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II- Potential mutagenic effect of dusting *Vicia faba* seeds with Malathion 1% powder or fumigating by Phosphine for storage

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ABSTRACT

This work was carried out to evaluate the potential mutagenic effect of the two stored seeds protectant agents "Malathion 1% powder and Phosphine fumes", commonly used in Egypt, to protect the stored beans from two storage *Bruchids* pests (*Callosobruchus maculates* F and *C. chinensis* L.) after six months of storing of *Vicia faba* seeds (Var. Giza 716) under treatment with (1, 2 & 4 times the effective concentrations) of each of the two agents. The evaluation took place on: germ cells (meiosis); some quantitative agronomic traits of F1&F2 generation plants and the changes in the storage protein banding pattern of M2 seeds, for each concentration comparing with concomitant negative control.

The obtained results emphasized that: "Phosphine fumes" is the more potential mutagenic agent, while "Malathion 1% powder" was the least. So for the need of agriculture Malathion 1% powder can be safely used as seeds' protectant agent for storage if used in the recommended concentration.

Key words: Malathion, Phosphine, meiosis, metric traits, protein electrophoresis.

INTRODUCTION

Since dusting with "Malathion 1% powder" and fumigation with "Phosphine fumes" have been recommended in Egypt long time ago till now, to be used as beans protectant agents against *Bruchids* pests for storage (Prog. Pest Cont., Min. Agric., A.R.E., 1980-2001 last ed. and Flyer of pest control- Min. Agric., ARE, 2014/2015), it becomes of importance to study their potential mutagenic effect.

Many investigators have suggested that the study of chromosomal aberration in mitotic and meiotic divisions (Amer *et al.*, 1989 and Haroun, 2010); changes in some quantitative agronomic traits of the F1&F2 plants (Coimbra *et al.*, 2004 and Tamas, 2010) and changes in storage protein banding patterns of the M2 seeds/ grains are suitable systems to detect the potential mutagenic effect caused by any compound (Gamal El-Din *et al.*, 1988 and Mendhulkar, 1993). In this respect, the potential mutagenic effect of these two stored seeds protectant agents were recently studied by Adam *et al.* (2016) on the mitotic division of *Vicia faba* root-tip meristems, in addition the ultrastructure changes of some cytoplasmic organelles were detected. They found that, "Phoshine fumes" was more potentially mutagenic effective and inducing deleterious effect on the cytoplasmic organelles, while "Malathion 1% powder" was the least.

The objective of the present study was to obtain further additional information about the potential mutagenic effect of Malathion and Phosphine at the cytogenetic; genetic and biochemical genetic levels on

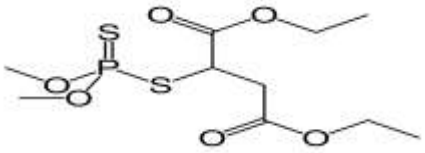
Vicia faba plants originated from the treated seeds.

MATERIALS AND METHODS

a) Treatment materials:

a.1) The synthetic organic insecticide "Malathion 1% powder":

Malathion, is an organophosphorus insecticide, also known as carbophos, maladision and mercaptothion. Its chemical formula is: $C_{10}H_{19}O_6PS_2$ and its skeleton is:



"Malathion 1% powder" (Mal.) was purchased from one of the respected store for agricultural insecticides. The effective concentration is 0.08g for 100g of stored seeds (Prog. Pest Cont., Min. Agric., A.R.E., 2001).

a.2) The synthetic inorganic fumigator insecticide "Phosphine":

Phosphine (phos.) or hydrogen phosphide PH_3 is colorless, flammable and toxic gas, it is a highly toxic seed fumigant that can be produced from the reaction of metal phosphides with water (http://en.wikipedia.org/wiki/Phosphine). Aluminum phosphide (API) is an inorganic phosphide, remains as the only economically viable fumigant for widespread use. It is mainly used as an indoor fumigant at crop transport or storage. It is available in pellet and tablets under the trade names (ash Phos, Gastoxin, Phostoxin ….etc.) (US Environ. Protect. Agency1992). Phostoxin, recently is the commonly used pesticide in Egypt by the farmers and sometimes in the agricultural communities, to protect the stored seeds and grains from the infestation by storage insects.

Phostoxin is kindly produced by Crop Res. Inst., Agric. Res. Centre, Giza, Egypt, in a form of aluminum phosphide powder compressed into hard round tablets sealed in foil and packed in tin can. The effective concentration was calculated, just as, recommended by the Min. of Agric. ARE, to be 0.003g for each 350 cm^3 well- closed glass jar (Prog. of Pest Cont. 2001 Last ed.).

Vicia faba seeds (V. Giza 716) were used in the conducted experiments.

b) Methods:

b.1) Treatment with the tested materials:

About 100 gram of nearly similar size selected *Vicia faba* seeds were used for each test dusting concentration treatment with Mal. Three samples for the different concentrations (1, 2 and 4) times the effective concentration (EC) which were previously studied by "*Adam et al*. (2016)" on the mitotic division of the root-tip and one sample for the control. Dusting treatment took place by shaking the seeds with the powder thoroughly inside well closed two layers of thick paper sacs. Samples were stored apart from each other for 6 months period at room temperature.

Also, 100 gm of *V. faba* seeds for each fumigation treatment with Phos. in three different concentrations (1, 2 and 4EC). Each sample was put in a tulle sac and well closed to be ready for fumigation treatment. Fumigation treatment took place by crushing a small part of the tablet of "Phostoxin" before weighting the determined amounts necessary for each concentration treatment. Quickly each of the weighed powder was put at the bottom of the glass jar, followed by a thick layer of seeds of about 8 cm height, then the samples in the tulle sac were put flattened on the surface of the seeds. The jars were tightly closed and stored in a dark place at room temperature for 6 months.

b.2) Observations and scoring:

i- Meiotic Studies:

The stored seeds of *Vicia faba* were thoroughly washed in water to remove any

traces of the tested materials adhered to the surface of the seeds and sown in 40 cm diameter pots at the beginning of November in the botanical garden of Faculty of Women for Arts, Science and Education, Ain Shams University. Irrigation when needed and fertilization was carried out as recommended. Six seeds were sown in each pot, six pots were used for each treatment as well as for the control. After ten days of planting, the number of plants survival were recorded / each pot. After 40 days from sowing, the flower buds were gathered for meiotic studies and three plants/ pot were left for studying the quantitative agronomic traits.

The flower buds were gathered and fixed immediately in carnoy's fixative for 24 h at 4°C, then after rinsed several times in tap water and stored in 70% ethyl alcohol in refrigerator till preparation of the slides. To examine the meiotic division, the slides were prepared temporary by the use of aceto-carmine smear method (Sharma and Sharma, 1980). Flower buds (commonly from 6 different plants) were investigated for each treatment using light microscope with camera (OLYMPUS BH). Abnormalities were counted in the 1st and 2nd meta- and ana- telophase stages of the pollen mother cells. Abnormalities scored were in the pollen mother cells, tetrads and pollen grains. According to their availability 50- 100 cells in each meiotic stage were investigated / plant and 1000 PGs. / plant were counted. The data collected were analyzed using t- test analysis to check the significant statistical differences between the control and the test plants.

ii- Metric traits:

Cultivation of the F1 generation plant took place as described before in the meiotic studies. Number of plants emerged / pot was recorded after 10 days from seed sowing. The plants heights were measured after 100 days from sowing. After harvesting, weight of seeds /plant and weight of 100 seeds/ plant were recorded. By the next year, the same experiment was repeated on first yield seeds to obtain the F2 generation plants. Number of plants emerged /pot was recorded after 10 days then thinning took place after two weeks from seeds sowing. Examinations of the same metric traits of the second generation were carried out. The data collected for F1&F2 generations were analyzed using t-test analysis.

iii- Biochemical genetic analysis:

Qualitative characterization of Protein using gel electrophoresis:

The total storage protein of M_2 seeds Legumes were analyzed using discontinuous polyacrylamide gel electro-phoresis in the presence of sodium dodecyle sulphate (i.e.) discont-SDS-PAGE from that described by Laemmli (1970). Protein GELANALIZER 2010 program is used for data analysis.

RESULTS AND DISCUSSION

1- At the cytogenetic level (meiosis):

Regarding meiosis as a critical process in the life cycle of the sexual plants; the normal meiosis ensures gamete viability while abnormal meiosis gives an indication of mutagenicity of the tested material.

Table (1) illustrates that dusting treatments with the different concentrations of Mal., induced highly significant concentration independent percentages of abnormal PMCs. / plant. The higher percentage was (5.81%), observed after treatment with 2EC. Fumigation treatments with Phos., were more destructive (i.e. the more potentially mutagenic agent).They affected concentration dependent, higher percentages of abnormal PMCs. / plant, it

reached (12.74%) of the scored PMCs. /plant, after treatment with 4EC of Phos.. The induction of highly significant increasing percentages of PMCs./plant were previously observed by: Morsi (2003) after mixing *Vicia faba* seeds with " Neem leaf powder" for storage ; Mohamed and El-Ashry (2012) after treatment of *Pisum sativum* seeds with "aqueous *Trigonella foenum graecum* extract" and storage of six months and Tamas Elena (2010), after soaking for 6 h of dry dormant *Vicia faba* seeds with different concentrations of the two mutagens diethylsulphate and dimethylsulphate .

Percentages of abnormal PMCs. were generally lower in the 2nd meiotic division than those of the 1st meiotic division, after all dusting treatments with Mal. and fumigation treatments with the higher concentrations of Phos (Table 1). The same effect was observed previously by Mohamed and El-Ashry (2012) and Al-Zahrani *et al*. (2012) and suggested this phenomenon to be a result of recovery in this cell age.

The two agents affected the induction of a wide range of abnormalities covering all stages of the meiotic divisions. The induced irregularities were grouped in the three categories: The 1st category is those of stickiness dominated; the 2nd category is those of disturbed dominated and the 3rd category is the chromosomal structural aberrations, which includes ana-telophases I &II with structural bridges and PMCs with fragments and/or breakage are present. In addition, there were two other types of abnormalities, normal contracted chromosomes which were observed at the metaphase I & II stages only after treatment with Phos. and PMCs. with micronuclei which were observed at the ana-telophase 1 & II stages after treatment with the two agents (Table 2). It was clear from data in Table (2) that the higher percentages of chromatin material liquefaction abnormalities were observed in the PMCs. after dusting treatment with 2EC of Mal. (3.78%) and after fumigation treatment with all concentrations of Phos. (4.76 , 3.98 and 4.71% successively). This group includes stickiness of the chromosomes and sticky bridges (e.g. Figs. 1-4). Stickiness of the chromosomes dominated.

Stickiness of the chromosomes was observed at all stages of the meiotic division, after all concentrations of dusting treatment with Mal. and fumigation treatment with Phos. Relative to those of Mal, fumigation treatment with Phos affected higher percentages of PMCs. with sticky chromosomes at all stages, after almost concentrations treatment. They were concentration dependent only, at the metaphase I and ana-telophase I stages.

The nature of stickiness had been discussed by many investigators. Hsu *et al.* (1965) speculated that, chromosome stickiness might be caused by an increase amount or an abnormal behavior of the RNA-containing particles which coat the metaphase and anaphase chromosomes. Bhat *et al*. (2007), attributed stickiness to some kind of gene mutation leads to incorrect coding of some non-histone proteins involved in chromosome organization.

Chromosome kinetic abnormalities group includes: disturbance of the chromosomes and lagging chromosomes (Figs. 5-11). Table (2) illustrates that, those with disturbed chromosomes consist the higher frequency after both dusting treatments with Mal. and after fumigation treatments with Phos.. Higher percentages were induced after fumigation with the different concentration treatments with Phos. relative to those of Mal., they were concentration dependent and reached (6.44%) of the scored PMCs. after treatment with 4EC .

II- Potential mutagenic effect of dusting *Vicia faba* seeds with Malathion 1% powder or fumigating by Phosphine for storage

Table (2) shows that two types of disturbance of the chromosomes were observed in the PMCs., after both treatments with Mal. and Phos.. They are: the normal disturbance of the chromosomes, which originated as a result of disturbance of the spindle and the splitting of the chromosomes, which originated as a result of splitting of the spindle. Generally, the normal disturbance dominated. It also illustrates that, too much higher percentages of disturbance of the chromosomes were recorded, after the different fumigation treatments with Phos. and they were concentration dependent.

Concern the disturbed configurations of the chromosomes, it is considered as an important indication of the failure of the spindle apparatus to organize and function in normal way (Pickett-Heaps *et al*., 1982). Ananthakrishanan *et al*. (2013) suggested that, disoriented metaphase may be due to spindle dissolution, which could be due to alteration of the gene controlling the spindle mechanism.

As shown in Table (2), dusting treatment with the EC of Mal. affected small percentage (0.67%) PMCs. with lagging chromosomes, decreased with the increase of concentration till they reached (0.39%). Higher percentages of PMCs with lagging chromosomes were observed after fumigation treatment with the higher used concentrations of Phos. Generally, Phos effected concentration dependent percentages of PMCs. with lagging chromosomes that were observed at all stages of the meiotic division, after treatment with almost concentrations of Mal. or all concentrations of Phos. The induction of laggard chromosomes could be attributed to irregular orientation of chromosomes and they were all due to the failure of spindle mechanism (Patil and Baht, 1992). In meioses, these lagging chromosomes (univalent or bivalent) which are characterized by retarded movement during anaphase, may be distributed randomly to either poles at anaphase I or II which result ultimately in aneuploidy (Amer and Mikhael, 1987; Natarajan *et al*., 1993) or may give for micronuclei at telophase II (Abdel-salam *et al.,* 1993a).

Chromosomal structural aberrations group includes two types: (a) Fragments and /or breakage and (b) Structural bridges (e.g. Figs. 12-17). Summation of the percentages of the two types of chromosomal structural aberrations relative to the scored PMCs. was the lower percentages, comparative to the other main groups. These percentages were very low, concentration dependent, after dusting treatments with Mal. Higher percentages were induced after the different fumigation treatment with Phos., but, they were concentration independent (Table 2).

PMCs with structural bridges were observed at the anaphase I & II stages and rarely at the telophase I & II, after both treatments. Dusting treatments with Mal., induced very small concentration dependent percentages of ana-telophase I PMCs with structural bridges. It's higher percentage was (0.33%) induced after treatment with 4EC, lowered to (0.08%) of the scored ana-telophase II PMCs after the same concentration treatment. Higher percentages of ana-telophase I PMCs. with structural bridges were recorded after the lower concentration treatment with Phos. (0.26% & 0.50%) comparative to those of Mal. (0.08% & 0.17%). At the ana-telophase II stage, their percentages lowered to (0.09% & 0.11%), but still higher than those of Mal., which were (0.00% & 0.08%) (Tables are not attached). Structural bridges could be due to the breakage and reunion of the broken ends (El-Khodary *et al*., 1990 a).

Similar to our observations (stickiness and disturbance of the

chromosomes, laggards and bridges) which were induced in the meiotic division after the different treatments of the seeds with the two agents, were previously observed by Morsi (2003); Tamas (2010) and Mohamed and El-Ashry (2012), and by Bhat *et al*. (2007) after treatment of the (8 hr) presoaked *Vicia faba* seeds to 6 hr treatment with the mutagen ethyl methane sulphate.

Relative to the scored meiosis, fragments was observed in low percentages, the higher percentages of PMCs. with fragments were recorded after treatment with 2EC & 4EC of Phos. (Table 2, e.g. Figs. 14-17). Agents with such effect are considered to exert clastogenic action on the chromosomes, which is regarded to affect the chromosomal DNA (Grant, 1978), i.e. Phos. is the more clastogenic agents and Mal. is the least. Generally, their percentages were higher at the metaphase I & II stages comparative to those of the anaphase I & II stages after different concentration of Phos. There weren't any fragments at ana-telophase II stage after treatment with all concentrations of Mal.

It is well known that, structural bridges and fragments lead to structural changes in the chromosomes. The percentages of (metaphases I & II PMCs with fragments + anaphases I & II PMCs with structural bridges and/or fragments) relative to their scored number. Table (3) illustrates that, treatment with Mal affected significant percentage only after treatment with 4EC and those of Phos affected significance after all concentration treatments.

Relative to the scored PMCs., the percentages of the meioses with micronuclei were very small after all treatments. They were observed at the ana-telophase I & II stages and were without clear trend with the increase of concentration after treatment with Mal. But, treatments with Phos affected concentration dependent increase in the percentages of PMCs. with micronuclei (Table 2 e.g. Fig. 18). The higher percentages at the ana-telophase II stage with micronuclei were observed after treatment with the higher used concentrations of Phos. Micronuclei may originate from a chromosome lagging or fragment at the anaphase I & II stages (Brown and Dyes, 1972). Micro-nucleus derived from a whole chromosome has higher probability to survive and undergo condensation in synchrony with the main nucleus than the micronuclei derived from a chromosome fragment (Gustavino *et al*., 1987). In the present study, the presence of micronuclei indicates that Phos may have a mutagenic effect which may lead to loss of the genetic materials (Auerbach, 1962). Micronuclei were previously observed by Bhat *et al.* (2007) and Tamas (2010).

Slight percentages of abnormal tetrads were observed in the F1 generation flower buds after treatment of the seeds with 1EC of Mal. Higher percentages were recorded after treatment with 2EC & 4EC of Phos, Pentads was the type of abnormality observed in the tetrad stage (Figs. 19 & 20). Pentads seemed to be formed as a result of the induced laggerds, multipolarity or break at the ana-telophase II stage.

Table (1) illustrates that, the two stored seeds protectant agents affected small percentages of abnormal pollen grains in the flower buds of the F1 generation plants. Their percentages were concentration dependent. Mal. affected significant percentage after treatment with the higher concentration (4EC). Higher effect was observed after fumigation treatment with Phos., it affected significant percentage after treatment with 2EC , increased to be highly significant after treatment with the higher used concentration (4EC). Small and deformed (with irregular margins) pollen grains were the types of abnormality observed (Figs. 21-24) Small pollen grains

seemed to be formed form pentads and deformed pollen grains from disturbance of the poles at the ana-telophase II stage (El-Ashry, 1986). Small and deformed pollen grains were scored and considered to be sterile pollen grains by Fisun and Rasgele (2009) and El-Ashry and Mohamed (2013). Similar effects were observed by Tamas (2010) and Fisun and Rasgele (2009).

2- <u>At the genetic level (metric traits)</u> :

Table (4) illustrates that, there was no significant reduction in the percentage of 10 days old seedling survival of the F_1 generation plants, after all concentration treatments with the two agents, decrease in these percentages were observed after treatment with the higher used concentration of Phos.. The percentages of seedling survival of the F_2 generation plants were lower after all concentration treatments with Phos and the higher concentration treatment with Mal. Significant reduction was observed only in the F_2 generation plants after treatment with the higher used concentration of Phos.. Reduction in the percentages of seedling survival were observed previously by: Bhat *et al*. (2007), Tamas (2010) after treatment of *Vicia faba* seeds with different mutagens and by Dixit *et al*. (2012) after treatment of *Nigella sativa* seeds with sodium azide. Bhat *et al*. (2007) attributed this reduction in the percentage of seedling survival of the F_1 plants to the cytogenetic damage and physiological disturbances leading to the death of the cells and those of the F_2 plants could be due to mutants induced in the F_1 plants.

Measuring plant's hight of the plants after 100 days from sowing showed that, dusting treatment with only 4EC of Mal. affected the significant reduction of plant's height of F1 generation plants which extended to the F2 generation plants. Highly significant reduction in plant's height were observed in the F1 generation plants after treatment with the higher used concentration of Phos.. Significant reduction of plant's height of the F_2 generation was observed after treatment with 2EC of Phos., increased to be highly significant after treatment with the higher used concentration (Table 4).

Similar effects were observed by Coimbra *et al*. (2004) after treatment of the hexaploid oat seeds with the two mutagenic agents (Co^{60} gamma rays and EMS), the data of the M_2 generation pointed to a decrease in the plant's height under increasing agent doses and by Haroun (2010) after treatment of the presoaked *Vicia faba* seeds with different concentrations of the insecticidal active "aq. *Kochia indica* fresh leaves and stems ext.". Our results indicates that, treatment of the seeds with the two stored seeds protectants for storage were responsible for the decrease in plant's height between the two generations F_1 and F_2 plants in *Vicia faba* variety Giza 716 at the higher concentrations. These findings are in agreement with those obtained by Grant (1982) that, pesticides as any agricultural chemicals may affect the genetic constitution of the progeny plants.

The variations in some metric traits were studied in the F_1 and F_2 generation plants with respect to: weight of seeds/plant and weight of 100 seeds /plant. (Table 5) revealed that, all concentrations treatment with the two agents induced dose dependent reduction in the yield traits. But, the statistically significant reduction in the weight of seeds/plant was observed only after treatment with the 4EC of Mal. in the F_1 & F_2 generation plants. While those of the weight of 100 seeds /plant were without significant effect at any of the concentrations treatment. The reductions in the yield traits were higher in the F_1 & F_2 generation plants after treatment with the different concentrations of Phos.

comparative to those of Mal. Phos. affected significant reduction of the weight of seeds/plant after treatment with 2EC increased to highly significant with the increase of concentration. (4EC) in the F_1 & F_2 generation plants. Phos. also affected significant reduction of the weight of 100 seeds/ plant only after treatment with the higher used concentration in the F_1 & F_2 generation plants contrary to that of Mal. (Table 5).

These effects are consistent with those of abnormal pollen grains. Amer *et al*. (1989) suggested that, the occurrence of such abnormal pollen grains affects the fertilization and seeds formation. Also, Haroun (2010) stated that, meiotic disorders affect normal disjunction which finally leads to form abnormal and nonfunctional gametes cannot complete any normal pollination or any sexual reproduction.

In the light of these findings, the capacity of the two stored seeds protectants against *Bruchids* pest used in the present study to induce significant reductions in the measured yield parameters at the higher concentrations could be an additional indication for their potential to induce mutational changes. It becomes clear now that, Mal. is the least potential mutagenic agent while, Phos. is the higher.

3- At the biochemical genetic level :

The changes in the total protein banding patterns of the second yield *Vicia faba* seeds, produced from treated parent seeds with Phos and Mal for storage as compared with the control, are illustrated in Figure (25) and Table (6). The changes includes: changes in the band relative mobility; sub-fractionation of some bands; appearance of some bands and disappearance of others. Similar effects were obtained previously by Morsi (2003) and Badr *et al.* (1995).

M2 *Vicia faba* seeds, produced from fumigated seeds with different concentrations of Phos, revealed variations in the protein profile (Table 6 , Lanes 3,4 and 5). Nine newly elaborated bands with different molecular weights were evident and nine of the protein bands represented by the control disappeared in the protein patterns, after all treatments. Sub-fractionation of three bands and great number of changes in the band relative mobility were detected. The summation of the qualitative variations (appearance + disappearance + subfractionation) of bands were twenty one, recorded in the protein banding patterns, after all concentration treatments with Phos. Generally, appearance of bands among M2 samples, was functional with the applied Phos. concentration.

M2 *Vicia faba* seeds, produced from dusted seeds with different concentration of Mal., revealed variations in the protein profile (Table 6, Lanes 6, 7 and 8). Eight newly elaborated bands with different molecular weights were evident and six of the protein bands represented by the control disappeared in the protein patterns, after all treatments. Subfractionation of two bands and great number of changes in the bands relative mobility were detected. Sixteen were summation of qualitative variations (appearance + disappearance + subfractionation) of bands were recorded in the protein banding patterns after all concentration treatments with Mal.. Generally appearance of bands and disappearance of bands, among M2 samples, were not functional with the applied Mal. concentration.

Table (6) illustrates that, treatment with the different concentrations of Phos induced the higher number of newly elaborated bands comparative to those of Mal. Phos also affected great increase in the number of newly elaborated bands after treatment with the higher used concentration

contrary to that of Mal.**.** The appearance of new bands could be explained on, the base of mutational event at the regulatory system of unexpected gene(s) that activate it (Abdel-Salam *et al.,* 1993 a and Ehsan, 2000). Also, the occurrence of additional bands may be the result of synthesis of new protein controlled by structural gene (Shehab *et al*., 1996). Mekki (2008) in her study on the effect of the three agricultural chemicals (GA, K2O &Tomaset) suggested that, this newly induced bands might be related to defense responses in the plants to protect them from different harmful effect caused by the different concentration of the agent. This suggestion is in accordance with our study about the fumigation treatments with Phos. that, appearance of bands among M2 samples, was functional with the applied Phos. concentration.

The different treatments with Phos. affected the higher number of the disappearance of bands and those of Mal. affected the least (Table 6). Both (Phos. and Mal.) did not show any dose response relationship. Muller and Gottschalk (1973) suggested that, changes in protein pattern did not show dose relationship may be due to mutational event. Disappearance of some bands could be traced back to the induction of two mutational types i.e. gene mutation and cytological aberrations (Barakat and Hassan, 1997). The induction of bridges, breaks, laggards and micronuclei would lead to a loss of some of the genetic materials. Therefore some of the electrophoretic bands might disappear due to deletion of their corresponding genes. It seems that, disappearance of bands of Phos. and Mal. may be due to the two mutational types (gene mutation and cytological aberration).

Changes in bands could be attributed to, the occurrence of point mutation in the concerned structural genes that create stop prior or post the original. They gave rise to the production of shorter or longer polypeptide chains (Brakat and Hassan, 1997).

Sub-fractionations of some bands at low molecular weights were recorded after all concentration fumigation treatments with Phos. and dusting treatment with EC & 2EC of Mal.. Sub-fractionation could be due to, the induction of cytological abnormalities that lead to gene duplication, followed by the occurrence of gene mutation at the regulatory system that suppress transcription of one or more of the duplicated genes that involved in the multistage biosynthetic pathway of certain seed storage protein product. Therefore two bands appeared, one of them represents the original protein product while the other is the intermediate one (Abdel-Salam *et al.,* 1993 b).

The observed variations in the M2 seed storage protein banding patterns are heritable changes, as the plants originated from the tested seeds were able to transfer these mutational events to the next generations. This conclusion is in accordance with (Muller and Gottschalk, 1973; Gamal El-Din *et al.,* 1988).

An association between the alterations in the seed protein electrophoretic pattern and occurrence of mutations in a number of morphological traits was clearly recognized by Mendhulkar (1993) in *Desmodium tortuosum* after treatment with chemical mutagens. This may be considered as a valid support for the use of the induced alterations in the seeds protein electrophoretic profile to identify mutants and to indicate the mutagenic potential of the tested agents. On this account, the revealed variations in this study showed that, Phos is the more potential mutagenic agent and Mal is the least (i.e. Mal. induced the more genetic stability in *Vicia faba* plants comparable to Phos).

CONCLUSION

From the above effects we can conclude that:-

- The synthetic insecticide Phos. was the more having mutagenic effect at the: cytogenetic ; genetic and biochemical genetic levels and the lower used concentration of Mal. was the least (i.e. the least affecting genetic stability of *Vicia faba* as an important crop plant). These findings are corroborating with those obtained previously by *Adam et. al.* (2016) on their effect on root-tip mitosis.
- For the needs of agriculture, the synthetic insecticide Phos., as seeds protectant agent for storage must be avoided and the synthetic insecticide Mal. can be used safely, if used in the recommended concentration.

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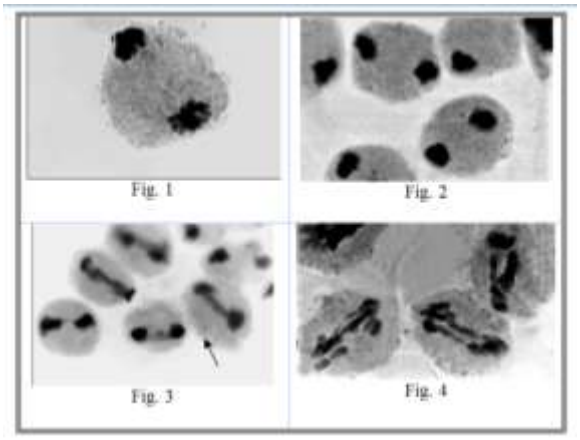

Figs. 1-4: Sticky anaphase I (Fig. 1), a group of sticky anaphases I (Fig. 2), a group of sticky anaphases I with sticky bridges & one with structural bridges (arrow) (Fig. 3) and a group of sticky anaphases I with severe sticky bridges (Fig. 4), of *Vicia faba* plants originated from: seeds dusted with 2 EC. (Fig. 1) and EC. (Fig. 3) of Mal.. EC. (Fig. 2) and 2 EC (Fig. 4) of Phos..

II- Potential mutagenic effect of dusting *Vicia faba* seeds with Malathion 1% powder or fumigating by Phosphine for storage

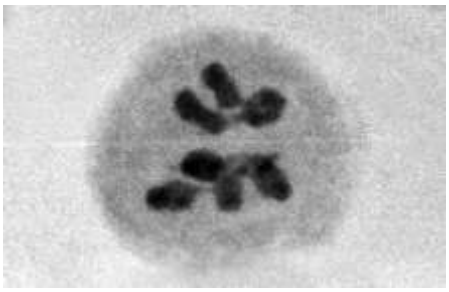

Fig. 5

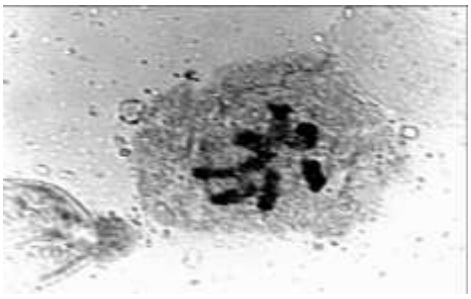

Fig. 6

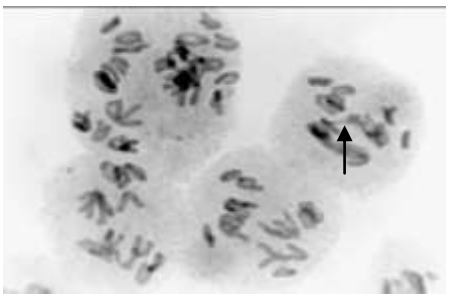

Fig. 7

Fig. 8

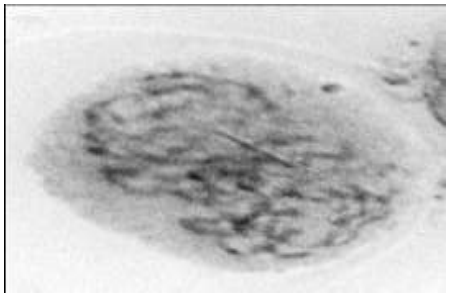

Fig. 9

Figs. 5-9: split metaphase I (Fig. 5), disturbed metaphase I with two univalents (Fig. 6), a group of anaphases I with disturbed chromosomes, and structural bridge "arrow" (Fig. 7), disturbed anaphase I (Fig. 8) and telophase I with disturbed poles and fragment (Fig. 9) in the flower buds of *Vicia faba* plants originated from: seeds dusted with EC. (Figs. 5, 7& 9) of Mal. and other fumigated with EC (Fig. 6) and 2EC (Fig. 8) of Phos..

Figs. 10&11: Anaphase I with lagging contracted chromosome top view (arrow) (Fig 10) and anaphase I with two lagging chromosomes (Fig. 11) in the flower buds of *Vicia faba*

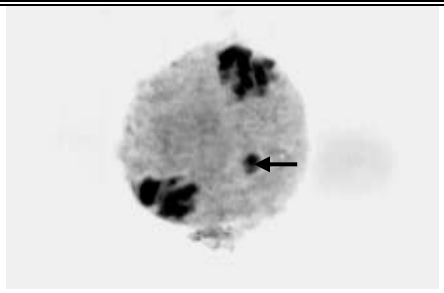

Fig. 10

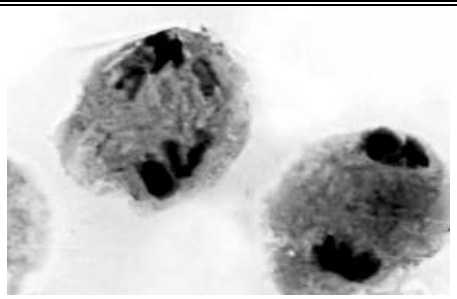

Fig. 11

plants originated from: seeds dusted with EC (Fig. 10) of Mal. and 2EC (Fig. 11) of Phos..

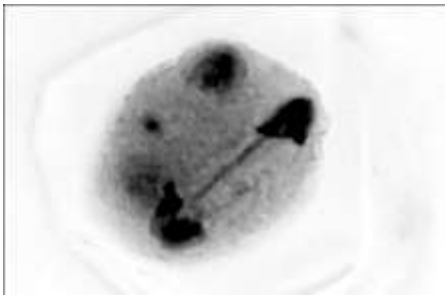

Fig. 12

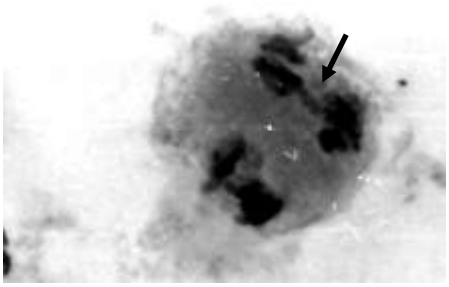

Fig. 13

Figs. 12&13: Slight sticky anaphase II with lagging chromosome and structural bridge (Fig. 12) and slight sticky anaphase II with structural bridge (black arrow) (Fig.13) in the flower buds of *Vicia faba* plants originated from: seeds dusted with 2EC (Fig. 12) of Mal. and other fumigated with 2EC (Fig. 13) of Phos..

II- Potential mutagenic effect of dusting *Vicia faba* seeds with Malathion 1% powder or fumigating by Phosphine for storage

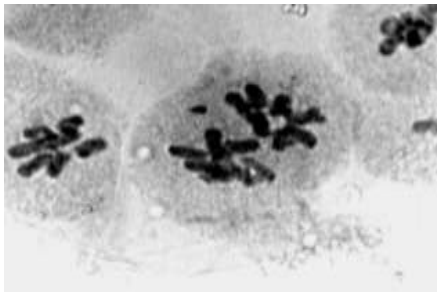

Fig. 14

Fig. 15

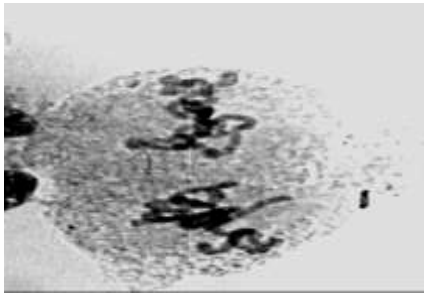

Fig. 16

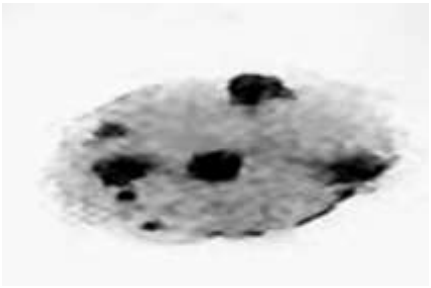

Fig. 17

Figs.14-17: Metaphase I with fragment (Fig. 14), slight sticky anaphase I with fragment (arrow)(Fig. 15), metaphase II with fragment (Fig. 16) and sticky anaphase II with many fragments and a breakage (Fig. 17) in the flower buds of *Vicia faba* plants originated from: seeds fumigated with EC (Figs. 14, 15& 16) and 2EC (Fig.17) of Phos..

Fig. 18

Fig. 18: Telophase II with two micronuclei in the flower buds of *Vicia faba* plants originated from fumigated seeds with 2EC of Phos..

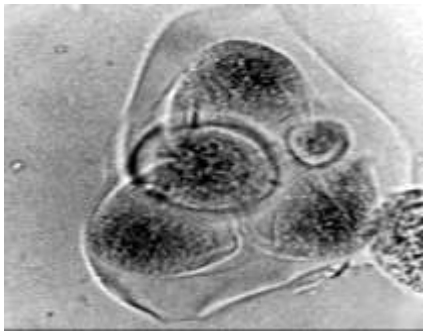

Fig. 19

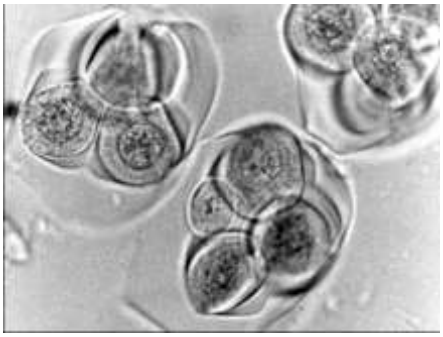

Fig. 20

Figs. 19& 20: Pentad in the spore tetrads of *Vicia faba* plants, originated from: seeds dusted with EC. (Fig.19) of Mal. and other fumigated with 2EC (Fig.20) of Phos..

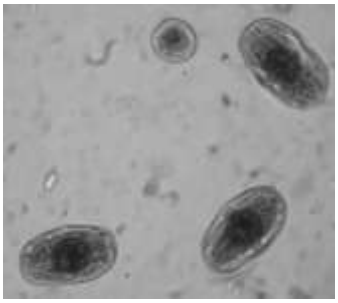

Fig. 21

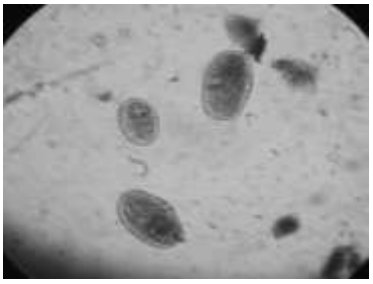

Fig. 22

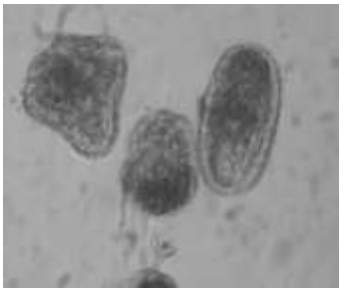

Fig. 23

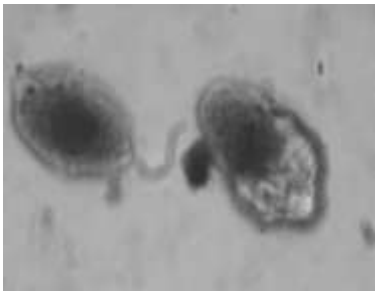

Fig. 24

Figs. 21-24: A small pollen grain (Figs. 21& 22) and deformed pollen grain (Figs. 23& 24) in *Vicia faba* plants, originated from: seeds dusted with 2EC (Fig. 21) and EC (Fig. 23) of Mal. and other fumigated seeds with EC (Fig. 22) and 2EC (Fig. 24) of Phos..

II- Potential mutagenic effect of dusting *Vicia faba* seeds with Malathion 1% powder or fumigating by Phosphine for storage

Table 1: Percentage of abnormal PMCs and abnormal PGs / *Vicia faba* Plant originated from seeds dusted with Mal. (a) and other fumigated with Phos. (b) .

Treatment	No. of inv. Plants.	Mean of PMCs/plant ± S.E.	1st Meiotic division		2nd Meiotic division		Pollen grains	
			No. Scor.	%	No. Scor.	%	No. scor.	Mean of % abn. P.G./plant ± SE
Control	6	0.86 ± 0.08	1800	1.06	1800	0.67	6000	0.00 ± 0.00
a) Malathion 1%								
1EC	6	3.86 ± 0.16**	1800	4.56	1800	3.17	6000	0.050 ± 0.022
2EC	6	5.81 ± 0.30**	1800	7.61	1800	3.89	6000	0.067 ± 0.033
4EC	6	2.11 ± 0.16**	1800	2.33	1800	1.89	6000	0.117 ± 0.048*
b) phosphine								
1EC	6	7.33 ± 0.28**	1710	7.25	1630	7.36	6000	0.050 ± 0.034
2EC	6	10.66 ± 0.50**	1540	11.17	1350	9.93	6000	0.083 ± 0.031*
4EC	5	12.74 ± 0.72**	1285	14.09	1090	10.46	5000	0.100 ± 0.032**

Values are means± S.E. of experiments *significant at P=0.05 **significant at P=0.01
EC: Effective concentration

Table 2: Percentage of the different types of abnormalities / scored PMCs. of *Vicia faba* plants, originated from seeds dusted with Mal. (a) and other fumigated with Phos.(b).

Treatment	No. scor. PMCs	% of the different types of abnormal PMCs/scored number												
		Chroma. mat. liquefac. abn.			Chrom. Kinetic abn.					Chrom. Struct. Aberr.			Contr. chrom.	Micro-nu.
					Dist.									
		Stic.	Sti. b.	Sum.	Dist.	Split.	m.pol.	Lag.	Sum.	Str. b.	Frag. & break.	Sum.		
Control	3600	0.72	0.00	(0.72)	0.03	0.00	0.00	0.08	(0.11)	0.00	0.03	(0.03)	0.00	0.00
a) Malathion 1%														
1 EC	3600	1.61	0.14	(1.75)	1.14	0.14	0.00	0.67	(1.95)	0.03	0.03	(0.06)	0.00	0.11
2 EC	3600	3.50	0.28	(3.78)	1.08	0.11	0.00	0.56	(1.75)	0.08	0.03	(0.11)	0.00	0.11
4 EC	3600	0.83	0.08	(0.91)	0.47	0.11	0.00	0.39	(0.97)	0.14	0.08	(0.22)	0.00	0.00
b) Phosphine														
1 EC	3340	4.55	0.21	(4.76)	1.62	0.06	0.00	0.36	(2.04)	0.12	0.30	(0.42)	0.00	0.09
2 EC	2890	3.88	0.10	(3.98)	4.02	0.14	0.04	0.93	(5.09)	0.21	0.93	(1.14)	0.17	0.21
4 EC	2375	4.67	0.04	(4.71)	5.39	0.04	0.00	1.01	(6.44)	0.08	0.84	(0.92)	0.04	0.30

Table 3: Percentage of metaphase I & II and anaphase I & II stages PMCs. with structural aberrations/ the scored number, of *Vicia faba* plants originated from seeds dusted with Mal. (a) and other fumigated with Phos. (b).

Treatment	Metaphase I & II + anaphase I & II PMCs.		
	No. scor.	No. with str. aberr. @	Mean ± S.E.
Control	2400	1	0.04 ± 0.04
a) Malathion 1%			
1EC	2400	2	0.08 ± 0.05
2EC	2400	4	0.17 ± 0.08
4EC	2400	8	0.33 ± 0.08*
b) Phosphine			
1EC	2140	14	0.64 ± 0.20*
2EC	1790	33	2.04 ± 0.29**
4EC	1475	22	1.69 ± 0.37**

@ Number of metaphase I&II PMCs. with fragments + number of anaphase I&II PMCs. with structural bridges & fragments. Values are means± S.E. of experiments *significant at P=0.05 **significant at P=0.01

Table 4: Percentage of plant survival of 10 days from sowing of F_1 and F_2 *Vicia faba* plants and mean values of plant height of F_1 and F_2 *Vicia faba* plants originated from seeds dusted with Mal.(a) and other fumigated with Phos.(b)

Treatment	% of plant survival / pot @		Plants height	
			100 days old	
	F_1 Mean ± S.E.	F_2 Mean ± S.E.	F_1 Mean ± SE	F_2 Mean ± SE
Control	100.00 ± 0.00	100.00 ± 0.00	69.60 ± 1.22	77.53 ± 1.52
a) Malathion 1%				
1 EC	100.00 ± 0.00	100.00 ± 0.00	68.00 ± 1.28	76.93 ± 1.34
2 EC	100.00 ± 0.00	100.00 ± 0.00	66.44 ± 1.37	77.47 ± 1.80
4 EC	100.00 ± 0.00	94.45 ± 5.55	63.50 ± 1.96*	72.35 ± 1.78*
b) Phosphine				
1 EC	100.00 ± 0.00	94.45 ± 5.55	70.35 ± 1.98	74.82 ± 2.21
2 EC	100.00 ± 0.00	94.45 ± 5.55	69.75 ± 1.57	71.75 ± 2.18*
4 EC	88.89 ± 7.03	72.22 ± 10.24*	63.00 ± 0.96**	65.77 ± 2.19**

@: 36 seeds were sown (6 seeds/pot). Values are means± S.E. of experiments
*significant at P=0.05 **significant at P=0.01

II- Potential mutagenic effect of dusting *Vicia faba* seeds with Malathion 1% powder or fumigating by Phosphine for storage

Table 5: The mean values of weight of seeds/ plant & weight of 100 seeds/ plant in F_1 and F_2 generations of *Vicia faba* plants originated from: seeds dusted with Mal.(a) and other fumigated with Phos. (b).

Treatments	Weight of seeds/plant		Weight of 100 seeds/plant	
	F_1 Mean ± SE	F_2 Mean ± SE	F_1 Mean ± SE	F_2 Mean ± SE
Control	15.67 ± 1.31	19.46 ± 1.27	75.67 ± 3.35	77.21 ± 2.89
a)Malathion 1%				
1EC	15.61 ± 1.20	18.61 ± 1.32	75.06 ± 3.61	76.37 ± 4.06
2EC	13.61 ± 0.95	17.56 ± 1.43	74.45 ± 2.33	76.01 ± 3.54
4EC	11.48 ± 0.95*	14.17 ± 1.60*	72.45 ± 1.46	70.56 ± 3.75
b) Phosphine				
1EC	13.00 ± 1.33	18.24 ± 2.01	72.71 ± 4.30	74.80 ± 3.99
2EC	10.74 ± 0.98*	14.36 ± 1.13*	69.79 ± 3.76	71.67 ± 3.38
4EC	6.61 ± 0.53**	11.31 ± 1.55**	63.87 ± 4.92*	65.93 ± 4.37*

Values are means± S.E. of independent experiments *significant at P=0.05 **significant at P=0.01

Table 6: Electrophoretic banding patterns of M2 *Vicia faba* seeds storage protein showing the effect of the different concentrations of the seeds protectants against *Bruchids* pests.

Row No	Lane No. / Treatment / MW (kd)	1 Mark.	2 Cont	3 Ph	4 2Ph	5 4Ph	6 M	7 2M	8 4M
1	151								
2	144								
3	124		+	+	+	(-)	+	+	+
4	119					⊕			
5	110					⊕	⊕		
6	101		+	(-)	(-)	(-)	(-)	(-)	(-)
7	97	+						⊕	⊕
8	89		+	+	+	(-)	+	(-)	(-)
9	85	+				⊕	⊕	+	⊕
10	80		+	(-)	+	(-)	+	(-)	(-)
11	77					⊕		+	+
12	74		+	(-)	(-)	(-)	(-)		
13	68	+		⊕			+		
14	67		+	(-)	+	+		+	+
15	62				⊕	⊕	⊕		
16	56					+		+	+
17	52		+	(-)	(-)		+		
18	46	+		⊕	⊕	⊕	⊕		⊕
19	44		+	+	+	+	+	+	(-)
20	42			⊕				⊕	
21	35				+	+	⊕	+	+
22	30	+	+	(-)			(-)		
23	28			+	+	+	+	⊕	+
24	27			+			+		
25	26		+	+	+	+	+	+	+
26	23	+	+			⊕	+	+	+
27	22			+	+				+
28	21	+			+	+	+	+	
29	20		+	+	-	+		+	
30	19		+	+	+	(-)	+	+	+
Total Bands No.			13	10	12	14	15	14	12

Ph: Phosphine M : Malathion 1%

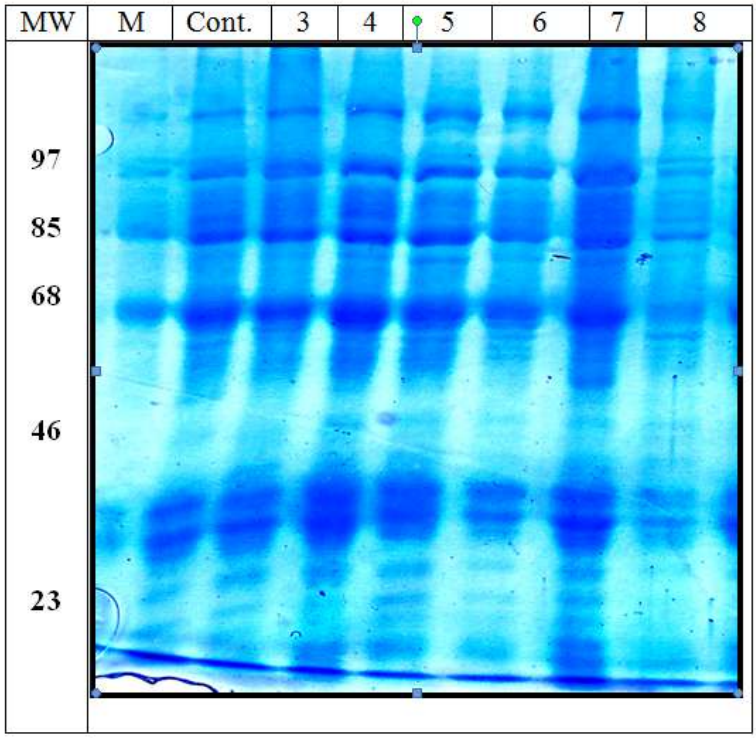

Fig. 25: Electrophoretic banding patterns of M2 *Vicia faba* seeds storage protein showing the effect of the different concentrations of the seeds protectants against *Bruchids* pests for storage.

Lane 1: marker **Lane 2: control**
Lane 3: 1 EC Phosphine **Lane 4: 2 EC Phosphine**
Lane 5: 4 EC Phosphine **Lane 6: 1 EC Malathion 1%**
Lane 7: 2 EC Malathion 1% **Lane 8: 4 EC Malathion 1%**

التأثير الوراثى الطفرى لتعفير بذور الفول البلدى بواسطة بودرة الملاثيون 1% او تبخيرها بواسطة غاز الفوسفين بغرض التخزين

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المستخلص

تهدف هذه الدراسه إلى استكمال تقييم التأثير الوراثى الطفرى لإثنين من المبيدات المصنعه: بودرة الملاسيون 1% , أقراص الفوستوكسين وهما المادتان الموصى بهما و الأكثر شيوعا فى جمهورية مصر العربيه لوقايه البقوليات المخزونه من الإصابه بآفه خنفساء البقول (Callosobruchus maculates F and C. chinensis L. s) وأجريت الدراسه بعد ستة اشهر من تخزين بذور الفول البلدى (صنف جيزه 716) والمعامله بمضاعفات (1, 2& 4) التركيز الأساسى من كلا المادتين تحت الإختبار بالإضافه الى بذور القياس الغير معامله. اشتمل هذا التقييم على دراسة كل من: الخلايا الجرثوميه,وبعض الخصائص الكميه لنباتات ومحصول الجيل الأول والجيل الثانى إضافة الى التغييرات الحادثه فى انماط التفريد الكهربى للبروتين المخزون بذور الجيل الثانى وذلك لكل من التركيزات الثلاثة المستخدمه مقارنه بالعينه القياسيه . واكدت النتائج أن غاز الفوسفين هو الأكثر تأثيرا طفريا بينما كانت بودرة الملاثيون هى الأقل. وأكدت النتائج ايضا أنه: تلبيه لمتطلبات الزراعه فإنه يمكن استخدام الملاسيون 1% لوقاية البذور المخزونه فى حال استخدام التركيز الموصى به.