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

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ABSTRACT

Experiments were carried out in laboratory and field to estimate the cytotoxic effects of two synthetic and two bio-fungicides on parents, F١ and F٢ faba bean plants. Lab. experiments showed that both synthetic inorganic and bio-fungicides have a lethal effect on all seeds so the recovery treatments were applied to study their effects on mitotic behaviour of *Vicia faba* parent and their F١ and F٢ plants. Data showed that mitotic index (MI) values of treated plants (seeds) were significantly lower than those of control plants. Seeds treated with ٤ gm/L Dithane at ١٢ h exhibited a low value of MI (٤.٨٧%) while that value of seeds treated with Rizolex (٣gm/L at ٢٤ h) was the lowest one (٣.٦٥%). The MI of the treatments of Blight Stop (١.٥٪ at ٢٤h) and Clean Root (١٠٪ at ٢٤h) were the lowest values (٧.٦٨% and ٦.٩٥% respectively). Values of MI of F١ seeds exhibited slight increasing than those of the parent treatments of both synthetic and bio-fungicides.

On the other hand, It was clearly observed that the treated plants exhibited significant total percentage of chromosomal aberrations (except treatments of ٦h at the lower two concentrations of both Dithane and Rizolex). The treatment of Dithane at ١٢h with the three concentrations induced the significant and highest percentage value of chromosomal aberrations (١٩.٧%and ١٩.٨%) when compared with that of 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 F١ and F٢ 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 (١٠.٠١٪ and ١٠.٠٦٪, respectively) than those of control and treated plants with bio-fungicides and there was a significant difference of the total abnormal (PMCs) in F١ plants between synthetic fungicides Dithane and Rizolex (٨.٦٨٪ and ١٠.٣٠٤٪ respectively) and that of the control (٣.٥٤٪), on contrast there was no significant effects in the percentage of total abnormal (PMCs) between bio-fungicides Blight Stop and Clean Root (٥.٤٩٤٪ and ٢.٥٨٧٪ respectively) and control plants. From the cytogentical point of view, results suggested that the use of bio-fungicides as an alternative agricultural material in spite of the synthetic pesticides may be more safety.

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*., ١٩٩٨; Menke, et *al*., ٢٠٠١ and Zaka, et *al*., ٢٠٠٢). 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, (١٩٧٠ and ١٩٧١)

The cytological and genetical effect of Dithane fungicides on onion (*Allium cepa*) were analysed by Mann (١٩٧٧). Most of treated onion 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 segregation errors were also observed by Mann (١٩٧٧). Similar types of abnormalities were also observed in *Vicia faba* and *Gossypzum barbadense* with the carbamate insecticide after treatment with organo-phosphorus insecticides (Amer and Farah, ١٩٧٤; ١٩٨٣ and ١٩٨٥).

The treatment of wheat grains with Sevin was done by Halwanker and patil (١٩٨٧) and induced mitotic abnormalities (chromosome breakage and laggards). Yi and Meng (٢٠٠٣) 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* (٢٠٠٦) reported that herbicide amiprophose-methyl (APM) induced metaphase synchronization division cells, multipolar, bridges, fragments and micronuclei in root meristem of rye and maize.

Amer, et *al* (١٩٨٧) found that spraying *Vicia faba* plants with cypermethrin solution induced a significant percentage of abnormal PMCs/plant after spraying the plants at flowering stage.

In the present work the study of cytogenetic effects of synthetic (inorganic) pesticides (Dithane M-٤٥ & Rizolex T-٥٠٪) 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-٤٥ & Rizolex-T ٥٠٪) while the second one contained two bio-fungicides (Blight Stop derived from *Trichoderma harzianum* & Clean Root derived from *Bacillus subtilis*).

١-LAB EXPERIMENTS

Seeds of pure strain of *Vicia faba* (v.Masr١) were kindly obtained from the Crop Research Institute, Agriculture Research Center (ARC), Giza, Egypt. The dry seeds were washed and soaked for ٢٤ h in a tap water. The germination was carried out at ٢٠ C^{o} in the dark. After two days, when the roots grew to ١.٥-٢ Cm long, treatments with distilled water as a control and three concentrations of each fungicide reagents were applied as a recovery experiments.

a- Synthetic fungicides

The chemical structure and concentrations of the inorganic (synthetic) fungicides is as fallow:-

١-Dithane M-٤٥; chemical formula [Manganese Ethylenebis (dithiocarbamate) polymeric complex with zinc salt]. It was applied with three concentrations, (١, ٢.٥ and ٤ gram /Litter D.W).

٢-Rizolex-T ٥٠٪; chemical formula is Tolclofos methyl: ٠, ٢, ٦ dichloro-p-toly ١٠,٠ dimethyl phosphorothioate and Thiram : tetra methyl thiuram disulfide and used with three concentrations, (١, ٣ and ٥gram /Litter D.W).

b- Organic Bio-fungicides.

١-Blight Stop is a culture filtrate from (*Trichoderma harzianum*) was used with concentrations ٠.٥, ١ and ١.٥٪ in D.W. (v/v)

٢-Clean Root is a culture filtrate from (*Bacillus subtilis*), and used with concentrations ٥, ١٠ and ١٥٪ in D.W. (v/v)

These bio-fungicides were prepared in the Central Lab of Organic Agriculture, Agriculture Research Center (ARC) according to the method of Brain and Hemming

(١٩٤٥) and Dowson (١٩٥٧) and the modified method of Abd-El-Moity and Shatla ١٩٨١.

Aceto-carmin squashed preparations were made from the treated fixed roots of at least ٥ plants of parent, F١ and F٢ plants in randomized complete design. At least ٨٠٠٠ cells were examined for each treatment (consisting of three seedlings).The all mitotic measurements were taken on parent, F١ and F٢ 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 (١٩٨٤) and MSTAT program (Version ٤) was applied.

٢-FIELD EXPERIMENT (SEASON ٢٠٠٦-٢٠٠٧)

The concentrations and methods of pesticide treatments in field experiment were carried out according to the recommendations of Egyptian Ministry of agriculture (٢٠٠٤-٢٠٠٥). 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 ٣: ١ (v / v).

Cytological preparations of PMCs were made using the Aceto-carmine smear methods. At least ٩٠٠ 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 F١ seeds were obtained from the above described methods. The data were statically analyzed (in RCBD) using MSTAT program (Version ٤).

٣-FIELD EXPERIMENT (SEASON ٢٠٠٧-٢٠٠٨)

F١ seeds were cultivated and the flowering buds were taken from them to make F١ meiotic analysis. The F١ plants were not treated with any pesticides. The F٢ seeds were a successfully taken to carry out the mitotic analysis of them in order to study the genetical transmission of mitotic aberrations.

Minia J. of Agric. Res. & Develop.
Vol.(٢٨) No. ٤ pp ٦٤٧-٦٧٠, ٢٠٠٨

RESULTS

١- LABORATORY EXPERIMENT

The laboratory experiment was carried out to estimate the cytotoxic effects of two synthetic and two bio-fungicides on parents, F١ and F٢ 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.

١.١. THE MITOTIC INDEX (MI)

١.١.١. MI in the parent seeds

Data in table (١) showed that 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 ٤ gm/L Dithane at ١٢ h exhibited the lowest value of MI (٤.٨٧%) while seeds treated with Rizolex (٣gm/L at ٢٤ h) have the lowest one (٣.٦٥%). 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 ٤gm/L at ١٢h (٢٦.٩٦%) and Rizolex ١gm/L at ١٢h (٣٢.٣٥%). The ana-telophase index values were insignificantly affected with treatments of both Dithane and Rizolex (except those of Rizolex with ١and ٣ gm at ١٢h).

Data in table (٢) showed 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 that of control. The MI of the treatments of Blight Stop (١.٥٪ at ٢٤h) and Clean Root (١٠٪ at ٢٤h) were the lowest values (٧.٦٨% and ٦.٩٥% 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).

Minia J. of Agric. Res. & Develop.
Vol.(٢٨) No.٤ pp ٦٤٧-٦٧٠, ٢٠٠٨

Table (١): 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		•	١١٧٥٩	٥٣.٥٣	١٨.٧٧	٢٧.٧٠	١٤.٩
Dithane	٦	١g /L	٨٢٤٩	٤٧.٤١	٢٢.١٠	٣٠.٤٩	١١.٢٤
		٢.٥ g / L	٩٨٦٧	٥٦.٧٢	١٦.٦٥	٢٦.٦٤	٩.٧٧٧
		٤ g / L	٧٥٠٨	٥٢.٢٠	٢٥.٢٨	٢٢.٥٢	٩.٤٧
	١٢	١g /L	١٩٠٨٣	٥٣.٥٨	٢٥.٥٤	٢٠.٨٨	٩.٤٢٣
		٢.٥ g / L	٧٦٢٤	٤٦.٣٤	٢٥.٩٩	٢٧.٦٨	٩.٣٦٣
		٤ g / L	١٩٩٦٢	٥١.٦٤	٢٦.٩٦	٢١.٤١	٤.٨٦٧
	٢٤	١g /L	٢٠٥٥٤	٤٩.٤٧	٢٠.٢٢	٣٠.٣١	٦.٩٩
		٢.٥ g / L	١٢١٤٠	٥٩.٨٠	١٦.٧٨	٢٣.٤٢	٥.٠٣٧
		٤ g / L	٢٣٢١٣	٥١.٧٥	١٣.٧٦	٣٤.٤٩	٤.٣٩٣
Rizolex	٦	١g /L	١٦٠٥٦	٥٨.٥٩	١٩.٠٨	٢٢.٣٣	٥.٤٢٣
		٣g /L	١٥٠٠٨	٥٢.١١	١٧.٩٣	٢٩.٩٦	٧.٢٠٧
		٥g /L	١٤٨٣١	٥٤.٠٣	٢١.٢٧	٢٤.٧٠	٥.٣٤٧
	١٢	١g /L	١٠٩٠٤	٥١.٥١	٣٢.٣٥	١٦.١٤	٨.١٧
		٣g /L	٢١٥٧٩	٥٣.٢٥	٢٧.٤٤	١٩.٣١	٥.٣٧٧
		٥g /L	٢٤٢٢٢	٥٤.٣٩	١٨.٧٢	٢٦.٨٩	٤.١٨
	٢٤	١g /L	١٩٦٦١	٤٤.٧٢	١٩.٢٩	٣٥.٩٩	٥.٤٩٣
		٣g /L	١٨١٩٨	٥١.٨٦	١٦.٩٦	٣١.١٨	٣.٦٥
		٥g /L	١٧٧٢٢	٤٨.٩٦	٢٠.٤٠	٣٠.٦٤	٥.٤٤٧
***L.S.D. =**				**٨.٤٢١**	**٦.٢١٣**	**٧.٧٣٢**	**٢.٩٨٤**

Table (٢) : 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		•	١١٧٥٩	٥٣.٥٣	١٨.٧٧	٢٧.٧٠	١٤.٩
Blight Stop	٦	٠.٥٪	٢٢٧٤٢	٥٢.٥١	٢٠.١٣	٢٧.٣٦	٩.٤٩
		١٪	٢٢٠٤١	٥٢.١٧	٢٣.٧٢	٢٤.١٢	١٢.٢٨
		١.٥٪	٢٠٥٧٤	٥٥.٨٣	٢٠.٩٤	٢٣.٢٤	٩.٤٩
	١٢	٠.٥٪	٢٥٠٥٣	٥٠.٣٩	٢١.٩٣	٢٧.٧٨	٨.٨٦
		١٪	٢٣٢٣٤	٥١.٤١	٢٢.٥٣	٢٦.٠٦	١٠.٠٤
		١.٥٪	٢٨٢٦٨	٥٤.٥٠	١٩.٨٤	٢٥.٦٧	٩.٠٥٣
	٢٤	٠.٥٪	٣٠٥٢٩	٥٤.٨٤	٢٣.١٩	٢١.٩٧	٨.٥٨٧
		١٪	٤٢١٧٣	٥٢.٠٢	٢١.٤٢	٢٦.٥٧	٧.٧٧
		١.٥٪	٣٨٦٢٧	٥٣.٣٣	٢٤.٧٤	٢١.٩٤	٧.٦٨
Clean Root	٦	٥٪.	١٥٥٨٧	٥٣.٤٩	١٨.٥٣	٢٧.٩٨	١٢.١٣
		١٠٪	١٥٣٢٤	٥٤.٣٩	١٩.١٩	٢٦.٤٢	١٠.٢
		١٥٪	١٥١٨١	٤٩.١٧	٢٣.٦٠	٢٧.٢٣	١١.٠٥
	١٢	٥٪	١٣٠٣١	٤٧.١١	٢٤.٧٥	٢٨.١٤	١٠.٨٨
		١٠٪	١٣٣٣٧	٤٧.٢٢	٢٥.٤٩	٢٧.٢٩	١٠.٧٩
		١٥٪	١٥٣٥٤	٤٩.٠٢	٢٥.٠٠	٢٥.٩٨	١٠.١١
	٢٤	٥٪	١٢٥٤٢	٤٧.٨٩	٢٦.٣٦	٢٥.٧٥	٨.٢٣
		١٠٪	١٣٧١٤	٥٧.٦٢	١٩.٥٣	٢٢.٨٥	٦.٩٥
		١٥٪	١٩٦٨٩	٤٨.٠٩	٢٢.١٠	٢٩.٨١	١٠.٤٤
***L.S.D. =**				**٨.٤٢١**	**٦.٢١٣**	**٧.٧٣٢**	**٢.٩٨**

* L.S.D for the two tables.

١.١.٢ MI of F١ and F٢

Data in table (٣) showed that values of MI of F١ seeds exhibited slight increasing than those of the parent treatments of both synthetic and bio-fungicides. On the other hand, the MI of F١ 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 F١ and F٢ plants were insignificantly changed when compared with control plants. F٢ plants exhibited the lowest MI value of Dithane treatments (٥.٩٦%). The MI values of F١ and F٢ plants were insignificantly changed on decreased from that of the control plants.

Table (٣): mitotic index obtained from treatments with four pesticides on faba bean F١& F٢ plants.

Treatments	F١					F٢				
	Total No. of cells	Pro.	Meta.	Ana. & telo.	MI	Total No. of cells	Pro	Meta.	Ana. &telo	MI
Control	١٥٣٨٨	٥٣.٤٥	١٧.٣٤	٢٩.٢١	١١.٧٣	١٧٢٧٣	٥٦.٣٨	١٩.١٤	٢٤.٤٨	١١.٤٩
Diathane	١٤٦٤٧	٥٢.١٢	٢٠.٢٣	٢٧.٦٥	١٠.٩٠	٢٣٩٣٧	٦٤.١٢	١٧.٦٨	١٨.٢٠	٥.٩٦
Rizolex	١٥٤٢٨	٥٥.١٢	١٩.١٦	٢٥.٧٢	٧.١٣	١٧٢٨٠	٥٥.٩٩	٢٠.٥٩	٢٣.٤١	٩.٤٢
Blight Stop	١٧٤١٦	٥٥.٠٤	١٩.٧٥	٢٥.٢٢	٨.٩٦	١٨٢٩٥	٥٩.٧٢	١٨.٢٣	٢٢.٠٥	١١.٠٢
Clean Root	١٦٠٧٩	٦١.٠٨	١٦.٤١	٢٢.٥١	٩.٥٦	١٩٢٤٢	٥٧.٤٣	١٨.١٠	٢٤.٤٨	١٠.٥٩
L.S.D.=		٨.٤٩	٣.٣٩	٧.٠٤	٣.٩٢		٩.٢٤	٥.٠٥	٥.٤١	٤.٩٨

١.٢. THE MITOTIC ABERRATIONS

١.٢.١. Mitotic aberrations of parent plants

Table (٤) showed the percentages of mitotic abnormalities in parent plants such as lagging chromosomes, Chromatid Bridge during anaphase, chromatin fragments during the different mitotic stages, outside chromatin and chromosome stickiness (Fig.١). The cells with micronuclei were scored in stages of the meristemic tissue including the interphase (Fig. ٢). It was clearly observed that the treated plants exhibited significant total percentage of chromosomal aberrations (except treatments of ٦h at the lower two concentrations of both Dithane and Rizolex). The treatment of Dithane at ١٢h with the three concentrations induced the significant and highest percentage value of chromosomal aberrations (١٩.٧%and ١٩.٨%) when compared with that of control plants. The concentrations ١gm/L at ١٢h and ٥gm/L at ٢٤h of Rizolex fungicide induced the significant and higher percentage of chromosomal abnormalities (١١.١٣% and ١٠.٩٤%) than that of control parents (١.٣١%). The percentage of micronuclei reached a maximum of (٢.١٣٪) after treatment for ٦hr with Dithane ٤g/L. It has been observed that percentages of micronuclei were significantly increased after treatments for ٢٤h 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.

Table (٥) shows 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 ٦ 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.

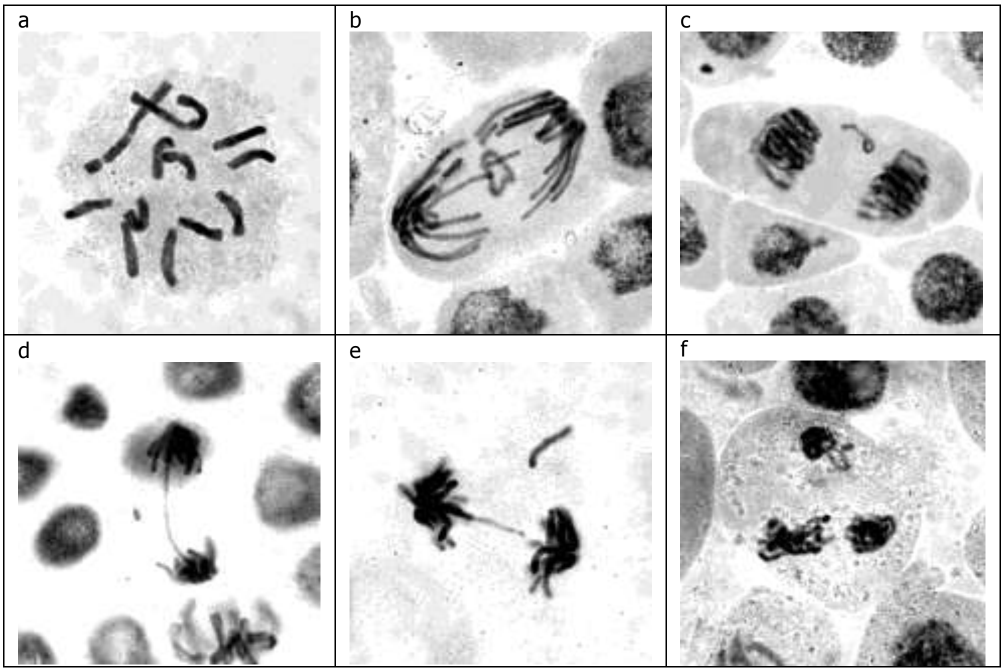


Fig (١) some of chromosome abnormalities at different mitotic stages of *Vicia faba* meristemic cells treated with four different fungicides: (a) normal metaphase, (b)lagging chromosome at anaphase,(c)lagging chromatid at telophase, (d) fragment and bridge at telophase, (e) lagging and bridge at telophase, (f) cell with tripoler telophase

Minia J. of Agric. Res. & Develop.
Vol.(٢٨) No.٤ pp ٦٤٧-٦٧٠, ٢٠٠٨

Table (٤): 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		٠	١١٧٥٩	٠.٤٦	٠.٢٥	٠.٣٦	٠.٠٦	٠.١٩	-	١.٣١	٠.٣٩
Dithane	٦	١	٨٢٤٩	٠.٦٤	٢.٠٤	٠.٢١	٠.٢١	٠.٣١	-	٣.٤١	٠.٩٦
		٢.٥	٩٨٦٧	٠.٤٦	٠.٧٤	٠.٧٤	٠.٤٥	٠.١٩	-	٢.٦٧	١.٢٨
		٤	٧٥٠٨	١.٥٥	١.٩٧	٠.٩٦	٠.٧٩	٠.٨٨	٠.٥٨	٦.٧٤	٢.١٣
	١٢	١	١٩٠٨٣	٢.٣٢	٦.٥٢	١.١٠	٠.٦٦	٠.٩٠	٣.٤٧	١٤.٩٨	٠.٤٦
		٢.٥	٧٦٢٤	٤.٧٥	٣.٩٦	٣.٣٤	٠.٤٣	٠.٩٠	٦.٤٥	١٩.٨٢	٠.٣٣
		٤	١٩٩٦٢	٥.٣٧	٣.٣٧	٤.١٤	١.٦٩	١.٦٨	٣.٤٦	١٩.٧٢	١.٢٧
	٢٤	١	٢٠٥٥٤	٢.٢٨	١.٦٢	٠.٨٤	٠.٢٧	٠.١٩	٠.٥٥	٥.٧٥	١.٠٢
		٢.٥	١٢١٤٠	٣.٤٢	١.٥٨	١.٢٠	٠.٥٠	١.٦٢	٠.٧٠	٦.٧٢	١.٣٢
		٤	٢٣٢١٣	٣.٣٥	٠.٩٣	١.٦٠	٠.٣٥	١.١٩	١.١٤	٨.٥٥	١.١٠
Rhizolex	٦	١	١٦٠٥٦	٠.٧٧	٠.٢٠	١.٤٨	٠.١١	٠.٣٥	-	٣.٠١	٠.٣٨
		٣	١٥٠٠٨	١.١٧	٠.٥١	٠.٦٥	٠.٢٠	٠.١٧	-	٢.٧٧	٠.٥٧
		٥	١٤٨٣١	٣.٠٦	٠.٢٥	١.٨٥	٠.١٤	٠.٤٠	-	٥.٨٢	٠.٤٦
	١٢	١	١٠٩٠٤	٣.٠٠	١.١٤	٢.٠١	٠.٤١	٠.١٨	٤.٣٩	١١.١٣	٠.٢٥
		٣	٢١٥٧٩	١.٩٦	١.٨٨	١.٤٣	٠.٥٥	٠.٢٧	٢.٨٥	٨.٩٤	٠.١٤
		٥	٢٤٢٣٢	١.٩٢	١.٦٦	١.٩٧	٠.٥١	٠.٦٧	٣.٢٢	٩.٩٧	٠.١٩
	٢٤	١	١٩٦٦١	١.٠٨	٠.٦٢	٠.٥٣	٠.٣٠	٠.٥٢	١.٦٩	٤.٧٣	١.٠٣
		٣	١٨١٩٨	١.٧٦	١.١١	٠.٧٠	٠.٥١	١.٤٩	٢.٧٤	٨.٢٩	٠.٨٨
		٥	١٧٧٢٢	٣.٧٠	١.٤٨	٢.٧٠	٠.٧٢	١.١٧	١.١٧	١٠.٩٤	١.٣٦
*L.S.D. =				١.٥٢	٠.٩٢	٠.٩٠	٠.٤٠	٠.٢٥	١.٢٩	٢.٦٥	٠.٥١٩

Table (٥): 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		٠	١١٧٥٩	٠.٤٦	٠.٢٥	٠.٣٦	١.٣١	٠.٣٩
Blight Stop	٦	٠.٥٪	٢٢٧٤٢	٠.٨٠	٠.٤٢	٠.٢٨	١.٦٨	١.٣٢
		١٪	٢٢٠٤١	٠.٦٣	٠.٦٨	٠.٤٤	١.٧٥	١.١٧
		١.٥٪	٢٠٥٧٤	٠.٦٧	٠.٨٤	٠.٤٩	٢.٠٥	١.٠٥
	١٢	٠.٥٪	٢٥٠٥٣	١.٤٣	٠.٨٠	٠.٤٩	٢.٨٣	٠.٦٩
		١٪	٢٣٢٣٤	٠.٦١	٠.٢١	٠.٦٤	١.٤٦	٠.٧٦
		١.٥٪	٢٨٢٦٨	١.٠٢	٠.٢٨	٠.٦٣	٢.٠٠	١.١٦
	٢٤	٠.٥٪	٣٠٥٢٩	٠.٣٧	٠.٧٤	٠.٥٤	١.٦٥	٠.٤٥
		١٪	٤٣١٧٣	٠.٤٥	١.٠٥	٠.٣٨	٢.٠٤	٠.٤٣
		١.٥٪	٣٨٦٢٧	٠.٦٨	٠.٩٧	٠.٣٣	٢.٠٨	٠.٧٥
Clean Root	٦	٥٪	١٥٥٨٧	٠.٥٠	٠.٢٧	٠.٥٣	١.٨٠	٠.٧٤
		١٠٪	١٥٣٢٤	٠.٥١	٠.٣٣	٠.٣١	١.٥٣	٠.٦٢
		١٥٪	١٥١٨١	٠.١١	٠.٣٩	٠.٢٤	١.٠٤	١.٠٢
	١٢	٥٪	١٣٠٣١	٠.٤٥	٠.٢٩	٠.٢٩	١.٣٢	٠.٩٨
		١٠٪	١٣٣٣٧	٠.٨١	٠.٨٧	٠.٦٤	٢.٣٨	٠.٨٧
		١٥٪	١٥٣٥٤	٠.٧٥	١.٢٢	٠.١٥	٢.١٧	٠.٨٦
	٢٤	٥٪	١٢٥٤٢	٠.٥١	١.٢١	٠.٤٧	٢.١٩	٠.٧٤
		١٠٪	١٣٧١٤	٠.٣٦	٠.٨١	٠.٦٦	٢.٠٠	٠.٥١
		١٥٪	١٩٦٨٩	٠.٦١	١.٢١	٠.٧٠	٢.٦٣	٠.٤١
*L.S.D. =				١.٥٢	٠.٩٢	٠.٩٠	٢.٦٥	٠.٥١٩

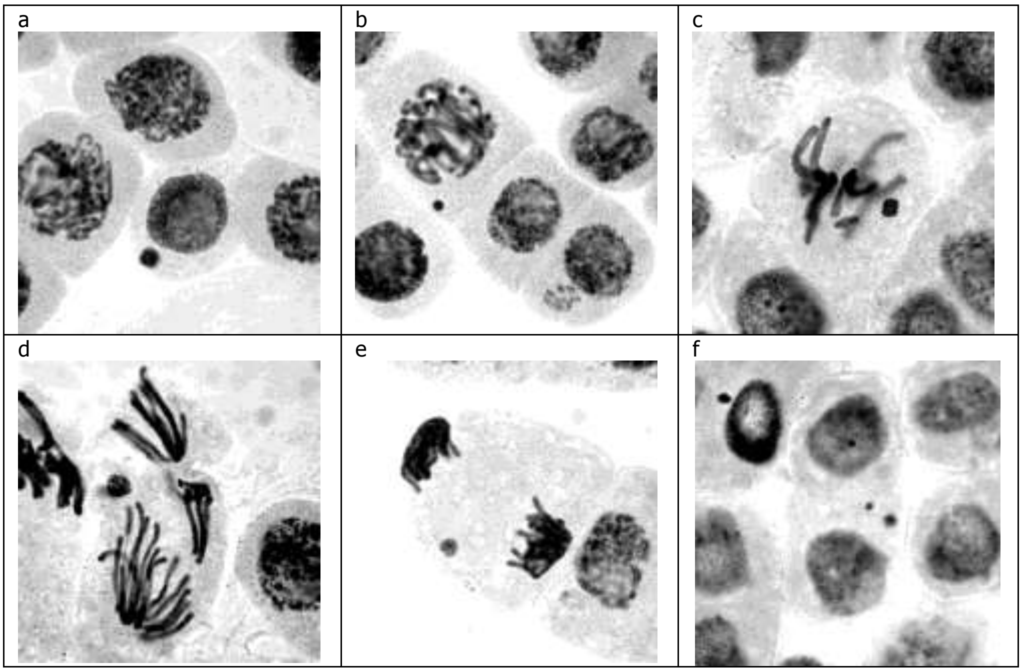


Fig (٢) Micronucleus at interphase and its appearance at other different mitotic stages of *Vicia faba* root cells treated with four different fungicides: (a) micronucleus at interphase, (b) micronucleus at prophase, (c) micronucleus at metaphase, (d)micronucleus at anaphase, (e) micronucleus at telophase, and (f) two micronuclie in interphase.

١.٢.٢. Mitotic aberrations of F١ and F٢ plants.

Data in table (٦) showed that values of total abnormal cells of F١ and F٢ exhibited highly decrease than those of the parent treatments (both synthetic and bio-fungicides). The significance of F١ data was determined by statistical analysis and showed that all F١ plants have lower abnormal cells than that of parent treatments. The F٢ plants of Dithane and Rizolex treatments exhibited significant and the highest value of micronuclei (٢.٤٨% and ٢.٠٨%) when compared with that of control and bio-fungicide plants (٠.٧%).

Table (٦) the mean percentage of obtained mitotic irregularities in faba bean F١& F٢ treated with four pesticides.

Treatments	F١					F٢				
	Lage	Bridge	Frag.	Total Abnor.	Micro	Lage	Bridge	Frag.	Total abn.	Micro.
Control	٠.٤٩	٠.٢٤	٠.١١	١.٢٦	٠.٠١	٠.٣٢	٠.١٢	٠.١٦	٠.٦٠	٠.٧٠
Diathane	٠.٤٩	٠.٦٤	٠.٤٣	١.٧٦	٠.١٤	٠.٤٩	٠.٣٠	٠.٢٣	١.١٨	٢.٤٨
Rizolex	٠.٣٥	٠.٦٩	٠.١٥	١.٤٤	٠.٣٣	٠.٧٨	٠.٤٤	٠.١١	١.٤٩	٢.٠٨
Blight Stop	٠.٤٢	٠.٢٧	٠.١٥	٠.٨٩	٠.١٣	٠.٣٦	٠.٠٩	٠.٠٥	٠.٥٠	١.٣٩
Clean Root	٠.٣٨	٠.٢٧	٠.٢٩	٠.٩٤	٠.٢٣	٠.٣٠	٠.٠٥	٠.٠٥	٠.٤٤	٠.٧١
L.S.D.=	٠.٣٧	٠.٦٦	٠.٤٣	٠.٧٤	٠.٣٥	٠.٤٨	٠.٢٢	٠.٣٢	٠.٥٢	٠.٨٠

Minia J. of Agric. Res. & Develop.
Vol.(٢٨) No.٤ pp ٦٤٧-٦٧٠, ٢٠٠٨

٢. FIELD EXPERIMENT

٢.١. Meiotic behavior

Chiasma frequency

Data in table (٧) 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 F١ 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 (٧): Chiasma frequency in parent and F١ plants:

Treatment	Parent		F١	
	Dickinesis	Metaphase I	Dickinesis	Metaphase I
Control	٢٠.٨٤	١٧.٧٤	١٩.٢٨	١٥.٧٠
Dithane	٢٠.٦٠	١٧.٨٧	١٨.٧٥	١٤.٩٠
Rizolex	٢١.٢٧	١٨.٠٧	١٨.٤٣	١٥.٢٠
Blight Stop	٢٠.٨١	١٧.٨٣	١٨.٤٧	١٥.٥٠
Clean Root	١٩.٩٧	١٧.٧٣	١٨.٦٧	١٥.٦٠
L.S.D =	٢.٦٠	٢.١٧	٠.٨٦	٠.٨٦

٢.٢. Meiotic aberrations of parent plants.

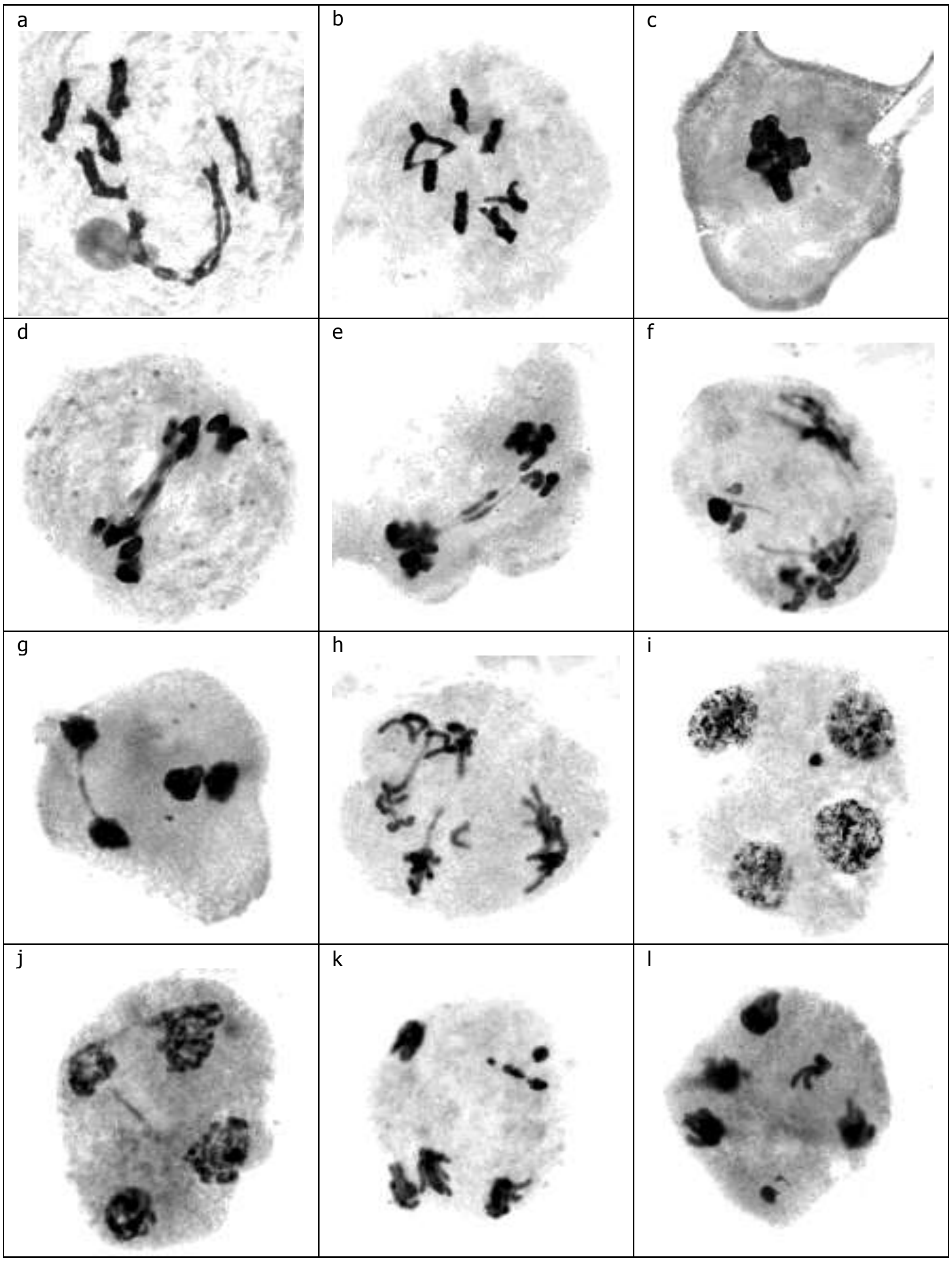
Table (٨) showed that treated parent plants with synthetic fungicides (Diathane and Rizolex) have a significant proportion of abnormal pollen mother cells (١٠.٠١ and ١٠.٠٦, respectively) than those of control and treated plants with bio-fungicides. However, there were no significant differences between total meiotic aberrations of treated plants with bio-fungicides and that of control. 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 (١٠.٢٦٪ uni and multivalents).

٢.٣. Meiotic aberrations of F١ plants

Table (٩) showed that there was a significant difference of the total abnormal (PMCs) in F١ plants between synthetic fungicides Dithane and Rizolex (٨.٦٨٪ and ١٠.٣٠٤٪ respectively) and that of the control (٣.٥٤٪), on contrast there

Minia J. of Agric. Res. & Develop.
Vol.(٢٨) No. ٤ pp ٦٤٧-٦٧٠, ٢٠٠٨

was no significant effects in the percentage of total abnormal (PMCs) between bio-fungicides Blight Stop and Clean Root (٥.٤٩٤٪ and ٢.٥٨٧٪ respectively) and control plants. The high percentage (١٣.٥٢٪) of uni and multivalent in meiosis I (metaphase I and Dickinesiss) was recorded after treatment with Dithane (synthetic fungicide).



Fig(٣) 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 (٨) 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	٢.٦٠٠	٠.٤٦٣	٠.١٧٧	٠.٤٦٣	٠.٢٨٠	٢.٣٣٠	-	٤.٣٩٦	٠.٦٨٣
Diathane	١٠.٢٦٠	١.٤٦٧	١.٢٤٠	٠.٩٥٣	٠.٤٢٣	١.٠٦٧	١.٧٠٧	١٠.٠١	٣.١٥٣
Rizolex	٣.١١٧	٢.٨٠٣	٠.٤٨٠	٠.٧٦٧	٠.٨٧٧	١.١١٣	١.٢١٧	١٠.٠٦	٢.٨٠٣
Blight Stop	٤.٢٩٧	٢.٢٠٠	٠.٦٢٧	٠.٥٩٣	٠.١٤٧	٠.٦٤٠	٠.١٩٣	٦.٢٧٧	١.٨٧٧
Clean Root	٥.٦٧٣	٢.٩٠٧	٠.٩٥٠	٠.٣٩٧	٠.٣٦٧	٠.٦٦٠	-	٧.١٤١	١.٨٦٠
L.S.D. =	٦.٤٨١	١.٧٧٩	١.١٦٥	٠.٧٧٤	٠.٧١٢	١.٢٧٨	١.١٣١	٤.٣٧٩	١.٦٩٠

Table (٩) Percentage of types of abnormalities occurring in the meiosis of Vicia faba F١ 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	٢.٣٩٣	٠.٦٩٠	٠.٩٣٠	٠.٦٣٧	٠.٦٣٧	٠.٢٥٠	٠.١٥٠	٣.٥٤٠	٠.٢٥٠
Diathane	١٣.٥٢٠	١.٥٠٠	١.٦٩٠	٠.٣٩٠	٠.١٩٠	٢.٤٤٣	١.٩٠٠	٨.٦٨	٠.٥٦٧
Rizolex	٣.٧٤٠	٢.١٢٠	٠.٧٠٠	١.٣٧٣	٠.٣٧٠	٠.٩٤٧	١.٨٧٧	١٠.٣٠٤	٢.٩١٧
Blight Stop	١.٣٤٧	٠.٥٤٧	٠.٨٩٧	١.٢٣٠	٠.٨٦٧	٠.٢٧٠	١.١٠٣	٥.٤٩٤	٠.٥٨٠
Clean Root	١.١٧٠	٠.٦٤٠	٠.٣٧٠	٠.٦٣٧	٠.١٤٠	٠.٠٧٣	٠.٥٨٧	٢.٥٨٧	٠.١٤٠
L.S.D. =	٤.٢١٩	٠.٧٣٧	٢.٦٣٣	٠.٧٣٧	٠.٧٩٤	١.٤٥٤	١.٢٠٧	٢.٤٧٨	٢.٢١٩

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, ١٩٧٠ and ١٩٧١). In the recent years some alternative bio- products were used as safe pesticides in agriculture (Abd-El-Moity and Shatla, ١٩٨١). In the present work the cytotoxic effects of bio-fungicides (Blight Stop and Clean Root) were examined in comparison with two partner inorganic (synthetic) fungicides (Diathane M-٤٥ and Rizolex T-٥٠٪). 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.* ٢٠٠٠ and Deysson, ١٩٦٨). The two major reasons are the inhibition of both protein synthesis (Kim and Bendixen, ١٩٨٧) and DNA amount and replication (Beu *et al.*, ١٩٧٦, Badr, ١٩٨٣). 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.*, ١٩٩٦). Results also revealed the transmitted depression effects of Rizolex in F١ seeds and Diathan in F٢ 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 F١ and F٢.

The mitotic chromosomal aberrations which included chromatin bridges, laggards, fragments, stickiness and micronuclei were also scored in the parent, F١ and F٢ plants in order to assess the cytogenetical damaging effects of both synthetic and bio-fungicides. Present results ensure that great values of damaging effects were induced either in treated parent with synthetic fungicides or in their F١ and F٢ 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.*, ١٩٩٠) and herbicide glean (Badr and Ibrahim, ١٩٨٧). 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, ٢٠٠٤).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, ١٩٧٨; Mansour, ١٩٨٤ and Patil and Bahat, ١٩٩٢).

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, ١٩٧٩ and Badr, ١٩٨٣). 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*, ١٩٧٤).. 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, ١٩٧٨ and Tomkins and Grant, ١٩٧٢).The stickiness of chromosomes may cause incomplete separation of daughter chromosomes as a result of cross- linkage of chromoproteins (Kong and Ma, ١٩٩٩). This led to subchromatid connections between chromosomes and thus they remained connected by bridges (McGill *et al*., ١٩٧٤; Klasterska *et al*., ١٩٧٦; Badr *et al.,* ١٩٩٢).

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.,* ١٩٨٦). The micronuclei forms in two ways: One is, the chromosomal fragments formed in the last G٢ could not act in phase with normal chromosomes, and are rejected to the outside of nuclei in interphase. The other is the occurrence of various forms of lagged chromosomes, non-equatorial plane aggregated chromosomes, and the chromosomal grouping (Li, ١٩٩٧). 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.,* ١٩٨٨; Grover and Kaur, ١٩٩٩). Micronuclei were considered an indication of a true mutation effect (Auerbach, ١٩٦٢), 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 F١ plants whereas the frequency of uni and multi-valents are significantly affected in Diathane treatment either in parent or in F١ 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 of the synthetic pesticides may be more safety.

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الملخص العربى

تأثير السمية الخلوية لبعض المبيدات المخلقة والحيوية على الفول البلدى

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فى هذا البحث 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 البيانات المتحصل عليها من التجارب المتكررة للمعامله المباشره ان كلا من المبيدات الفطريه المخلقه (الغير عضويه) و الحيويه لها تاثير 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 البيانات أن معدل الانقسام الميتوزى (MI) لمعظم النباتات (البذور) التى عوملت بثلاثه تركيزات مختلفه من المبيدين الفطريين الدياثان و الريزولكس فى اوقات تعريض مختلفه كان لها معنويه منخفضه عن تلك الخاصه الكنترول. ولقد اظهرت المعامله بالديثان 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٪). ولقد اظهرت 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). واظهرت 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 الديثان 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 (١٠.٠٦%) عن تلك الخاصه بالكنترول وكذلك أعلى من 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 وكذلك عن النباتات الجيل الأول الناتجة من الأباء المعاملة بالمبيدات الفطرية الحيوية.كذلك لم تكن هناك نسبة عالية من الشذوذات الميوزية فى نباتات الجيل الاول الناتجة من الأباء المعاملة بالمبيدات الحيوية بالمقارنة بالكنترول بل ربما انخفضت عنها فى بعض المعاملات. وبذلك يمكن القول من وجهة النظر الوراثية السيتولوجية أن البديل الحيوى للمبيدات الكيماوية ربما يكون أكثر أمنا فى الإستخدامات الزراعية