Cytogenetic effect of Insecticide Telliton and Fungicide Dithane M-45 on Meiotic Cells and Seed Storage Proteins of *Vicia faba*.

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Abstract: The genotoxic effects of insecticide Telliton and fungicide Dithane M-45 were examined on meiotic cell divisions and changes in the M2 seed storage protein banding pattern of *Vicia faba* plants. The percentage of abnormal pollen mother cells, (PMCs) increased as the concentration of both pesticides increased. All concentrations and treatment periods of both pesticides, induced a number of chromosomal aberrations in PMCs as stickiness, bridges, laggards, disturbed, micronuclei and multinucleate. A marked change was observed in the M2 *V. faba* seed storage protein banding pattern. These changes included alterations in band intensity, relative mobilities, disappearance of some bands and appearance of new other ones. These results showed that Telliton has more mutagenic effects than Dithane M-45. [Journal of American Science. 2011;7(1):19-25]. (ISSN: 1545-1003).

Key words: *Vicia faba*, chromosomal abnormalities, insecticide, fungicide and SDS -PAGE protein.

1. Introduction:

Pesticides are used all over the world, their use has increased spectacularly because it has greatly improved agricultural yield through inhibition of diseases by acting against pests in the field and during storage of agricultural products Taylor *et al.*, (1997). A number of pesticides are used to protect agricultural products from diseases, weeds and insects, but residues of these chemicals lead to environmental pollution and pose threat to people and animals. Although chemical control creates several problems, use of pesticides is still maintaining its popularity for obtaining effective results. Now, After increased application of many new agrochemicals on a large scale in the egyptian agriculture and other countries has led some workers to investigate the possible genetic material and storage proteins alterations Badr (1988), Abdel Salam *et al*., (1993a & b), Hassan (1996), George and Ghareeb (2001) Asita and Makhalemele (2009). Telliton known as profenophos is the insecticide commonly used in delta Egypt region in agricultural fields. Dithane M-45, also known as mancozed fungicide belongs to a class of chemicals as ethylene bisdithiocarbamate (EBDC).The EBDC is fungicide used to prevent crop damage in the field and to protect harvested crops from deterioration during storage or transport. Chromosomal aberrations have been considered as a reliable indicator of mutagenic activity, since there have been evidence for a correlation between chromosomal damage and toxic effects of a number of pesticides Badr (1983), Askin (2006), Shehata *et al*., (2008), Ozturk (2008) and Fisun and Goc Rasgele (2009). On the other hand, Abdelsalam *et al.*, (1993b) and Hassan *et al*., (2002) used electrophoretic banding patterns of seed storage proteins for monitoring the mutagenic effects of pesticides and other chemicals.

The present work was planned to study the mutagenic effect of the insecticide Telliton and fungicide Dithane M-45 as revealed by meiotic abnormalities and changes in M2 seed storage protein banding patterns of *V. faba* as a biological system.

2. Materials and Methods:

Vicia faba L. variety Giza 3, kindly procured from Crop Research Institute, Agricultural Research Center, Giza, Egypt.

1- Meiosis

Vicia faba plants at the flowering stage were sprayed with different concentrations of the insecticide Telliton, (*0*-4-bromo-2-chlorophenyl *0*-ethyl s-propyl phosphorothioate) 1.5, 3, and 6 ml/L and the fungicide Dithane M-45(ethylene-bis dithiocarbamate) 150, 300 and 600 mg/L. These, pesticides selection were purely on the basis of the frequent use in the agricultural fields by the farmers of Delta, Egypt. A negative control plants were sprayed with distilled water. Eight flower buds from eight different plants were gathered through durations of 24 h., 48 h. and 10 days.

For meiotic studies the appropriate flower buds were collected and fixed in Carnoy's solution (ethyl alcohol absolute and glacial acetic acid in the ratio 3:1) for 24 h. and then transferred to 70% ethyl alcohol and kept in refrigerator. The cytological analysis were carried out by using 2% aceto-carmine stain as described by Darlington and La Cour(1976). The data recorded for different treatments were statistically analyzed using *t*-test for determine significant differences between these treatments.

2-Electrophoresis of water soluble and non soluble proteins:

Water soluble and non soluble proteins were performed on vertical slab (20 cm x 20 cm x 0.2 cm) using the gel electrophoresis apparatus (Manufactured by LABCONCO) according to Laemmli (1970). The dry M2 seeds of *V. faba* plants, whose parents were sprayed with these pesticides, were decoated and milled to fine powder. Soluble proteins were extracted overnight using 0X Tris-Hcl buffer of pH 6.8. Centrifugation was performed at 10000 rpm for 10 min., then the non soluble proteins were extracted from the belt by add IX Tris-Hcl buffer pH 6.8 for 24 h. and then centrifuged at 10000 rpm for 10 min., then 40 µl supernatant of soluble and non-soluble proteins were loaded in SDS-slab gel of 15% acrylamide containing 10% SDS. Gel was run at a current of 15 mA for 1 hour followed by 25 mA for 4-5 h. Molecular weights of different bands were calibrated using the wide range protein marker ranged from 10 -200 KDa according to Matta *et al.*, (1981).

3. Results and Discussion:

I-Cytological studies:

A wide spectrum of chromosomal abnormalities were recorded in eight flower buds from different plants after treatment with different concentrations of Telliton (1.5, 3 & 6 ml/L) and Dithane (150, 300 & 600 mg/L). The number of meiotic cells of treated and control plants are presented in Tables (1& 2). The insecticide Telliton give the number of chromosomal abnormalities higher than fungicide Dithane. Both pesticides caused a hollow range of meiotic abnormalities. The number of abnormal pollen mother cells (PMCs) formed in the flower buds of *V. faba* plants was obvious with all concentrations of pesticides and in all stages and durations.

Data in Tables 1& 2 shows that the percentages of abnormal PMCs in the first division were greater than those recorded in the second division after spraying with both pesticides. The most frequent types of abnormalities were observed stickiness, laggards, bridges, disturbed, micronuclei and multinuclei after being treated with all concentrations of both pesticides. These results demonstrated in Tables (1&2) and Fig.1 revealed that the abnormalities were present in metaphase, anaphase and telophase stages of the meiosis with all treatments. The induction of meiotic abnormalities appears to be a common effect of most pesticides (Fisun & Goc Rasgele, 2009).

The stickiness and disturbed stages were the most common abnormalities found in all phases of the meiosis after treatments with all doses of both pesticides (Fig.1). The number of sticky cells increased in all stages of meiotic divisions as the concentration of both pesticides increased during durations of 24 h, 48 h and 10 days. Our results are in agreement with the results of Badr (1988); Pandey *et al.*, (1994); Singh *et al.* (2007) and Srivastava & Singh (2009). Abdelsalam *et al.*, (1993b) they suggested that the chromosome stickiness may results from breakage and exchange between chromatin fibers on the surface of adjoining chromosomes.

The second type of abnormalities is the laggard that occurred at metaphase cells. They could be attributed to the failure of the spindle apparatus to organize and function in a normal way Pickett-Heaps *et al*., (1982). These laggards may be distributed randomly to either poles at anaphase I or II which result ultimately in aneuploidy (Amer & Mikhael, 1987; Amer & Ali, 1988) or may give for micronuclei at telophase II (Abdelsalam *et al*., 1993 a). The induction of laggard chromosomes could be attributed to irregular orientation of chromosomes (Patil and Bhat, 1992).

In addition to the previous common abnormalities, it was observed more on meiotic division including bridges, micronuclei and multinucleate. Bridges were induced under the treatment with both pesticides. They could be due to the breakage and reunion (El-Khodary *et al*., 1990) or due to the general stickiness of chromosomes (Haliem, 1990).While, micronuclei and multinucleate were also recorded with low percentages after treatment with both pesticides and our results are in agreement with the results of Badr (1988) and Pandey *et al.*, (1994). Finally, the induction of these chromosomal abnormalities were pointed to the mutagenic potential of the applied concentrations of these pesticides.

II-Biochemical studies:

At the biochemical genetic level, water soluble and non-soluble protein, Table (3 & 4) and Fig. (2) represent the mutagenic effects of both pesticides, Telliton and Dithane on the banding pattern of M2 seed storage proteins of *V. faba* plant. These changes include alterations in band intensity, relative mobilities, disappearance of some bands and appearance of some new other bands.

Alterations in bands intensity could be attributed to change in the structure or performance of genes and thus they produce changes in the gene expression of the regulator genes used in the regulatory system of the structural genes Hassan 1996. The increase in band(s) intensity could be attributed to gene(s) duplication that resulted from cytological abnormalities induced by applied pesticides. The presence of laggards and bridges support this conclusion. This conclusion is in agreement with

Gamal El-Din *et al*., (1988).Also, they noticed that increasing the number of genes encoding for the different protein subunits through doubling of chromosome number from 12 to 24 in *V. faba* caused an increase in band intensity.

Changes in relative mobility of these bands are probably due to point mutation that leads to production of shorter or longer polypeptide chains. These changes in the soluble proteins are probably due to the occurrence of gene duplication mutation more than point mutation that takes place in one or more of the duplicated genes that encoding the protein subunit of that band Abdelsalam *et al*., (1993b). Also, these alterations in bands intensities or densities and relative mobility are in agreements with Hassan (1996), George and Ghareeb (2001) and Hassan *et al*., (2002).

The disappearance of some bands in soluble and non soluble proteins of *V. faba* to the inherited effects of the both pesticides, Telliton and Dithane could be explained on the basis of mutational event at the regulatory genes that prevent or attenuate transcription (Muller & Gottschalk, 1973).Induction of laggards, bridges and micronuclei by these pesticides may lead to the loss of genetic materials. Therefore, some electrophoretic bands were disappeared due to the loss of their corresponding genes (Abdelsalam *et al.,* 1993b). They also reported that the reduction of chromosome complement in *V. sativa* (2n=6) lead to the complete disappearance of the convicilin like band. The present results therefore may point out a mutagenic potential of both pesticides, Telliton and Dithane as indicated by observing a large number of the meiotic abnormalities and the heritable changes in the M2 seed storage protein banding patterns.

Table (1): Numbers and percentages of abnormal PMCs in the1st & 2nd meiotic divisions, percentages of types and mean of meiotic abnormalities after spraying of *V.faba* plants with Telliton insecticide for (24, 48 hours & 15 days).

Time	Conc. In Ml/L	No. of counted PMCs	No. of abnormal PMCs	% abnormal In 1st division	% abnormal In 2nd division	Types and percentages of meiotic abnormalities						Mean of % abnormal PMCs ± SE
						Stick.	Lag	Brid.	Dist.	Micronuclei	Multinuclei	
24 h	Cont.	7684	12.00	0.19	0.11	25.00	16.66	8..33	50.00	-	-	0.16±0.03
	1.5	5761	479	8.15	8.49	29.23	13.36	7.72	47.59	0.83	1.25	8.31±1.04
	3	4677	718	18.14	12.68	35.93	12.39	6.82	42.06	1.11	1.67	15.35±1.32
	6	3471	924	28.43	24.89	42.09	11.04	6.17	35.60	1.62	3.46	26.62±1.12
48h	1.5	3023	567	10.55	8.20	31.75	13.76	7.41	43.56	1.59	1.94	9.38±1.12
	3	2968	656	13.00	9.10	31.09	14.18	7.32	42.84	1.98	2.59	11.05±1.22
	6	2741	798	17.10	12.01	35.34	14.16	6.52	39.59	1.50	2.88	14.55±1.34
10 days	1.5	4672	390	4.64	3.70	28.97	12.05	7.43	50.25	0.51	0.77	4.17±0.94
	3	4195	506	7.44	4.67	33.00	13.04	6.32	44.66	1.78	1.19	6.06±1.01
	6	3841	565	8.25	6.46	35.75	12.92	6.55	41.42	1.42	1.95	7.36±1.32

Stick: stickiness Lag.: laggards Brid.: bridge Dist.: disturbed PMCs : pollen mother cells

Table (2): Numbers and percentages of abnormal PMCs in the1st & 2nd meiotic divisions, percentages of types andmean of meiotic abnormalities after spraying *V. faba* plants with Dithane fungicide for (24, 48 hours & 15 days).

Time	Conc. In mg/L	No. of counted PMCs	No. of abnormal PMCs	% Abnormal In 1st division	% Abnormal In 2nd division	Types and percentages of meiotic abnormalities						Mean of % abnormal PMCs ± SE
						Stick.	Lag	Brid.	Dist.	Micronuclei	Multinuclei	
24 hr	Cont.	7684	12.00	0.19	0.11	25.00	16.66	8..33	50.00	-	-	
	150	6142	384	3.58	2.67	25.26	14.06	10.67	47.14	0.52	1.82	0.16±0.03
	300	5639	463	4.58	3.64	24.62	16.41	11.66	43.84	1.08	2.37	3.12±0.90
	600	5173	628	6.57	5.56	29.30	18.78	11.46	36.15	1.91	2.39	4.11±1.10
48hr	150	5672	298	2.96	2.29	26.17	14.42	12.08	43.28	1.67	2.35	6.07±1.20
	300	5113	315	3.23	2.93	26.66	16.50	13.33	40.63	0.95	1.90	2.63±0.80
	600	4370	362	4.53	3.75	26.79	16.85	13.81	37.56	1.65	4.42	3.08±1.02
10 days	150	6176	256	2.14	2.01	23.04	14.84	12.11	45.31	0.78	3.91	4.14±1.32
	300	6214	291	2.43	2.25	24.39	15.81	13.05	41.24	1.72	3.78	2.07±1.00
	600	5365	310	3.31	2.46	26.45	16.45	15.81	36.77	1.94	2.58	2.34±1.36

Stick: stickiness, Lag.: laggards, Brid.: bridge, Dist: disturbed, PMCs : pollen mother cells.

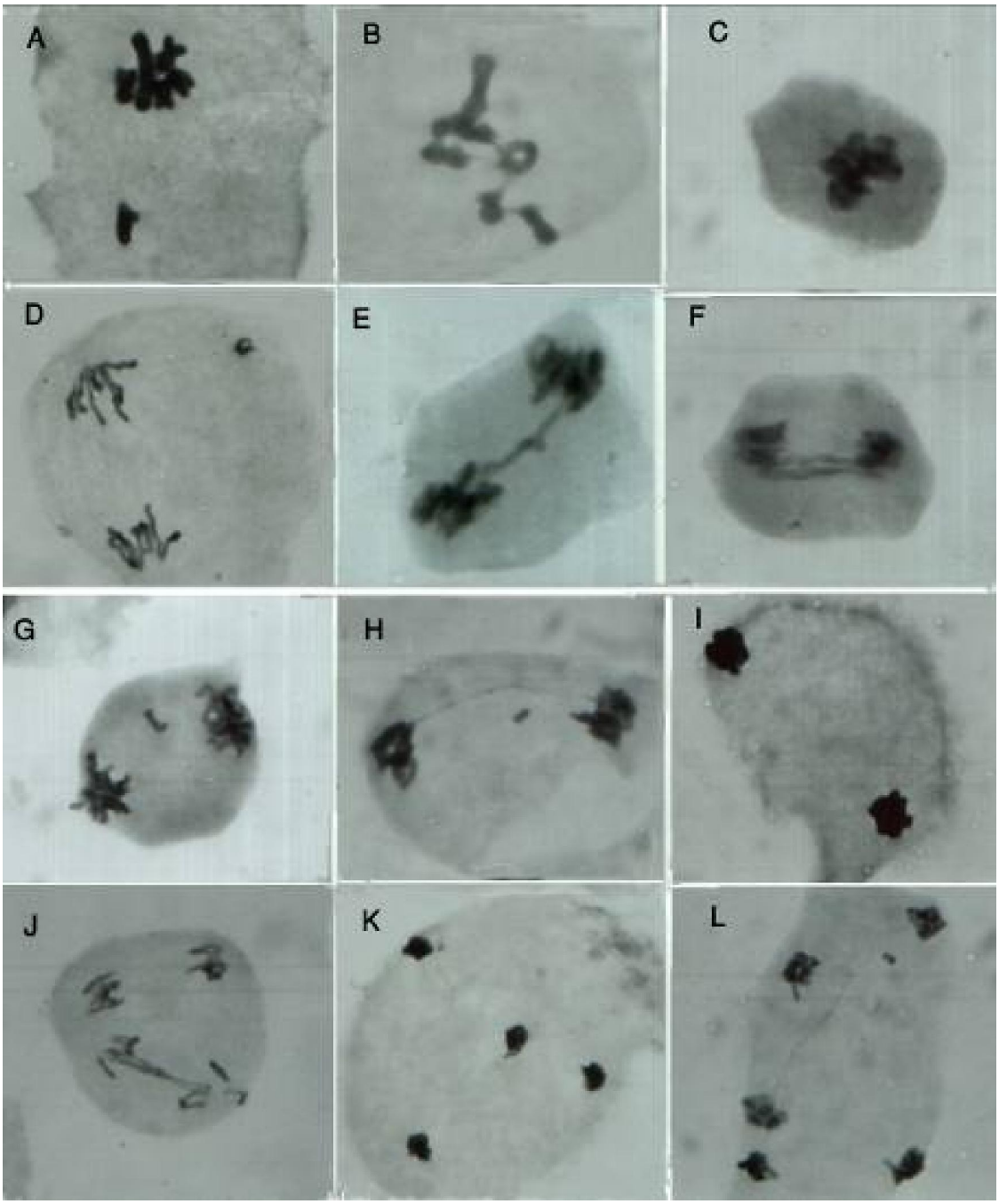

Fig (1): Types of chromosomal abnormalities produced after treatments with different concentrations of Telliton and Dithane. A- metaphase I with laggard. B- metaphase I with ring. C- metaphase I with sticky. D- anaphase I with laggard. E- anaphase I with bridge. F- anaphase I with double bridges. G- telophase I with laggard. H- telophase I with broken bridge and laggard. I- telophase I with sticky .J- anaphase II with double bridges. K- disturbed telophase 2. L- telophase II with multinucleate and micronucli.

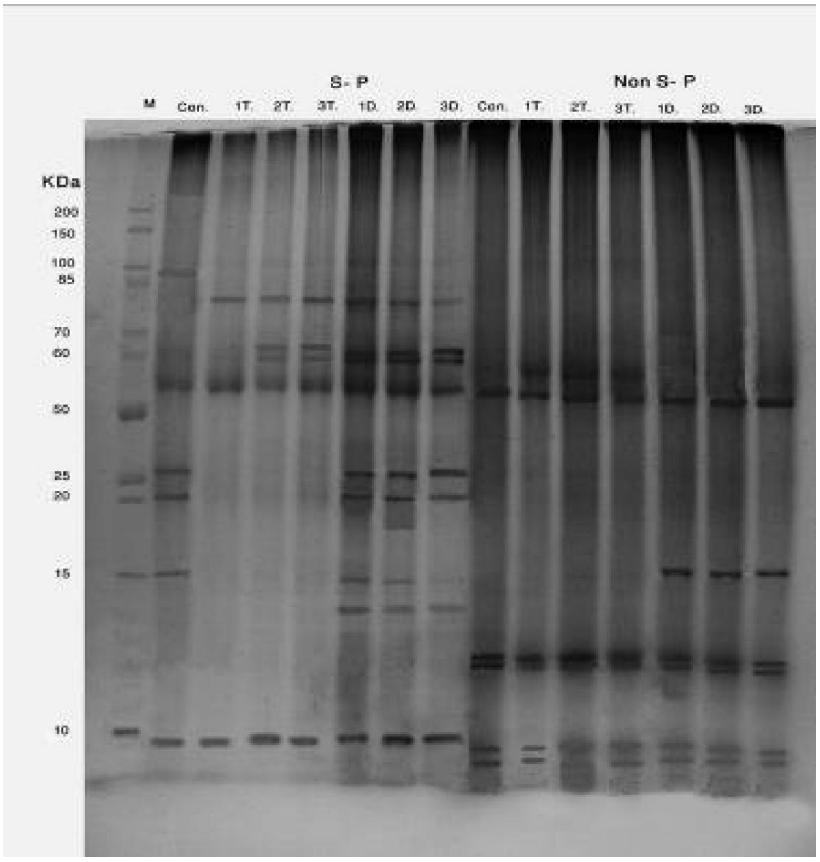

Fig (2): SDS–PAGE banding patterns of water soluble (s-p) & non-soluble (non s-p) proteins for *V. faba* after sprayed with three concentrations of Telliton (T) and Dithane (D) pesticide.

Table (3): Electrophoretic of water soluble protein banding patterns of *V. faba* seed storage protein showing the effects of three concentrations of Telliton (T) & Dithane (D) pesticides.

No. of band	MW	RF	Con.	1T	2T	3T	1D	2 D	3 D
1	102.939	0.139	+2	-	-	-	-	-	-
2	92.218	0.172	-	+2	+2	+3	+2	+2	+
3	69.238	0.258	-	-	+2	+2	+3	+3	+3
4	65.207	0.276	-	-	+2	+2	+3	+3	+3
5	56.501	0.319	+2	+2	+	+2	+3	+3	+2
6	34.965	0.463	+2	-	-	-	+2	+2	+2
7	30.196	0.507	+2	-	-	-	+2	+2	+2
8	19.320	0.641	+	-	-	-	+	+	-
9	14.171	0.734	-	-	-	-	+	+	+
10	7.325	0.932	+2	+	+2	+2	+2	+2	+2

- : Missed +: Fait +2: Dark +3: Very dark

Table (4): Electrophoretic of water non-soluble protein banding patterns of *V. faba* seed showing the effects of three concentrations of Telliton (T) & Dithane (D) pesticides.

No. of band	MW	RF	Con.	1 T	2 T	3T	1D	2D	3D
1	63.107	0.287	-	+2	+2	+2	-	-	-
2	55.478	0.327	+2	+2	+2	+2	+2	+2	+2
3	19.982	0.644	-	-	-	-	+3	+3	-
4	13.359	0.769	+2	+2	+2	+2	+2	+2	+2
5	12.485	0.790	+2	+2	+2	+2	+2	+2	+2
6	8.056	0.926	+2	+2	+	+	+	+	+
7	7.505	0.948	+2	+2	+	+	+	+	+

- : Missed + : Fait +2: Dark +3: Very dark

4. Conclusion

From the present data it may be concluded that treatment of *V. faba* plants with different concentrations of Telliton and DithaneM-45 showed positive chromotoxic effects in PMCs and changes in the M2 seed storage protein banding patterns. These effects included chromosomal abnormalities such as stickiness, laggards, bridges, disturbed, micronuclei and multinuclei. While at the biochemical level, the obtained data showed several changes in M2 seed storage protein banding pattern included alterations in band intensity, alterations in the relative mobilities of some bands, disappearance of some bands and appearance of new other bands of protein banding patterns, as compared with the negative control.

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