

Cytogenetic Effects of Insecticide Cymax5% on the Root Tip Cells and Meiotic cells of *Vicia faba*

Abstract

The genotoxic effects of insecticide cymax5% was examined on the root tip meristem cells and meiotic cell divisions of the two Varieties of *Vicia faba* plants (Giza 843 and Giza 716) .Mitotic index decreased and chromosomal aberrations increased by increasing of the concentration of insecticide. The chromosomal anomalies include stickiness, fragment, star-shaped, bridge and c- metaphase .The percentage of abnormal pollen mother cells, (PMCs) increased as the concentration and treatment period increased. The most common abnormalities were stickiness, disturbed, laggard, bridges, ring and micro- multinuclei. These results showed that the types of abnormalities were more or less common in both the varieties but the percentages of aberrations were comparatively higher in Variety Giza 716 indicating its greater sensitivity to Cymax5%.

1. Introduction

Pesticides are chemical substances that are used all over the world against plant pests and diseases. The use of pesticides in commercial agriculture has led to an increase in farm productivity (Krol et al., 2000). Pesticides are essential in modern agricultural practices; but, due to their biocidal activity and potential risk to the consumer, the control of pesticide residues in foods is a growing source of concern for the general population (Torres et al., 1996).Teratogenic, carcinogenic and toxic properties of pesticides have been reported in the literature. Pesticides acted through a common mechanism of toxicity and conducted cumulative risk assessment (Bernard &Gordon,2000). (Jalilian et al. ,2000) reported the cytogenetic effect of the pesticides. The presence of their residues in fruits and vegetables can be a significant route to human exposure (Anonymous, 1990).

Cymax effect by contact, intestinal tract and it is highly effective at all larval stages, whether modern hatching or advanced larvae thus their life cycle is not completed. Cymax affects about 25% of the eggs and this lead to stopping hatching process. Cymax inhibits the formation of chitin layer and the worming process stops. Therefore, it cannot move from the larval age at the time of the treatment to the next

larval age. The worms stop feeding within hours and the crops damage stops and dies within two days. Cymax is a modern chemical group that has significant impact on insects [especially winged wing worms] that have acquired immunity from carbamate compounds, phosphorous and pyrethroid inhibitors.

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) .The present work was planned to study the mutagenic effect of the insecticide Cymax 5% as revealed by mitotic abnormalities and meiotic abnormalities of *Vicia faba* as a biological system.

2. Materials and Methods

Broad bean (*Vicia faba* L.) seeds [Variety Giza 843 and Giza 716] were used for the cytological and molecular studies as test plants. They were obtained from the Agricultural Research Center, Field Crops Research Institute, Giza.

(A)Mitotic studies:-

The seeds of *Vicia faba* .L [Variety, Giza 843 and Giza 716] were germinated in tap water in petri dishes at room temperature .Water had being changed twice a day till the roots were 2-3 cm long .The germinating seedling were then transferred to aqueous dilutions of the insecticide, where roots were immersed in the insecticide solutions containing 2, 4 and 8ml/L of the insecticide for 1 hour. Control roots simultaneously treated with tap water.

Immediately after each treatment, roots were cut away and fixed in Carnoy's fixative for 24 hours. After fixation, roots were rinsed in tap water for several times and- if not to be examined at once-, stored in 70% ethanol under refrigeration.

Feulgen squash technique was carried out for the microscopical observations of root tip cells. This technique is summarized in the following:-

After fixation and rinsing of roots, they were hydrolysed in hydrochloric acid solution (1N) at 60°C for 7-8 minutes. Roots were then rinsed several times with tap

water and stained in Feulgen reagent (Leuco-basic fuchsin, Coleman, 1938) for 1-2 hours in the dark. Root tips (1-2 mm) were teased out in a drop of acetic acid (45%), covered with a cover slip, and squashed between blotting paper folds. The prepared slides were examined under the light microscope (Sharma and Sharma, 1980), where 5-7 roots for each treatment were completely analyzed. The frequencies of the different stages of mitosis, as well as the frequencies of different mitotic abnormalities were determined.

(B) Meiotic studies:-

To study the effects on meiotic division, the flower buds of *V.faba* plants were treated as follows:-

On reaching the suitable size the flower buds were sprayed with different concentrations of the insecticide Cymax 5% 2, 4, 8 ml/L. A negative control plant was sprayed with distilled water. Eight flower buds from eight different plants were gathered through durations of 24h., 48h. 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 24h. and then transferred to 70% ethyl alcohol and kept in refrigerator.

To examine the meiotic division, the slides were prepared as follows:-

Anthers of the flower buds were isolated on a slide in a drop of iron acetocarmine stain (Belling, 1926) and covered with a cover-slip. The contents of the anthers were squashed by pressing the slide and cover between blotting paper folds. The slide was warmed gently over a spirit flame for few seconds. The cover-slip edges were sealed with paraffin wax to keep the prepared material unspoiled for few days.

3. Results and Discussion

1-Cytological studies:

1.1-Mitotic Investigation:-

The treatment of *Vicia faba* root meristematic tissue with Cymax 5% resulted in a highly reduction in the rate of cell division (MI) as compared to the control. Figure [1] indicates that mitotic index decreased with increasing insecticide concentration. Fig.[1] show that the direct treatment scored a maximum MI(5.66) after treatment with 2ml/l Cymax 5% for one hour [the lowest concentration] as compared to that of the control(10.44).The minimum scored MI was (1.22) after treatment with 8ml/l Cymax 5% for one hour [the highest applied concentration].

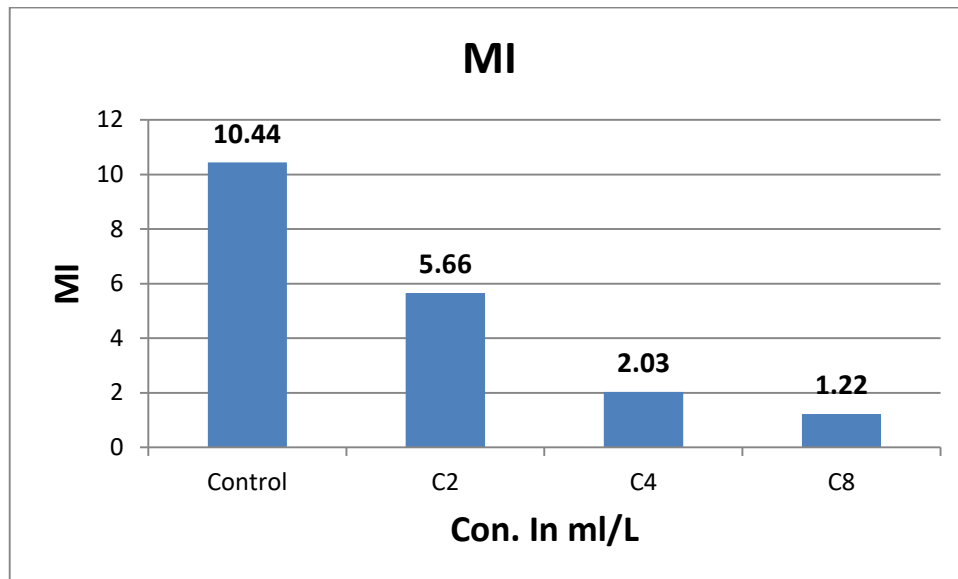


Fig. [1] Mitotic index (M.I.) in *Vicia faba* L.[Variety Giza843]control and treated root tip cells after treatment with Cymax 5%

As shown in fig.[1] the mitotic index in *Vicia faba* L.[Variety Giza843] root tip cells decreased with increasing the insecticide concentration(c₂, c₄ and c₈ of Cymax 5%).

Chromosomal aberrations were observed to be increased in frequency with increasing the concentration of insecticide. The types of observed chromosomal aberrations include fragmentation, sticky chromosomes, star-shaped, C -metaphase and chromatin bridges.

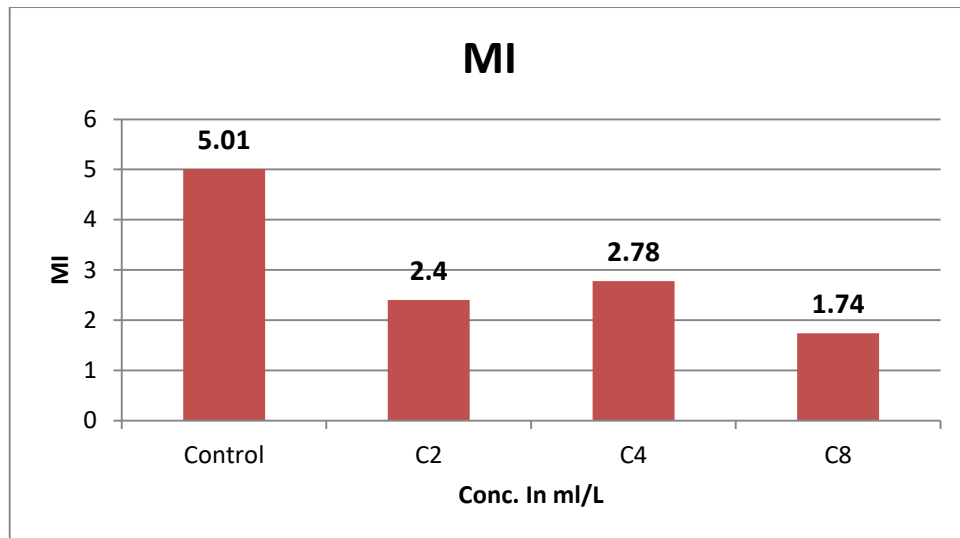


Fig.[2] Mitotic index (M.I.) in *Vicia faba* L.[Variety Giza716]control and treated root tip cells after treatment with Cymax 5%

Chromosomal aberrations were observed to be increased in frequency with increasing the concentration of Cymax 5%. The types of observed chromosomal aberrations include fragmentation, sticky chromosomes, star-shaped and chromatin bridges as shown in Fig.[3]. As shown in fig.[2] the mitotic index in *Vicia faba* L.[Variety Giza843] root tip cells decreased with increasing the insecticide concentration (C₂, C₄ and C₈ of Cymax 5%). Fig.[2] show that the direct treatment scored a maximum MI (2.78) after treatment with 4ml/l Cymax 5% for one hour as compared to that of the control (5.01). The minimum scored MI was (1.74) after treatment with 8ml/l Cymax 5% for one hour



Fig.[3] Control stages of mitosis are (A) prophase, (B) metaphase, (C) anaphase, (D) telophase. Disturbed stages of mitosis in root tip cells of *Vicia faba* L. (Variety Giza843) are shown as chromatin bridge (E), stickiness (F), star-shaped (G), laggard (H) and C-metaphase (I).

1.2-Meiotic Investigations:-

1.2.1-Meiotic abnormalities of *Vicia faba* L.(Variety Giza843) treated with Cymax5%:-

A wide spectrum of meiotic abnormalities was recorded in eight flower buds from different plants after treatment with different concentrations of Cymax5%(2, 4& 8ml/L).The number of meiotic cells of treated and control plants are presented in Figure (3).The number of abnormal pollen mother cells (PMCs) formed in the flower buds of *V.faba* plants was obvious with all concentrations of pesticide and in all stages and durations. Fig.(3) shows that the percentage of abnormal PMCS was increased by the increasing of Cymax5% concentration in the most treatments. Also, the same was observed by increasing the duration period from 24 to 48h in all treatments; however this trait was decreased in the 10 days duration period in the most treatments as a result of recovery in this period as shown in Fig.[4]. The most frequent types of abnormalities observed were: stickiness, disturbance, laggards, bridges, ring, micronuclei and multinuclei after Cymax5% treatments as shown in Fig.[5]. In the control plant, the number of abnormal PMCs was 38 cells. The induction of meiotic abnormalities appears to be a common effect of most pesticides (Shehata et al., 2008; Fisun and Goc Rasgele 2009).

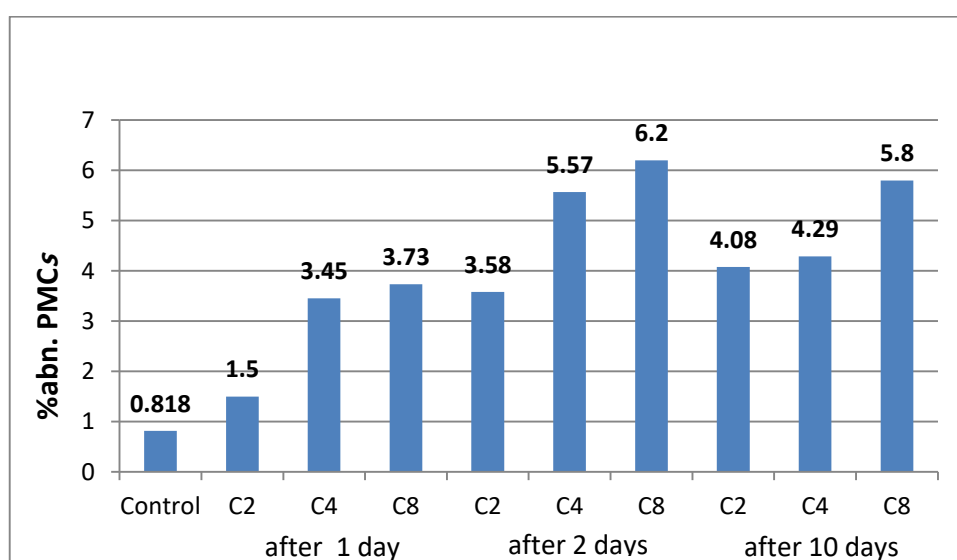


Fig. [4]: Percentage of total abnormalities (PTA) in *Vicia faba* pollen mother cells after treatment with different concentrations of Cymax 5%.

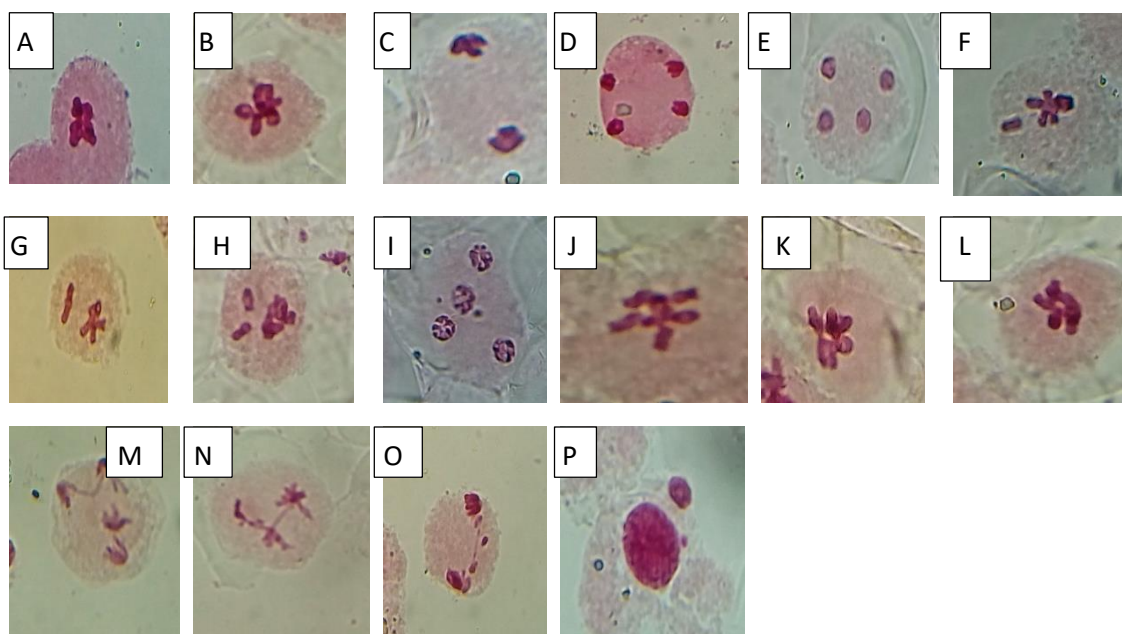


Fig.(5):- Types of chromosomal abnormalities after treatments with different concentrations of cymax5%. A&B: stickiness in metaphase I ,C: - stickiness in anaphase I,D: - stickiness in anaphase II, E: - stickiness in telophase II,F, G & H: - laggard in metaphase I,I: - disturbed telophase II,J, K&L:-ring in metaphase I,M: - chromatid bridge in anaphase II, N&O:-chromatid bridge in anaphase I, P: - micronuclei in interphase.

The stickiness and disturbed stages were the most common abnormalities found in all phases of the meiosis after treatments with all doses of the pesticide .The highest percentage of total abnormalities (PTA) was 6.2% after the treatment of the Variety Giza 843 with 8ml/L of Cymax 5% for 48 hours.

Stickiness is the first type of abnormalities is the stickiness found in most phases of meiosis after different treatments of Cymax5 %. The number of sticky cells increased in all stages of meiotic divisions as the concentration of pesticide increased during durations of 24h, 48h and 10 days. Our results are in agreement with the results of Badr(1988);Laemmli 1970; Matta et al. 1981;Shehata et al. 2008;Fisun and Goc Rasgele(2009) ; Pandey et al.,(1994) ;Singh et al.,(2007) ;Srivastava & Singh (2009)and Abdelsalam et al.,(1993b).

Laggard is 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 ; Atef et al.2011). These laggards may be distributed randomly to either pole at anaphase I or II which

result ultimately in aneuploidy (Amer & Mikhael, 1987; Amer & Ali, 1988) or may give 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). Bridges were induced in some treatments .They could be due to the breakage and reunion,Asita and Makhalemele (2009) and El-Khodary et al.,1990) or due to the general stickiness of chromosomes(Haliem,1990 and Ozturk,2009). Micro and multinuclei were recorded in all treatments and our results are in agreement with the results of Badr(1988)and Pandey et al. (1994). Finally the inductions of these chromosomal abnormalities were pointed to the mutagenic potential of the applied concentrations of Cymax5%.

1.2.2-Meiotic abnormalities of *Vicia faba* L. (Variety Giza716) treated with Cymax5%:-

Similar to the Variety Giza 843, the Variety Giza 716 showed a wide spectrum of meiotic abnormalities after treatment with different concentrations of Cymax 5 % (2, 4&8 ml/L)..The number of abnormal pollen mother cells (PMCs) formed in the flower buds of *V.faba* plants was obvious with all concentrations of pesticide and in all stages and durations. Fig.(5) shows that the percentages of abnormal PMCs were increased by the increasing of Cymax5% concentration in the most treatments. Also, this trait was increased by increasing the duration period from 24 to 48h in all treatments; however this trait was decreased in 10 days period duration in the most treatments as a result of recovery in this period as shown in Fig.[6]. Results obtained here revealed that although the types of abnormalities were more or less common in both the varieties but the frequency of aberrations were comparatively higher in Variety Giza 716 indicating its greater sensitivity to Cymax5%.

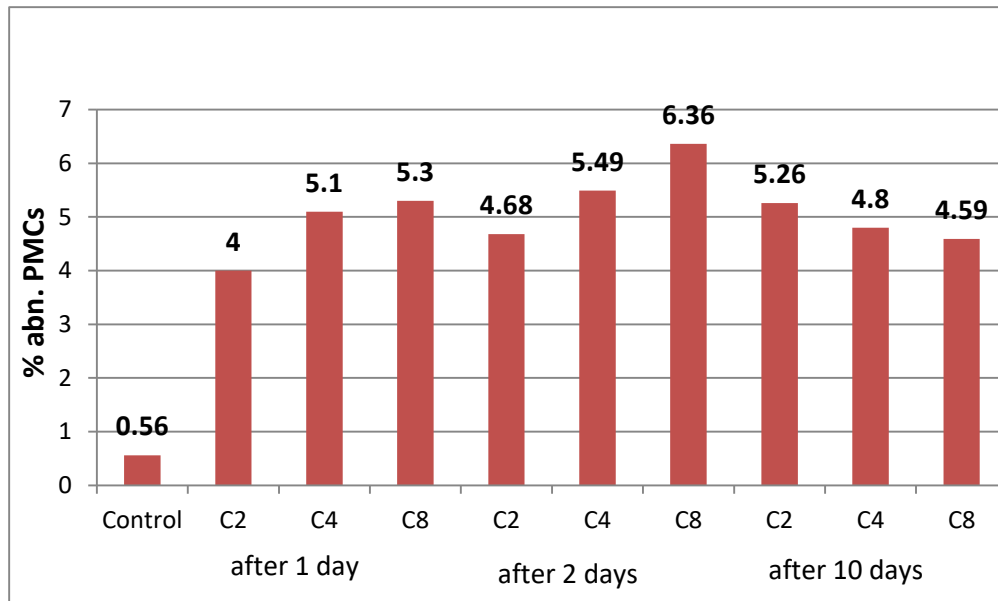


Fig. [16]: Percentage of total abnormalities (PTA) in *Vicia faba* pollen mother cells after treatment with different concentrations of Cymax 5%.

4. References

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