Meiotic Behavior In Treated *Vicia Faba* Plant With Diprofos Drug

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Abstract: The cytotoxicity of the corticosteroid drug "diprofos" (0.50, 1, 2 and 4ml /100ml) were examined on *Vicia faba* meiotic behavior. *Vicia faba* plants at the flowering stage spraying with the obvious diprofos concentrations to study the meiotic behavior after 24h. and 48h. from spraying. All diprofos treatments showed highly significant increased of abnormal pollen mother cells (PMC_s) which increased as the concentration and period duration increased. Abnormalities % at the second meiotic division were lower than those recorded in the first meiotic division in most treatments as a result of recovery in this cell age. On the other hand, the abnormalities were present in metaphase and anaphase stages at the first and second meiotic division. Stickiness and disturbed chromosomes were the most common abnormalities found in all phases of meiosis after diprofos treatments. In addition, laggard, bridges, breaks and micronuclei, which recorded with low percentages in some treatments. Results strongly suggest that diprofos drug has a cytotoxic effect on *Vicia faba* plant.
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1.Introduction

Corticosteroids are contra-indicated in patients with peptic ulcer, osteoporosis, psychoses, or sever psychoneuroses. Because of the interference with inflammatory and immunological response, corticosteroids should not be usually given in presence of cute infections. Life vaccines should be avoided in patients receiving corticosteroids. Corticosteroids should not be given during pregnancy or lactation. prolonged use of beta-methasone in high doses may cause pituitary suppression, acute adrenal insufficiency, fluid and electrolyte disturbance, hyperglycemia and glucosuria, increased susceptibility to infections, peptic ulcers, osteoporosis, arrest of growth, cushin's habitus (moon- face,buffalo hump), behavioral disturbance and cataract,Henein, (2004). Chromosomal aberration test (CA) is one of the sensitive and important tests for evaluating genetic hazard of drugs and / or carcinogens because CA is known to have a serious impact on human health. There is a clear association between chromosomal aberration and certain types of cancer. Many drugs such as; Trifluo perazine, aspirin, indometacin, theophylline, novalgin, oxicam, ampicilin, piroxicam, carbenilin, phenoparetal, clomiditens, nitroimidazol, acetyl salicylic acid, bohemine roscovitine, fenaton, diazebam, megazol, penta brominaled and nitroso-methylurea were studied in different biological systems (e.g., mice, salmonella, yeast, *Drosophila melanogaster*, human lymphocytes, *Allium cepa* and *Helianthus annus,*)El- Bayoumi et al., 1984; Atef et al., 2011). The present study aimed to evaluate the mutagenic effect of diprofos drug with different concentration at exposure times on *Vicia faba* meiotic division behavior.

2.Materiais And Methods:

2.1.Materials:

2.1.1.Sample:

Vicia faba plants (Var. Giza 40) at the flowering stage were used in this study

2.1.2.Tested drug:

The teated drug was Diprofos drug It is produced by Schering Plough Company, USA. This drug is present in ampoule form,each one ml ampoule contains 7 mg betamethasone.

2.2.Methods:

2.2.1.Treatment:

- *Vicia faba* plants were sprayed with four medical preparations of Diprofos drug.The recommended dose for this drug depends on the disease type. Generally, Dosage for adults: short term (1-2) ml daily for the first few days, subsequently reducing the daily dosage by 0.50-1 ml every 2-5 days.
- Four diprofos concentrations were used:

0.50, 1, 2, 4 ml/100 ml water.

- Control plants were sprayed with distilled water.

Ten flower buds from ten different plants were gathered after (24 and 48) hours from spraying for study.

2.2.2. Meiotic studies:

The appropriate flower buds were collected and fixed in carnoy's solution (ethyl alcohol absolute and glacial acetic acid with ratio 3:1 for 24h., and then transferred to 70% ethyl alcohol and kept in refrigerator. The cytological analysis were carried. by

using 2% aceto-carmine stain)as described by Darlington and La Cour,1979).

2.2.3.Statistical analysis:

The data recorded for different treatments were statistically analyzed using t-test for determining significantly of differences between these treatments.

3.Results And Discussion

3.1-Total Meiotic Abnormalities Percentages:

A wide spectrum of meiotic abnormalities were recorded in ten flower buds from different plants after different treatments with diprofos. Data in (table1) shows that total abnormal PMCs % was highly significant increased as the diprofos concentration increased and by the increasing of period duration from 24h. to 28h. in all treatments. Whereas, the total abnormalities ranged from (26.34% - 51.87 %) at the 24 h. period, while it ranged from (35.00% - 60.60 %) at the 48 h. period. The induction of meiotic abnormalities appears to be a common effect of most chemicals by many investigators (Ann et al, 2012; Rina et al., 2012; Madhuri et al, 2012; Sefa et al, 2012).

Table(1): Percentages of abnormal PMC$_s$in the 1st & 2nd meiotic divisions and total mean of meiotic abnormalities after spraying of *Vicia faba* plant with diprofos for 24 and 48 hours.

Conc, Ml/100ML	Times hours	% abnormal In PMCs meiotic division			% abnormal in Ist division			% abnormal in 2nd division		
		dividing Cells No.	abnormal Cells No.	Abn.% PMCs ± S.E	Dividing Cells No	abnormal Cells No	abn.%	dividing Cells No.	abnormal Cells No.	Abn.%
Control		510	18	3.44 ± 1.07	276	6	2.17	234	12	5.13
0.50	24	540	148	26.34 ± 2.02**	186	42	22.58	354	106	29.94
	48	531	186	35.00 ± 1.04**	209	74	35.41	322	112	34.78
1	24	500	153	30.44 ± 0.85**	265	120	45.28	235	33	14.04
	48	559	202	36.09 ± 0.51**	310	138	44.52	249	64	25.70
2	24	515	246	47.81 ± 0.90**	209	120	57.42	306	126	41.18
	48	491	244	49.59 ± 0.55**	251	144	57.37	240	100	41.67
4	24	462	240	51.87 ± 2.62**	246	130	52.85	216	110	50.93
	48	475	288	60.60 ± 0.74**	285	176	61.75	190	112	58.95

PMCs: pollen mother cells. * significant (0.05), * * highly significant (0.01) (T- Test).

3.2- First and Second Meiotic Abnormalities Percentage

Table (1) revealed that the percentages of abnormalities in the first meiotic division increased as diprofos concentration increased and by the increasing of the duration period in the most treatments, which it ranged from (22.58% – 57.42%) and (35.41% – 61.75%) at the duration period 24h. and 48h. respectively.

Also, this trait in the second meiotic division increased as the increasing of both diprofos concentration and period duration except for diprofos concentration 1ml / 100ml which it showed the lower percentage in second meiotic abnormalities; 14.04% and 25.70% at the duration period 24h. and 48h. Respectively. While the highest value of this trait were 50.93% and 58.95% at the 4ml/100ml diprofos concentration at the duration period 24h. and 48h. respectively.

On other hand, the percentages of the abnormalities in the second meiotic division were lower than those recorded in the first meiotic division in all treatments except for the (0.50ml/100ml) diprofos at the 24h. as a result of recovery in this cell age (table 1).

Results from table (2) and figure (1) revealed that abnormal% (met, ana and telo) phases in the second meiotic division ranged from: (5.53%% – 21.05%, 5.53% – 21.05% and 0.56% – 11.11%) respectively lower than those recorded in the first meiotic division that ranged from: (5.38% – 27.37%, 13.98%% – 27.37% and 3.23% – 12.20%) respectively as a result of recovery in this cell age except for two treatments: 0.50 ml/100ml at 24 h. and 1ml/100ml at 24h. for abnormal (meta and telo) phases respectively. However, abnormalities didn't show in prophase 1 but it observed in prophase 2 with percentage (3.11% – 13.68%). These results were in agreement with many researches, (Priti et al., 2009; Haroun,2010; Asmahan and Salha, 2012).

Table (2): Abnormal meiotic phases percentages in *Vicia faba* plants after spraying with diprofos for 24 and 48hours

Conc. Ml/100 ML	Time Hours	First Meiotic Division			Second Meiotic Division			
		Abn. Metaphase %	Abn. Anaphase%	Abn. Telophase%	Abn. Prophase%	Abn. Metaphase%	Abn. Anaphase%	Abn. Telophase %
Control		1.45	0.72	----	----	5.13	----	----
0.50	24	5.38	13.98	3.23	3.39	14.69	11.30	0.56
	48	17.22	14.35	3.83	3.11	11.18	18.01	2.48
1	24	20.38	18.87	6.04	4.26	5.53	4.26	----
	48	22.58	18.06	3.87	3.21	8.84	12.85	0.80
2	24	23.92	25.84	7.66	6.54	18.30	10.46	5.88
	48	27.09	24.70	5.58	6.67	15.83	11.67	7.50
4	24	17.89	22.76	12.20	8.33	16.67	14.81	11.11
	48	27.37	27.37	7.02	13.68	21.05	16.84	7.37

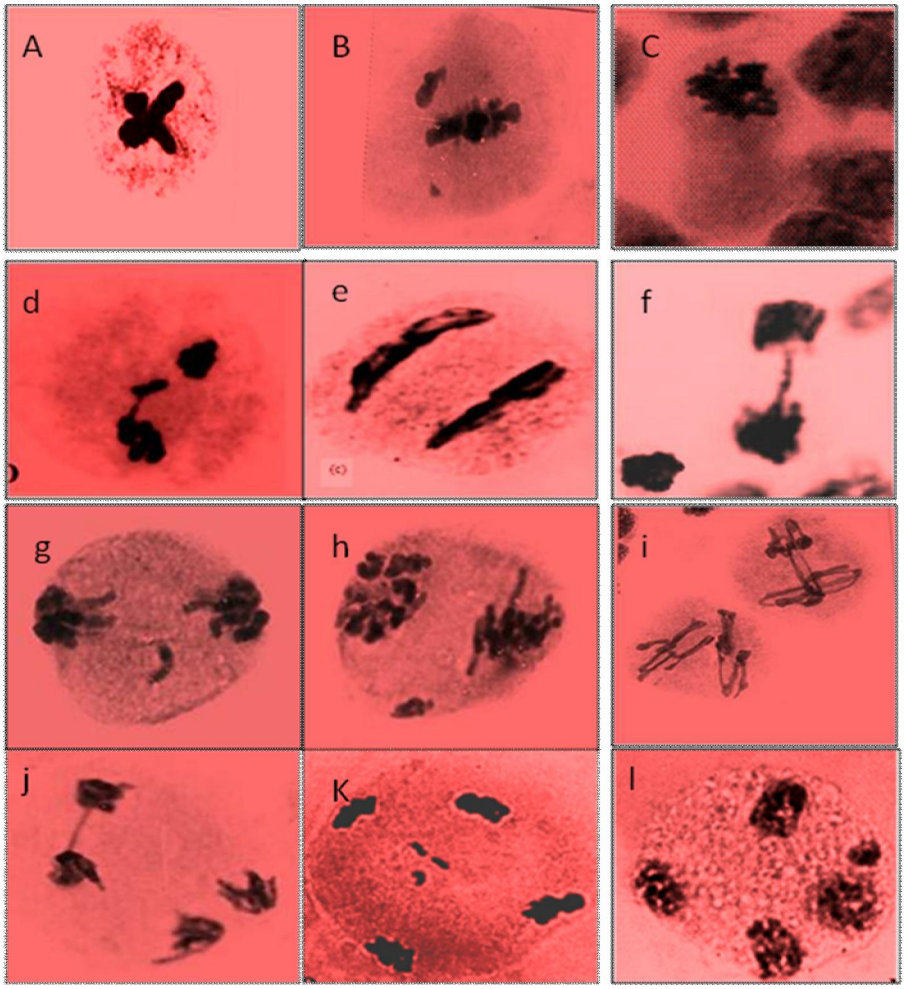

Fig.1: Different types of abnormalities in treated *Vicia faba* plant after diprofos treatments. a: Sticky(M_1); b: Sticky, Lagging and break (M_1); c: Sticky and disturbed (M_1); d: Sticky and Lagging (A_1); e: Sticky(M_2); f: Sticky and bridges(A_1); g: Lagging (T_1); h: micronuclei (P_2); i: disturbed (M_2); j: bridges (A_2); k: Lagging (T_2); l: micronuclei(T_2).

{M_1,A_1,T_1 : first (meta, ana)phase);P_2, M_2,A_2,T_2: second (pro, meta, ana, telo)phaseFig.1: Different types of abnormalities in treated *Vicia faba* plant after diprofos treatments. a: Sticky(M_1); b: Sticky, Lagging and break (M_1); c: Sticky and disturbed (M_1); d: Sticky and Lagging (A_1); e: Sticky(M_2); f: Sticky and bridges(A_1); g: Lagging (T_1); h: micronuclei (P_2); i: disturbed (M_2); j: bridges (A_2); k: Lagging (T_2); l: micronuclei(T_2).

{M_1,A_1,T_1:first(meta,anaphase);P_2,M_2,A_2,T_2:second(pro,meta,ana,telo)phase}

Table (3): Percentages of different meiotic abnormalities in the 1st and 2nd meiotic divisions after spraying *Vicia faba* plants with diprofos for 24 and 48 hours.

Conc. Ml/100ML	Time Hours	1st Meiotic Division					2nd Meiotic Division				
		Stick-iness	Disturb-ed	Lagg-ards	Bridges	Breaks	Stick-iness	Disturbed	Lagg-ards	Bridges	Micro-nuclei
Control		----	1.18	----	----	----	0.39	1.96	----	----	----
0.50	**24**	2.59	5.19	----	----	----	10	9.26	----	----	0.37
	48	4.90	9.04	----	----	----	12.05	7.53	----	----	1.51
1	**24**	12	10.80	0.40	0.80	----	2.60	4	----	----	----
	48	11.45	11.81	0.36	1.07	----	8.59	2.86	----	----	----
2	**24**	15.53	6.99	0.39	0.39	----	18.25	3.88	1.94	----	0.39
	48	22.81	3.67	1.63	1.22	----	15.89	2.04	0.81	0.81	0.81
4	**24**	26.84	----	0.43	0.87	----	22.08	____	0.43	0.43	0.87
	48	30.74	1.26	2.11	1.68	1.26	18.11	1.26	1.68	0.84	1.68

3.3-Types of different meiotic abnormalities:

Stickiness and disturbed chromosomes were the most common abnormalities found in all phases of meiosis after diprofos treatments (table 3- fig:1). Number of sticky cells increase as the diprofos concentration increased or per longed the period duration in the most treatments.

On the other hand, sticky cells in the second meiotic division decreased than those recorded in the first meiotic division at these diprofos treatments: 1 and 4ml / 100ml (at 24h. and 48h.) and 2ml / 100ml at 24h. (Table 3). Sticky chromosomes were shown in various meiotic division (Fig.1:a,b,c,d,e and f). Our results are in agreement with the results of many investigators(Ann et al, 2012; Rina et al., 2012; Madhuri et al, 2012; Sefa et al, 2012; Asmahan, and Salha 2012), who suggested that chromosome stickiness may results from breakage and exchange between chromatin fibers on the surface of adjoining chromosomes.

The second type of abnormalities is the disturbed which observed in metaphase and anaphase in all treatments (Fig.1: c, f and i), and the percentage of this trait ranged from: (1.26% – 11.81%) and (1.26% – 9.26%) in the first and second meiotic division respectively (Table 3).

This abnormality was observed by many researcher (Maju et al.,1984; El-Ashmawy et al.,1992; Obreeht et al.,1996; Giri et al.,1999; Alia,et al.,2001; Poli et al., 2002; Evandri et al.,2003; Arkhipchuk et al., 2004; Sadiq and Al-Quraishe 2004; Usciat et al., 2004; Singh et al.,2005; Ganguly et al.,2010; Haroun, 2010; Gabriele et al.,2010; Min et al.,2010; Saulo et al., 2010; Atef et al., 2011) after many chemicals treatments, they suggested that the chromosomes disturbed may results from the effect of the chemical treatments on proteins constituting the spindle apparatus.

In addition to previous common abnormalities, it was observed anther abnormalities on meiotic division including: laggards, bridges, breaks and micronuclei, which recorded with low percentages in some treatments (Table 3). Laggard chromosomes were observed in some treatments in (meta, ana, and telo) phases in the first and second meiotic division (Fig.1, b, d, k and g,). Laggard at metaphase could be attributed to failure of the spindle apparatus to organize and function in a normal way (Haroun, 2010). These Laggards may be distributed randomly to either poles at both anaphase and telophase (I and II) which result ultimately in aneuploidy (Ann, *et al*, 2012) or may give for micronuclei at telophase (Rina, *et al*., 2012).The induction of Laggard chromosomes could be attributed to irregular orientation of chromosomes (Min, *et al*.,2010).

On the other hand, breaks appeared in metaphase I only in 4ml 100ml at 48h.(Table 3, Fig.1: b). While, bridges were induced in some treatments, (Table 3, Fig.1:f and j) and they could be due to the breakage and reunion (Atef, *et al*.,2011) or due to the general stickiness of chromosomes (Sefa, *et al*., 2012). While, micronuclei was also recorded in some treatments (Table 3, Fig.1: h and l) and our results are in agreement with the results of (Haroun, 2010).

Finally, the induction of these chromosomal abnormalities were pointed to the mutagenic potential of the diprofos drug.

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