 faba bean plants. Data obtained from the direct treatment repeated experiments showed that both synthetic inorganic and bio-fungicides have lethal effects on all seeds so the recovery treatments were applied to study their effects on mitotic behaviour of *Vicia faba* parent and their F1 and F2 plants. Data showed that mitotic index (MI) values of almost all plants (seeds) treated with the three different concentrations of Dithane and Rizolex fungicides at different exposuring times were significantly lower than those of control plants. Seeds treated with 4 gm/L Dithane at 12 h exhibited the lowest value of MI (4.87%) while that value of seeds treated with Rizolex (3g/L at 24 h) was the lowest one at all (3.65%). In general, most treatments with two bio-fungicides exhibited significantly lower MI values than that of control. The MI of the treatments of Blight Stop (1.5% at 24h) and Clean Root (10% at 24h) were the lowest values 7.68% and 6.95% respectively. Values of MI of F1 seeds exhibited slight increasing than those of the parent treatments of both synthetic and bio-fungicides. On the other hand, the MI of F1 plants of the Rizolex (synthetic fungicide) were significantly lower than that of the control and it was the lowest one at all.

It was clearly observed that the treated plants exhibited significant total percentage of chromosomal aberrations except treatments of 6h at the lower two concentrations of both Dithane and Rizolex. The treatment of Dithane at 12h with the three concentrations induced a highest percentage value of chromosomal aberrations (19.7%and 19.8%) when compared with control plants. There was no significant increase in the percentages of total chromosomal abnormalities among the two bio-fungicides (Blight Stop and Clean Root) and that of the control. The values of total abnormal cells of F1 and F2 exhibited highly decrease than those of the parent treatments (both synthetic and bio-fungicides). There were no significant differences between treated and control plants in chiasma frequency/cell for all used fungicides. The treated parent plants with synthetic fungicides (Diathane and Rizolex) have a significant proportion of abnormal pollen mother cells (10.01% and 10.06%, respectively) than those of control and treated plants with bio-fungicides and there was a significant difference of the total abnormal (PMCs) in F1 plants between synthetic fungicides Dithane and Rizolex (8.68% and 10.304% respectively) compared with control (3.54%), On contrast, there was no significant effects in the percentage of total abnormal (PMCs) between bio-fungicides Blight Stop and Clean Root (5.494% and 2.587% respectively) and control plants. It could be concluded that from the cytogentical point of view the use of bio-fungicides as an alternative agricultural material in spite of the synthetic pesticides may become very safe.

INTRODUCTION

Higher plants provided a useful genetic system for screening and monitoring environmental pollutants. Mutagenic activity of chemicals has been analyzed with different plant systems such as *Allium cepa*, *Vicia faba, Arabidopsis thaliana* and *Hordeum vulgare*. Chromosomal aberration assays, mutation assays and cytogenetic tests were performed in these plant systems (Conte, et *al*., 1998; Menke, et *al*., 2001 and Zaka, et *al*., 2002). Pesticides are agrochemicals, which used for protecting plants in all growth stages of plants. The mutagenic and teratogenic effects of the pesticides on human gene pool were tested by Grant (1970 and 1971)

The cytological and genetical effect of dithane fungicides on *Allium cepa* were analysed by Mann (1977) and reported that Most of treated plants displayed abnormal chromosome behaviour. The abnormalities comprised stickiness, heteromorphic bivalents, bridges (with and without fragments) and micronuclei. Furthermore, in a few pollen mother cells, univalent and segregational errors were also observed. Similar types of abnormalities were also observed in *Vicia faba* and *Gossypzum barbadense* with the carbamate insecticide (Rogor) after treatment with organo-phosphorus insecticides (Amer and Farah, 1974; 1983 and 1985).

The treatment of wheat grains with Sevin induced mitotic abnormalities, chromosome breakage and laggards (Halwankar and Patil, 1987). Yi and Meng (2003) reported that bisulfite-sulfite solution induced mitotic delay and decreased the mitotic index in *Vicia faba* and *Allium cepa* roots and increase the rate of chromosomal abnormalities. Wang at *el* (2006) reported that herbicide amiprophose-methyl (APM) induced metaphase synchronization division cells, multipolar, bridges, fragments and micronuclei in root meristem of rye and maize. Moreover, Amer, et *al* (1987) found that spraying *Vicia faba* plants at flowering stage with cypermethrin solution induced a significant percentage of abnormal PMCs/plant .

In the present work the study of cytogenetic effects of synthetic (inorganic) pesticides (Dithane M-45 & Rizolex T-50%) in comparison with those of bio- fungicides (Blight Stop & Clean Root) was carried out on faba bean plants.

MATERIAL AND METHODS

This study was carried out in Department of Genetics, Faculty of Agriculture, Minia University in order to determine the cytotoxic effects of two different groups of agro-chemicals, which used as fungicides on *Vicia faba*. The first group is composed of two synthetic fungicides (Dithane M-45 & Rizolex-T 50%) while the second one contained two bio-fungicides (Blight Stop derived from *Trichoderma harzianum* & Clean Root derived from *Bacillus subtilis*).

1- LAB EXPERIMENTS

Seeds of pure strain of *Vicia faba* (v.Masr1) were obtained from the Field Crops Research Institute, Agriculture Research Center (ARC), Giza, Egypt. Seeds were washed and soaked for 24 h in a tap water. The germination was

carried out at 20 C^{o} in the dark. After two days, when the roots reached to 1.5-2 cm long, treated with distilled water as a control and three concentrations of all fungicide reagents were applied as a recovery experiments.

a- Inorganic (Synthetic) fungicides

1-Dithane M-45; chemical formula [Manganese Ethylenebis (dithiocarbamate) polymeric complex with zinc salt]. It was applied with three concentrations, 1, 2.5 and 4 g /L D.W.

2-Rizolex-T 50%; chemical formula is Tolclofos methyl: 0, 2, 6 dichloro-p-toly 10, 0 dimethyl phosphorothioate and Thiram : tetra methyl thiuram disulfide and used with three concentrations, 1, 3 and 5g /L D.W.

b- Organic Bio-fungicides.

1-Blight Stop is a culture filtrate from (*Trichoderma harzianum*) was used with concentrations 0.5, 1 and 1.5% in D.W.

2-Clean Root is a culture filtrate from (*Bacillus subtilis*), and used with concentrations 5, 10 and 15% in D.W.

These bio-fungicides were prepared in the Central Lab of Organic Agriculture, Agriculture Research Center (ARC) according to the method of Brain and Hemming (1945) and Dowson (1957) and the modified method of Abd-El-Moity and Shatla 1981.

Aceto-carmin squashed preparations were made from the treated fixed roots of at least 5 plants of parent, F1 and F2 plants in randomized complete design. At least 8000 cells were examined for each treatment (consisting of three seedlings).All mitotic measurements were taken on parent, F1 and F2 plants. The mitotic index was calculated as the percentage of dividing cells to the total number of cells examined. The frequency of each mitotic phase was calculated as the percentage of cells in that stage to the total number of dividing cells. The same slides were analyzed for the percentage and types of the chromosomal abnormalities in cells at each mitotic phase as well as non-dividing cells. The analysis of variances was made according to Gomes and Gomes (1984) using MSTAT program (Version 4) was applied.

2-FIELD EXPERIMENT (SEASON 2006-2007)

The concentrations and methods of pesticide treatments in field experiment were carried out according to the recommendations of Egyptian Ministry of agriculture (2004-2005). The treatments were distributed in plots in Randomized Complete Block Design (RCBD). The flowering buds of the plants were gathered and fixed in a fresh fixative solution of ethanol / glacial acetic acid (3: 1 ,v / v).

Cytological preparations of PMCs were made using the Aceto-carmine smear methods. At least 900 dividing cells were examined and chromosomal abnormalities in both first and second meiotic divisions were scored. The rest of plants were allowed to produce seeds. Cytological data of F1 seeds were obtained from the above described methods. The data were statistically analyzed (in RCBD) using MSTAT program (Version 4).

3-FIELD EXPERIMENT (SEASON 2007-2008)

F1 seeds were cultivated and the flowering buds were taken from them to make F1 meiotic analysis. The F1 plants were not treated with any pesticides. The F2 seeds were a successfully taken to carry out the mitotic analysis of them in order to study the genetical transmission of mitotic aberrations.

RESULTS

1- LABORATORY EXPERIMENT

The laboratory experiment was carried out to estimate the cytotoxic effects of two synthetic and two bio-fungicides on parents, F1 and F2 faba bean plants. Mitotic index and mitotic irregularities were measured for three concentrations of each fungicide. The control measurements were taken from the plants treated with water. In general; data obtained from the direct treatment in repeated lab. experiments showed that both synthetic inorganic and bio-fungicides have a lethal effects on all seeds so the recovery treatments were applied to study their effects on mitotic behaviour of *Vicia faba* L. parent and their F1 and F2 plants.

1.1. THE MITOTIC INDEX (MI)

1.1.1. MI in the parent seeds

Data in table (1) showed that MI values of almost all treated plants with Dithane and Rizolex fungicides at different exposuring times were significantly lower than those of control plants. Seeds treated with 4 g/L Dithane at 12 h exhibited the lowest value of MI (4.87%) while that value of seeds treated with Rizolex (3g/L at 24 h) was the lowest one at all (3.65%). The prophase index was not affected by treatment of any of the studied synthetic fungicides. The metaphase index was also not affected by these treatments except those of Dithane 4g/L at 12h (26.96%) and Rizolex 1gm/L at 12h (32.35%). The ana-telophase index values were insignificantly affected with treatments of both Dithane and Rizolex (except those of Rizolex with 1and 3 g at 12h).

Table (1): Mitotic index obtained from treatments with three concentrations of two inorganic pesticides on faba been parent plants.

Treatments	Time	Conc.	Total No. of cells	Prophase	Metaphase	Ana. & Telophase	M I
Control		0	11759	53.53	18.77	27.70	14.9
Dithane	**6**	1g /L	8249	47.41	22.10	30.49	11.24
		2.5 g / L	9867	56.72	16.65	26.64	9.777
		4 g / L	7508	52.20	25.28	22.52	9.47
	12	1g /L	19083	53.58	25.54	20.88	9.423
		2.5 g / L	7624	46.34	25.99	27.68	9.363
		4 g / L	19962	51.64	26.96	21.41	4.867
	24	1g /L	20554	49.47	20.22	30.31	6.99
		2.5 g / L	12140	59.80	16.78	23.42	5.037
		4 g / L	23213	51.75	13.76	34.49	4.393
Rizolex	**6**	1g /L	16056	58.59	19.08	22.33	5.423
		3g /L	15008	52.11	17.93	29.96	7.207
		5g /L	14831	54.03	21.27	24.70	5.347
	12	1g /L	10904	51.51	32.35	16.14	8.17
		3g /L	21579	53.25	27.44	19.31	5.377

		5g /L	24222	54.39	18.72	26.89	4.18
	24	1g /L	19661	44.72	19.29	35.99	5.493
		3g /L	18198	51.86	16.96	31.18	3.65
		5g /L	17722	48.96	20.40	30.64	5.447
L.S.D. =				8.421	6.213	7.732	2.984

Data in table (2) showed that the mitotic index values of treated parent seeds with three concentrations of two bio-fungicides (Blight Stop and Clean Root) at three exposuring times. In general, most treatments exhibited MI values that were significantly lower than control. The MI of the treatments of Blight Stop (1.5% at 24h) and Clean Root (10% at 24h) were the lowest values (7.68% and 6.95% respectively). There were no significant effects of any concentration at any exposuring time of these bio-fungicides on the percentages of the mitotic phases (prophase, metaphase and ana-telophase).

Table (2): Mitotic index obtained from treatments with three concentrations of two bio-pesticides on faba been parent plants.

Treatments	Time	Conc.	Total No. of cells	Prophase	Metaphase	Ana. & Telophase	M I
Control		0	11759	53.53	18.77	27.70	14.9
Blight Stop	6	0.5%	22742	52.51	20.13	27.36	9.49
		1%	22041	52.17	23.72	24.12	12.28
		1.5%	20574	55.83	20.94	23.24	9.49
	12	0.5%	25053	50.29	21.93	27.78	8.86
		1%	23234	51.41	22.53	26.06	10.04
		1.5%	28268	54.50	19.84	25.67	9.053
	24	0.5%	30529	54.84	23.19	21.97	8.587
		1%	43173	52.02	21.42	26.57	7.77
		1.5%	38627	53.33	24.74	21.94	7.68
Clean Root	6	5%.	15587	53.49	18.53	27.98	12.13
		10%	15324	54.39	19.19	26.42	10.2
		15%	15181	49.17	23.60	27.23	11.05
	12	5%	13031	47.11	24.75	28.14	10.88
		10%	13337	47.22	25.49	27.29	10.79
		15%	15354	49.02	25.00	25.98	10.11
	24	5%	12542	47.89	26.36	25.75	8.23
		10%	13714	57.62	19.53	22.85	6.95
		15%	19689	48.09	22.10	29.81	10.44
L.S.D. =				**8.421**	**6.213**	**7.732**	**2.98**

1.1.**2 MI of F1 and F2**

Data in table (3) showed that values of MI of F1 seeds exhibited slight increasing than those of the parent treatments of both synthetic and bio-fungicides. On the other hand, the MI of F1 plants of the Rizolex (synthetic fungicide) were significantly lower than that of the control and it was the lowest one at all. The index values of the mitotic phases (prophase, metaphase and ana-telophase) of F1 and F2 plants were insignificantly changed when compared with control plants. F2 plants exhibited the lowest

MI value of Dithane treatments (5.96%). The MI values of F1 and F2 plants were insignificantly changed on decreased from that of the control plants.

Table (3): Mitotic index obtained from treatments with four pesticides on faba bean f1and F2 plants.

Treatments	F1					F2				
	Total No. of cells	Pro.	Meta.	Ana. & telo.	MI	Total No. of cells	Pro	Meta.	Ana. & telo	MI
Control	15388	53.45	17.34	29.21	11.73	17273	56.38	19.14	24.48	11.49
Diathane	14647	52.12	20.23	27.65	10.90	23937	64.12	17.68	18.20	5.96
Rizolex	15428	55.12	19.16	25.72	7.13	17280	55.99	20.59	23.41	9.42
Blight Stop	17416	55.04	19.75	25.22	8.96	18295	59.72	18.23	22.05	11.02
Clean Root	16079	61.08	16.41	22.51	9.56	19242	57.43	18.10	24.48	10.59
L.S.D.=		**8.49**	**3.39**	**7.04**	**3.92**		**9.24**	**5.05**	**5.41**	**4.98**

1.2. THE MITOTIC ABERRATIONS

1.2.1. Mitotic aberrations of parent plants

Table (4) showed the percentages in parent plants with mitotic abnormalities such as lagging chromosomes, Chromatid Bridge during anaphase, chromatin fragments during the different mitotic stages, outside chromatin and chromosome stickiness (Fig.1).

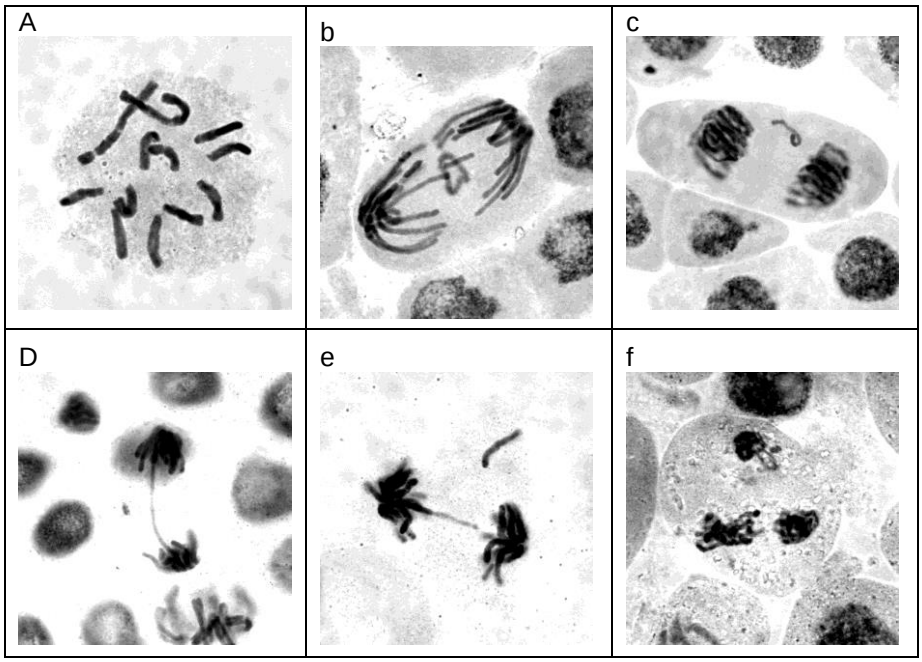


Fig (1) some of chromosome abnormalities at different mitotic stages of *Vicia faba* meristemic cells treated with four different fungicides: (a) normal metaphase, (b)lagging chromosome at

anaphase,(c)lagging chromatid at telophase, (d) fragment and bridge at telophase, (e) lagging and bridge at telophase, (f) cell with tripoler telophase

Table (4): The mean percentages of obtained mitotic irregularities in faba bean plants treated with two inorganic pesticides.

Treatments	Time	Conc.	Total No.	Lag.	Brid.	Frag.	Out.	Stick.	Tripoler	Total abn.	Micro.
Control		0	11759	0.46	0.25	0.36	0.06	0.19	-	1.31	0.39
Dithane	6	1	8249	0.64	2.04	0.21	0.21	0.31	-	3.41	0.96
		2.5	9867	0.46	0.74	0.74	0.45	0.19	-	2.67	1.28
		4	7508	1.55	1.97	0.96	0.79	0.88	0.58	6.74	2.13
	12	1	19083	2.32	6.52	1.10	0.66	0.90	3.47	14.98	0.46
		2.5	7624	4.75	3.96	3.34	0.43	0.90	6.45	19.82	0.33
		4	19962	5.37	3.37	4.14	1.69	1.68	3.46	19.72	1.27
	24	1	20554	2.28	1.62	0.84	0.27	0.19	0.55	5.75	1.02
		2.5	12140	3.42	1.58	1.20	0.50	1.62	0.70	6.72	1.32
		4	23213	3.35	0.93	1.60	0.35	1.19	1.14	8.55	1.10
Rhizolex	6	1	16056	0.77	0.20	1.48	0.11	0.35	-	3.01	0.38
		3	15008	1.17	0.51	0.65	0.20	0.17	-	2.77	0.57
		5	14831	3.06	0.25	1.85	0.14	0.40	-	5.82	0.46
	12	1	10904	3.00	1.14	2.01	0.41	0.18	4.39	11.13	0.25
		3	21579	1.96	1.88	1.43	0.55	0.27	2.85	8.94	0.14
		5	24222	1.92	1.66	1.97	0.51	0.67	3.22	9.97	0.19
	24	1	19661	1.08	0.62	0.53	0.30	0.52	1.69	4.73	1.03
		3	18198	1.76	1.11	0.70	0.51	1.49	2.74	8.29	0.88
		5	17722	3.70	1.48	2.70	0.72	1.17	1.17	10.94	1.36
L.S.D. =				1.52	0.92	0.90	0.40	0.25	1.29	2.65	0.519

The cells with micronuclei were scored at all stages of the meristemic tissue including the interphase (Fig. 2). It was clearly observed that the treated plants exhibited significant total percentage of chromosomal aberrations (except treatments of 6h at the lower two concentrations of both Dithane and Rizolex). The treatment of Dithane at 12h with the three concentrations induced the significant and highest percentage value of chromosomal aberrations (19.7%and 19.8%) when compared with control plants. The concentrations 1g/L at 12h and 5g/L at 24h of Rizolex fungicide induced the significant and higher percentage of chromosomal abnormalities (11.13% and 10.94%) than that of control parents (1.31%). The percentage of micronuclei reached a maximum of (2.13%) after treatment for 6hr with Dithane 4g/L. It has been observed that percentages of micronuclei were significantly increased after treatments at 24h with both Dithane and Rizolex. In general, the percentage of laggards, bridges, fragments, outside chromosome and stickiness were increased by increasing concentrations and duration of treatments.

Data in table (5) showed that there were no significant increase in the percentages of total chromosomal abnormalities among the two bio-fungicides (Blight Stop and Clean Root) and that of the control. Some treatments induced significant value of micronuclei than that of control plants (e.g. blight stop at 6 h). In general, the percentages of abnormal mitotic cells and the induction of micronuclei were higher in the parent plants treated with synthetic (inorganic) than those of bio-fungicides.

Table (5): The mean percentages of obtained mitotic irregularities in faba bean plants treated with two bio-pesticides

Treatments	Time	Conc.	Total No. of cells	Lag.	Bridges	Frag.	Total abnor.	Micron.
Control		0	11759	0.46	0.25	0.36	1.31	0.39
Blight Stop	6	0.5%	22742	0.80	0.42	0.28	1.68	1.32
		1%	22041	0.63	0.68	0.44	1.75	1.17
		1.5%	20574	0.67	0.84	0.49	2.05	1.05
	12	0.5%	25053	1.43	0.80	0.49	2.83	0.69
		1%	23234	0.61	0.21	0.64	1.46	0.76
		1.5%	28268	1.02	0.28	0.63	2.00	1.16
	24	0.5%	30529	0.37	0.74	0.54	1.65	0.45
		1%	43173	0.45	1.05	0.38	2.04	0.43
		1.5%	38627	0.68	0.97	0.33	2.08	0.75
Clean Root	6	5%	15587	0.50	0.27	0.53	1.80	0.74
		10%	15324	0.51	0.33	0.31	1.53	0.62
		15%	15181	0.11	0.39	0.24	1.04	1.02
	12	5%	13031	0.45	0.29	0.29	1.32	0.98
		10%	13337	0.81	0.87	0.64	2.38	0.87
		15%	15354	0.75	1.22	0.15	2.17	0.86
	24	5%	12542	0.51	1.21	0.47	2.19	0.74
		10%	13714	0.26	0.81	0.66	2.00	0.51
		15%	19689	0.61	1.21	0.70	2.63	0.41
L.S.D. =			**1.52**		**0.92**	**0.90**	**2.65**	**0.519**

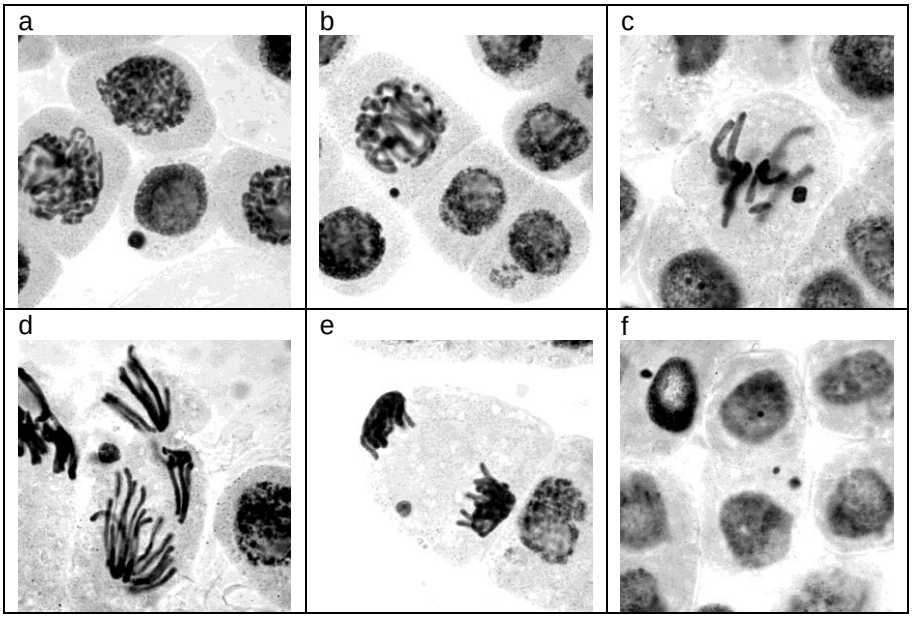


Fig (2) Micronucleus at interphase and its appearance at other different mitotic stages of *Vicia faba* root cells treated with four different fungicides: (a) micronucleus at interphase, (b) micronucleus at prophase, (c) micronucleus at metaphase, (d)micronucleus at anaphase, (e) micronucleus at telophase, and (f) two micronuclie in interphase.

1.2.2. Mitotic aberrations of F1 and F2 plants.

Data in table (6) showed that values of total abnormal cells of F1 and F2 exhibited highly decrease than those of the parent treatments (both synthetic and bio-fungicides). The significance of F1 data was determined by statistical analysis and showed that all F1 plants have lower abnormal cells than that of parent treatments. The F2 plants of Dithane and Rizolex treatments exhibited significant and the highest value of micronuclei (2.48% and 2.08%) when compared with that of control and bio-fungicide plants (0.7%).

Table (6): The mean percentage of obtained mitotic irregularities in faba bean F1& F2 treated with four pesticides.

Treatments	F1					F2				
	Lage	Bridge	Frag.	Total Abnor.	Micro	Lage	Bridge	Frag.	Total abn.	Micro.
Control	0.49	0.24	0.11	1.26	0.01	0.32	0.12	0.16	0.60	0.70
Diathane	0.49	0.64	0.43	1.76	0.14	0.49	0.30	0.23	1.18	2.48
Rizolex	0.35	0.69	0.15	1.44	0.33	0.78	0.44	0.11	1.49	2.08
Blight Stop	0.42	0.27	0.15	0.89	0.13	0.36	0.09	0.05	0.50	1.39
Clean Root	0.38	0.27	0.29	0.94	0.23	0.30	0.05	0.05	0.44	0.71
L.S.D.=	**0.37**	**0.66**	**0.43**	**0.74**	**0.35**	**0.48**	**0.22**	**0.32**	**0.52**	**0.80**

2. FIELD EXPERIMENT

2.1. Meiotic behavior

Chiasma frequency

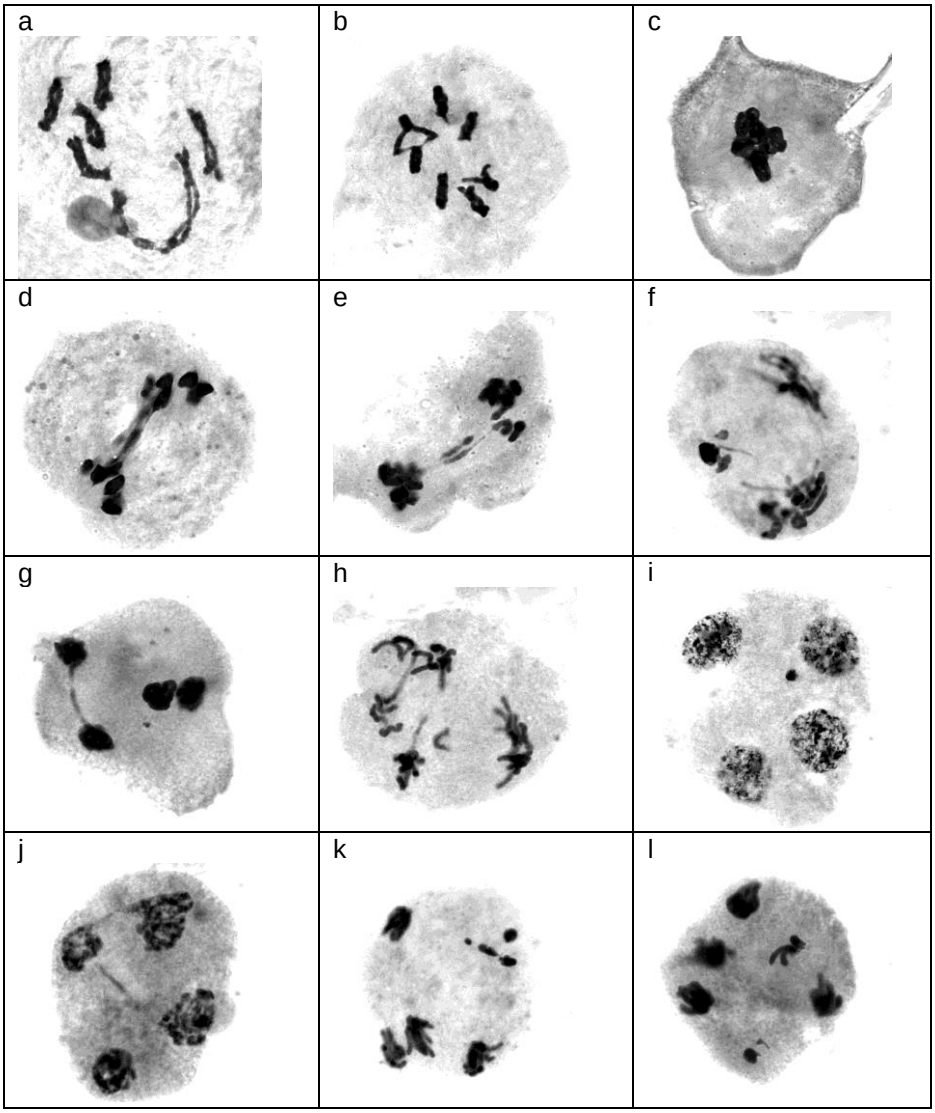
Data in table (7) showed the mean frequency of chiasmata per cell at both diakinesis and metaphase I after treatments with synthetic and bio-fungicides in parent and their F1 plants. There were no significant differences between treated and control plants in chiasma frequency/cell for all used fungicides. It was clearly observed that the mean frequency of chiasmata/cell was higher at diakinesis than that of metaphaseI in all tested plants.

Table (7): Chiasma frequency in parent and F1 plants:

Treatment	Parent		F1	
	Dickinesis	Metaphase I	Dickinesis	Metaphase I
Control	20.84	17.74	19.28	15.70
Dithane	20.60	17.87	18.75	14.90
Rizolex	21.27	18.07	18.43	15.20
Blight Stop	20.81	17.83	18.47	15.50
Clean Root	19.97	17.73	18.67	15.60
L.S.D =	**2.60**	**2.17**	**0.86**	**0.86**

2.2. Meiotic aberrations of parent plants.

Table (8) showed that treated parent plants with synthetic fungicides (Diathane and Rizolex) have a significant proportion of abnormal pollen mother cells (10.01 and 10.06, respectively) than those of control and treated plants with bio-fungicides. However, there were no significance differences between total meiotic aberrations of treated plants with bio-fungicides and that of control.



Fig(3) Meiotic abnormalities in *Vicia faba* PMCs treated with four different fungicides: (a) and (b) normal diakinesis and metaphase I , (c) stickiness at metaphase I, (d) bridge in anaphase I, (e)double bridges in anaphase I,(f) outside in metaphase II, (g) telophase II with bridge, (h) anaphase II with lagging and bridge, (i) micronucleus at telophase II, (j) double bridge at telophase II, (k) micronuclei at anaphase II, and (l) lagging at telophase II.

Table (8): Percentage of types of abnormalities occurring in the meiosis of Vicia faba parent plants after treated by four pesticides. The percentages of uni and multi-valent were not included in the total abnormalities.

Treatments	Uni & multivalent	Lag	Bridge	Frag.	Outside	Stick.	Tripolar	Total abnor.	Micro.
Control	2.600	0.463	0.177	0.463	0.280	2.330	-	4.396	0.683
Diathane	10.260	1.467	1.240	0.953	0.423	1.067	1.707	10.01	3.153
Rizolex	3.117	2.803	0.480	0.767	0.877	1.113	1.217	10.06	2.803
Blight Stop	4.297	2.200	0.627	0.593	0.147	0.640	0.193	6.277	1.877
Clean Root	5.673	2.907	0.950	0.397	0.367	0.660	-	7.141	1.860
L.S.D. =	6.481	1.779	1.165	0.774	0.712	1.278	1.131	4.379	1.690

The observed meiotic abnormal (PMCs) included laggards, bridges, outside chromosome, stickiness, tripoler cells and micronuclei. In addition, the micronuclei values were also highly significant at PMCs of treated plants with synthetic fungicides than that of control whereas there were insignificant differences between treated plants with bio-fungicides and control plants. The treated plants with Diathane (synthetic fungicide) exhibited significant asymmetric synapsis of meiotic chromosomes (10.26% uni and multivalents).

2.3. Meiotic aberrations of F1 plants

Table (9) showed that there was a significant difference of the total abnormal (PMCs) in F1 plants between synthetic fungicides Dithane and Rizolex (8.68% and 10.304% respectively) and that of the control (3.54%), on contrast there was no significant effects in the percentage of total abnormal (PMCs) between bio-fungicides Blight Stop and Clean Root (5.494% and 2.587% respectively) and control plants. The high percentage (13.52%) of uni and multivalent in meiosis I (metaphase I and Dickinesiss) was recorded after treatment with Dithane (synthetic fungicide).

Table (9): Percentage of types of abnormalities occurring in the meiosis of Vicia faba F1 plants after treated by four pesticides. The percentages of uni and multi-valent were not included in the total abnormalities.

Treatments	Uni & multivalent	Lag.	Bridge	Frag.	Outside	Stick.	Tripolar	Total abnor.	Micro.
Control	2.393	0.690	0.930	0.637	0.637	0.250	0.150	3.540	0.250
Diathane	13.520	1.500	1.690	0.390	0.190	2.443	1.900	8.68	0.567
Rizolex	3.740	2.120	0.700	1.373	0.370	0.947	1.877	10.304	2.917
Blight Stop	1.347	0.547	0.897	1.230	0.867	0.270	1.103	5.494	0.580
Clean Root	1.170	0.640	0.370	0.637	0.140	0.073	0.587	2.587	0.140
L.S.D. =	4.219	0.737	2.633	0.737	0.794	1.454	1.207	2.478	2.219

DISCUSSION

The safe agriculture is very important to the health and life of people worldwide. Using the agrochemicals particularly those synthetic pesticides is very dangerous and may cause tumors, cancers and teratogenic

abnormalities (Grant, 1970 and 1971). In the recent years some alternative bio- products were used as safe pesticides in agriculture (Abd-El-Moity and Shatla, 1981). In the present study the cytotoxic effects of bio-fungicides (Blight Stop and Clean Root) were examined in comparison with two partner inorganic (synthetic) fungicides (Diathane M-45 and Rizolex T-50%). A repeated preliminary laboratory experiments revealed the impossibility of studying the cytotoxic effects of the mentioned pesticides in direct treatments because of their lethal and damaging effects on *Vicia faba* root tips. However, the *in vitro* recovery treatments with both synthetic and bio-fungicides in three concentrations at three times on *Vicia faba* root tips revealed that mitotic index (MI) and sometimes the mitotic stage ratios are deeply affected. So the inhibition of mitotic division in plants has been attributed to a number of factors (Shehata *at el.* 2000 and Deysson, 1968). The two major reasons are the inhibition of both protein synthesis (Kim and Bendixen, 1987) and DNA amount and replication (Beu *et al.,* 1976, Badr, 1983). DNA content and the mitotic index in the root meristem are negatively correlated. The higher proportion of cells entering mitosis in the meristem of plants with a lower amount of DNA is not the result of alterations of the duration of the mitotic cycle, which was found to be quite comparable with largely differing genome sizes (Minelli *et al.,* 1996). Results also revealed the transmitted depression effects of Rizolex in F1 seeds and Diathan in F2 leading to think that the cytotoxic effects of these synthetic fungicides not only accumulative but may also genetically and cytologicaly inherited. It should be noted that the two studied bio-fungicides has no heritable and/or cytological transmission of damaging effects to F1 and F2.

The mitotic chromosomal aberrations which included chromatin bridges, laggards, fragments, stickiness and micronuclei were also scored in the parent, F1 and F2 plants in order to assess the cytogenetical damaging effects of both synthetic and bio-fungicides. Results obtained herein assure that great values of damaging effects were induced either in treated parent with synthetic fungicides or in their F1 and F2 plants. In general, there were no scored numerical aberrations in any fungicide treatments. The rate of chromosomal aberrations induced by bio-fungicides treatments was considerably lower than that of synthetic fungicides. The structural chromosomal aberrations were the most common abnormalities in both mitosis and meiosis of the present materials. Similar results were obtained in *Vicia faba* after using the two pesticides Malathion and Tamaron (Ebad *et al.,* 1990) and herbicide glean (Badr and Ibrahim, 1987b). The chromosomal aberration might be induced by the following ways: Firstly, chemical compounds directly affect DNA and lead to chromosomal aberration. Secondly, chemical compounds could disturb the synthesis of DNA and protein, or translation of RNA, so that no materials relating to the chromosomal movement could be formed, and the chromosomal aberration occurred eventually. Thirdly, chemical compounds can prevent the re-establishment of chromosome under normal conditions through interfering with the normal repairing of some damages to the new re-fusions, such as the rearrangement of chromosomal bridges, loops and fragments (Qian, 2004).Laggards and bridges represented the most common types of both

mitotic and meiotic abnormalities. The induction of laggard could be attributed to the failure of the spindle apparatus to organize and function in a normal way rather than inhibition of these spindle fibers and this may lead to irregular orientation of chromosomes (Grant, 1978; Mansour, 1984 and Patil and Bahat, 1992).

Induction of chromosomal and chromatin bridges at anaphase and telophase stages was also observed after treatment with the different four fungicides. These bridges may result from chromosome stickiness (Abraham and Koshy, 1979 and Badr, 1983). Due to such stickiness the separation of daughter chromosomes becomes incomplete even in the presence of spindle fibers and thus they remain connected by chromatin bridges (Kabarity *et al*, 1974).. Bridges may also result from breakage of chromosomes followed by proximal chromatid reunion, which evidently results in dicentric chromosomes and from characteristic anaphase bridges (Grant, 1978 and Tomkins and Grant, 1972).The stickiness of chromosomes may cause incomplete separation of daughter chromosomes as a result of cross- linkage of chromoproteins (Kong and Ma, 1999). This led to subchromatid connections between chromosomes and thus they remained connected by bridges (McGill *et al*., 1974; Klasterska *et al*., 1976; Badr *et al.,* 1992).

Micronuclei are true mutagenic aspects with many lead to a loss of genetic material. This mutagenic effect was estimated as a percentage of micronuclei formed in interphase (Ronchi *et al.*, 1986). The micronuclei forms in two ways: the first , the chromosomal fragments formed in the last G2 could not act in phase with normal chromosomes, and are rejected to the outside of nuclei in interphase. The second is the occurrence of various forms of lagged chromosomes, non-equatorial plane aggregated chromosomes, and the chromosomal grouping (Li, 1997). In general, the induction of micronuclei in root meristmic cells is the manifestation of chromosome breakage and disturbance of the mitotic process due to spindle abnormalities (Dash *et al.,* 1988; Grover and Kaur, 1999). Micronuclei were considered an indication of a true mutation effect (Auerbach, 1962), thus, the high percentage of micronuclei induced by the studied fungicides indicate their mutagenic ability. The chiasma frequency per cell was not affected by the treatments with both synthetic and bio-fungicides in parent and F1 plants whereas the frequency of uni and multi-valents are significantly affected in Diathane treatment either in parent or in F1 plants. These data might due to the disturbance in genetic control of pairing.

It could be concluded that from the cytogentical point of view the use of bio-fungicides as an alternative agricultural material in spite of the synthetic pesticides may become very safe.

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CYTOTOXIC EFFECTS OF SOME SYNTHETIC AND BIO-FUNGICIDES ON *Vicia faba* L. PLANT

تأثير السمية الخلوية لبعض المبيدات المخلقة والحيوية على نبات الفول البلدى

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في تجربة حقلية و معملية تم دراسة السميه الخلوية لاثين من المبيدات الفطريه المخلقه (الكيمائية) واثنين من المبيدات الحيويه (راشح فطر الترايكودرما وبكتيريا الباسيلس) على الاباء والجيل الاول والجيل الثانى لنباتات الفول البلدى. وأظهرت النتائج المتحصل عليها من التجارب المتكررة للمعامله المباشره ان كلا من المبيدات الفطريه المخلقه (الغير عضويه) و الحيوية لها تأثير قاتل على كل البذور لذلك تم تطبيق معاملات الإشفاء (recovery) لدراسه تاثيرها على السلوك الميتوزى للاباء والجيل الاول والجيل الثانى لنباتات الفول البلدى. ولقد اظهرت النتائج أن معدل الانقسام الميتوزى (MI) لمعظم النباتات التى عوملت بثلاثه تركيزات مختلفه من المبيدين الفطريين الدياثان و الريزولكس فى اوقات تعريض مختلفه كان لها معنويه منخفضه عن تلك الخاصه بالكنترول. ولقد وجد أن المعامله بالدياثان بتركيز ٤جرام/ لتر لمده ١٢ ساعه اقل قيمه للـ MI (٤,٨٧%) بينما معامله البذور بالريزولكس ٣جرام/ لتر لمده ٢٤ ساعه اعطت اقل قيمه للـ MI(٣,٦٥%). وعامة أظهرت اغلب المعاملات بالمبيدات الحيويه معنويه منخفضه لقيم MI عن تلك الخاصه بالكنترول. وكانت قيم MI للمعاملات البليت ستوب (١,٥% لمده ٢٤ ساعه) و الكلين روت (١٠% لمده ٢٤ ساعه) الاقل (٧,٦٨% و ٦,٩٥% على التوالى). على الجانب الأخر اظهرت قيم الـ MI لبذور الجيل الاول زياده بسيطه عن تلك الناتجه من معاملات الاباء لكل من المبيدات الفطريه المخلقه والحيويه. من الناحيه الاخرى, أظهرت النتائج أن قيم الـ MI لنباتات الجيل الاول الخاصه بالريزولكس(مبيد فطرى مخلق) كانت منخفضة معنوياعن تلك الخاصه بالكنترول وكانت الاقل قيمه.

ولقد لوحظ أن النباتات المعامله (ماعدا المعاملات ٦ ساعات للتركيزين المنخفضين لكلا من الدياثان والريزولكس) اظهرت زيادة معنويه فى النسبه الكليه للشذوذات الكروموسوميه عن الكنترول. واحدثت المعامله بالدياثان لمدة ١٢ ساعه للثلاثة تركيزات زيادة معنويه فى نسبه الشذوذات الكروموسوميه(١٩,٧% و ١٩,٨%) عند مقارنتها بالكنترول. وكانت هناك زياده غير معنويه فى النسبه الكليه للشذوذات الكروموسوميه بين كل من المبيدين الحيويين (البليت ستوب و الكلين روت) بالكنترول. ولقد اظهرت القيم الكليه للخلايا الشاذة فى الجيل الاول والثانى انخفاض عالى عن تلك الخاصه بمعاملات الاباء (كلا من المبيدات الفطريه المخلقه والحيويه).

ولقد اظهرت النتائج أن هناك اختلافات غير معنويه بين النباتات المعامله بكل المبيدات الفطريه المستخدمه والكنترول فى تكرارات الكيازما لكل خليه. واعطت نباتات الاباء المعامله بالمبيدات الفطريه المخلقه نسبه عالية المعنويه من الخلايا الاميه لحبوب اللقاح الشاذه ١٠,١١و ١٠,٠٦% على التوالى عن تلك الخاصه بالكنترول و النباتات المعامله بالمبيدات الفطريه الحيويه. وأظهرت نباتات الجيل الأول الناتجة من الأباء المعاملة بالمبيدات الفطريه المخلقه الدياثان والريزولكس (٨,٦٨% و ١٠,٣٠٤% على التوالى) نسبة عالية المعنوية من الشذوذات الميوزية عن نباتات الكنترول(٣,٥٤%). على العكس من ذلك لم يكن هناك تأثير فى نسبه الشذوذات الميوزية بين المعاملة بالمبيدات الحيوية البليت ستوب و الكلين روت (٥,٤٩٤و % ٢,٥٨٧) على التوالى

من وجهة النظر الوراثية السيتولوجية يمكن أن يخلص إلى أن استخدام المبيدات الحيوية يكون أكثر أمنا فى الإستخدامات الزراعية

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