**RESEARCH ARTICLE**

Laila Mekki
Aziza Soliman El-kholy
Hanan Sayed Ahmed
Abdelfattah Badr

# EVALUATION OF CYTOGENETIC AND GENOTOXIC POTENTIAL OF CRUDE AQUEOUS EXTRACT OF *PEGANUM HARMALA* L. SEEDS ON M2 GENERATION OF *VICIA FABA* PLANTS

**ABSTRACT:**

The cytotgenetic and genotoxic potentials of aqueous extract of *Peganum harmala* L. seeds were evaluated in the M2 generation of *Vicia faba* plants, following parent plants seeds exposure to different concentrations 12.5, 25, 50, and 100% of the aqueous extract for 3, 6, 12 and 24 hr., using three endpoints i.e. cytogenetic abnormalities, seed proteins and DNA simple sequence repeats (SSRs) fingerprinting. Chromosomal abnormalities were observed in the M2 plant roots due to actions on the spindle apparatus leading to c-mitosis, vagrant chromosomes and laggards, sticky chromosomes and anaphase-telophase bridges, disordered anaphase, as well as micronuclei in interphase cells. Some of these abnormalities may result in stable structural modifications that are transmissible across generations. The SDS-PAGE analysis showed distinctive qualitative and quantitative alterations in number of polypeptide bands, molecular weights, and intensities of polypeptide bands. The production of different proteins entails a vast array of DNA binding proteins that act in various combinations to either activate or repress gene expression. Twenty-five SSR alleles were detected in the control and M2 plants using 11 SSR primer pairs. The number of alleles in the control plants was much lower than the number of alleles in the M2 plants. The changes in SSR fingerprinting might be connected to structural rearrangements in the chromosomes and DNA caused by different types of DNA damages. Such changes validate the mutagenic potential that was indicated by abundance of chromosomal abnormalities and alterations in the SDS-PAGE analysis of seed proteins.

**KEY WORDS:**

Cytogenetic, Genotoxic, *Peganum harmala*, PAGE, SSR, *Vicia faba*

**CORRESPONDENCE:**

Laila Mekki

Botany Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

**E-mail:** lhmmekki_dr@hotmail.com

Aziza Soliman El-kholy*

Hanan Sayed Ahmed**

Abdelfattah Badr***

* Botany Department, Faculty of Science, Kafr Elsheikh University, Kafr Elsheikh , Egypt

** Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt

*** Botany and Microbiology Department, Faculty of Science, Helwan University, 11790 Cairo, Egypt

**ARTICLE CODE: 03.02.16**

## INTRODUCTION:

Plants have been considered as materials for alternative medicine and sources of pharmaceutical drugs. The World Health Organization (WHO) traditional medicine 2014-2023 strategy acknowledged that for many millions of people, herbal medicines, traditional treatments, and traditional practitioners are the main source of health care, and sometimes the only source of care (WHO, 2013). Despite the therapeutic advantages of medicinal plants, some of their constituents may be potentially toxic, mutagenic, carcinogenic, or teratogenic potentials (Gadano *et al*., 2006). Therefore, medicinal plants must be tested with regard to quality, safety and efficiency, like conventional drugs (Simaan, 2009). In the last decades, cases of poisoning due to herbal medicines have occurred in many countries (Zhou *et al*., 2013). The experience gained from traditional use of herbals is efficient to detect immediate or near-immediate relationship between administration and toxic effects but is quite unlikely to detect medium -

to long-term toxicities; thorough investigations of herbal medicines (toxicity assessments, active pharmacovigilance) that appear to be essential for their safe use (Efferth and Kaina, 2011). Genotoxicity is an especially insidious toxicity that may result in carcinoma development years after exposure; it can arise from multiple compounds, with or without metabolic activation (Efferth and Kaina, 2011; Zhou *et al*., 2013).

*Peganum harmala* L. is a highly branched perennial, herbaceous, glabrous medicinal plant, which grow in semi-arid range land and sandy soils, especially along the Mediterranean region in North Africa and the Middle East (El-Bahri and Chemli, 1991). The main medicinal part of the plant in the modern phytotherapy is the seed (Aghili, 2009) which is traditionally used as an abortifacient agent (Mahmoudian *et al.*, 2002). In traditional medicine, a decoction of powdered seeds is used as vermifuge, narcotic and removal of kidney stones, leaves and blossoms are used for rheumatism and stomachache (Batanouny, 1999). *Peganum harmala* seed extract composition is very complex, containing several alkaloids particularly β-carbolines such as: harmine, harmaline, harmalol and Harman (Cao *et al.*, 2007). Cao *et al.* (2005) reported that harmine and its 9-substituted derivatives exhibited remarkable DNA intercalation activities and the potency of intercalation into DNA were enhanced significantly by introducing an appropriate substituent into position-9 of β - carboline nucleus. Previous results of research by Mekki (2014) on the effects of ethanol and water extracts of *P. harmala* seeds indicated a cytotoxic and genotoxic potential of the aqueous and ethanolic extracts on M1 generation of *Vicia faba* plants and at the molecular level simple sequence repeats (SSRs) showed fingerprinting variation following exposure of the seeds in the M1generation of *V. faba* plants providing indications for the genotoxic potential of *P. harmala* extract (Mekki *et al*., 2015).

Accordingly, the main objective of the present study was to investigate the possible genotoxic and cytogenetic effects of the crude aqueous extract of *P. harmala* seeds on M2 generation of *V. faba* plants. Genotoxic and cytotoxic effects were tested using chromosome abnormalities of root tips, SDS - PAGE of seed protein and polymorphism of SSRs profiling.

## MATERIAL AND METHODS:

Plant materials: Seeds of the medicinal plant *Peganum harmala* L. were collected from Wadi Gebal in Saint Katherine, Sainai, Egypt in the end of the summer of 2011. Voucher specimens are preserved at the herbarium of Botany Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.

**Extract preparation and *V. faba* seed treatments:**

Aquatic extract of *P. harmala* seeds was prepared according to Al-Mizrakchi (1998); 100 g of ground dry seeds were infused in 500 ml of distilled water for 24 hours at room temperature with occasional agitation of the infusion using magnetic stirrer. Then the infusion was filtered, and the residue was infused again for another 24 hours in 500 ml of distilled water and the residue was discarded. The aqueous extract was left to dry in Petri dishes at room temperature, and the resulting powder was used for preparing the different concentrations (12.5, 25, 50, and 100%). The aqueous extract of *P. harmala* seeds were used to treat the seeds of *V. faba* for 3, 6, 12, and 24 hr. Three replicates of 30 *V. faba* seeds each were sown for each treatment in the Botanical Garden of Suez Canal University in October 2012 to raise the M1 generation. One set of *V. faba* seeds was kept untreated as control. Harvested seeds of M1 generation were planted and M2 generation was produced in 2013. The M2 seeds were germinated and used in the present study to investigate cytogenetic abnormalities, seed proteins electrophoretic variations and DNA simple sequence repeats (SSRs) fingerprinting.

**Cytogenetic procedures:**

Cytogenetic studies were performed as described by Mekki (2014); the M2 seeds were pre-soaked for 12 hr. in distilled water and germinated on water-moistened filter paper in a Petri dish until the roots reached a length of about 1.5 cm. Young roots were excised, washed in distilled water and fixed in freshly prepared fixative solution of acetic acid and alcohol (1:3 v/v) for 24 hr. Squash technique using Feulgen reagent was used for mitotic analysis. Normal and abnormal cells were scored and mitotic index (MI), percentage of nuclear, chromosomal and total abnormalities were calculated in five freshly prepared slides for each treatment. The results were statistically analyzed and the significance of variation between treatments was evaluated by using the one-way analysis of variance (ANOVA) using SPSS 12.0 software.

**Protein extraction and electrophoresis:**

For the extraction of total protein, mature M2 seeds were powdered and 100 mg seeds powder were homogenated in 1 ml of 0.125 M tris borate buffer pH 8.9 with 2% SDS. The extracts were centrifuged at 10000 rpm for 20 min and supernatants were stored at - 20ºC until used. Samples were denatured before being loaded on gel in sample buffer containing 5% 2-mercaptoethanol and heated at 100ºC for 4 min. One dimensional SDS-PAGE was performed using 12.5%

polyacrylamide gel (Laemmli, 1970) using a standard molecular weight marker in a Cole Parmer vertical gel electrophoresis apparatus (Model SE400). The gel was stained with Comassie blue and visualized in white fluorescent light. The gel was then photographed with Kodak digital camera ModelAF3X optical aspheric lens 9.2 mega bixel and molecular weight of protein bands was calculated using the Lab-Image software version 2.7 produced by Kapelan (2003).

**DNA extraction:**

The DNA was extracted from M2 young leaves of germinated seedlings using the DNA-easy Plant Mini kit (Qiagen, USA, Cat. # 69104) as described in the instruction manual. The DNA integrity was detected by using 1.5% agarose gel. DNA concentration was adjusted to 25 ng/µl using nano-drop for the PCR reaction.

**SSR Primers:**

Eleven SSR primer pairs were selected to reveal SSR polymorphism among the M2 generation of plants following the treatment of parent seeds with different concentrations (12.5, 25, 50, and 100%) of water extract of *P. harmala* seeds for 3, 6, 12, and 24 hours based on the level of polymorphism in faba bean germplasm as reported in previous studies particularly those by Burstin *et al*. (2001) and Tar'an *et al*. (2005). The sequence of the used primer pairs (Fermentas, EU) are listed in table 1. For SSR polymorphism, a total of 25 µl reaction mix was prepared (12.5 µl Maxima Hot Start PCR Master Mix, 0.5 µl of each of the primer pair, 1 µl template DNA and nuclease-free water to 25 µl. Polymerase chain reaction was made for amplification of SSR loci according to the procedure described by (Burstin *et al*., 2001) with modifications in the quantity of genomic DNA and annealing temperature, using a standardized PCR program with initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation for 1 min at 94°C, annealing according to each primer Tm-5 for 45 sec, and 1 min at 72°C for extension and a final extension of 5 min at 72°C and then at 4°C till removal of PCR tubes within 12 hours. A negative control sample was used in which the template DNA was replaced by dd water. Ten µl of PCR SSR products were electrophoresed in 1.8% agarose gels. The samples were loaded first followed by loading 3 µl of the DNA ladder in a separate lane as a molecular size marker. Electrophoresis was made for 1 hour at 120 volt and 100 Am. The gel was then removed and viewed under UV light using illumination box and photographed by using digital camera (Kodak AF 3X). Band molecular size were measured by Lab image program version 2.7 produced by Kapelan (2003).

Table 1. List of the 11 SSR primers used in this study and their forward and reverse sequences.

| Locus1 | Forward primer sequence (5s use | Reverse primer sequence (5s use |
|---|---|---|
| VfG 13 | TTTCAGCAAACTAGAACCAATC | GGCATTCAGTTTTTACCTTGTA |
| VfG 33 | TTCTTTGGTCCTCTCTCTATC | GCACTGTTGTTGCTGATACAA |
| VfG 93 | GGTTTTGAATAGAAATGCAA | AAGATGTGTCAATATTGTTTT |
| VfG 193 | AGCGATGGTGCTCATGCTTA | TCTCTCACGGAATCACATCTTT |
| VfG 283 | AGAGTCCCAAAGAGTGGGTT | CCAAAGGCAAAAATGAGGGCTT |
| VfG 313 | ATAAGAGAGAACGAGGGAGAA | TTATGGTGGGACGTCTTACAT |
| VfG 344 | GCACTCGAAGGAATTAATTTT | GAACAGTTGTTTCGTGTCGTA |
| VfG 413 | AGCCCATGGTTCAAATGCAA | GCAGTCATGCCACTGCTTA |
| VfG 444 | GATGTTGTTGGTGTTGTTTA | CAATTAGGAGCAAAATCAGA |
| VfG 693 | ATTGGGGAGGATGAAGGTT | TTCCATTTTCCGTTCTCTCT |
| VfG 873 | AGGGCCAGCGTGATCCAATA | TGGGTTGGGATCTTTTGGTTG |

**RESULTS:**

**Cytogenetic results:**

All concentrations of the tested extract showed concentration-dependent increase and duration-dependent decrease in the mitotic activity measured as mitotic index (MI) when compared to the control. The lowest MI value (10.8) was found after 24hr. treatment with 12.5% of the seed extract while the highest MI value (15.3%) was recorded after treatment with 100% concentration of the aqueous extract for 3 hr. (Table 2).

The chromosomal and nuclear abnormalities showed significant increase with increasing concentration compared to the control. The highest proportions of chromosomal and nuclear abnormalities were 27.72% and 10.39%, respectively that were recorded after treatment with the highest concentration (100%) for the shortest duration of 3hr. On the other hand the total abnormalities increased with increasing the concentration and the duration at the low concentrations of 12.5% and 25%. However, at higher concentrations (50% and 100%) and the treatment for longer duration, the percentage of total chromosomal abnormalities decreased (Table 2). The most encountered

chromosomal abnormalities observed in this study were sticky -chromosomes, anaphase-telophase bridges, laggard chromosomes, and disordered anaphases. The most common nuclear abnormality was micronucleus, while the binuclei and nuclear buds were observed at less frequency. Some of these abnormalities are in figure 1.

Table 2. The values of MI, % of nuclear abnormalities (micronuclei, binuclei and nuclear buds), % of chromosomal abnormalities and the total % of abnormalities (nuclear and chromosomal) in M2 generation of *V. faba* plants root tips germinated from treated seeds with different concentrations of crude aqueous extract of *P. harmala* seeds.

| Treatment code | Treatment | Mean MI | % of Total CA | % of Total NA | % of Total abnormalities |
|---|---|---|---|---|---|
| C | Control | 11.8 | 2.04 | 0.68 | 2.72 |
| 1 | 12.5% for 3h. | 12.5 | 10.48* | 3.17* | 13.65* |
| 2 | 12.5% for 6h. | 12.1 | 20.35* | 4.19* | 24.5.4* |
| 3 | 12.5% for 12h | 11.3 | 20.65* | 7.31* | 27.96* |
| 4 | 12.5% for 24h | 10.8 | 25.30* | 7.34* | 32.64* |
| 5 | 25% for 3h | 14.1 | 16.42* | 7.05* | 23.47* |
| 6 | 25% for 6h | 13.8 | 22.01* | 6.58* | 28.59* |
| 7 | 25% for 12h | 12.7 | 23.26* | 7.00* | 30.26* |
| 8 | 25% for 24h | 11.0 | 18.95* | 4.94* | 23.89* |
| 9 | 50% for 3h | 15.0 | 18.43* | 5.42* | 23.85* |
| 10 | 50% for 6h | 14.66 | 16.49* | 6.18* | 22.67* |
| 11 | 50% for 12h | 13.5 | 19.76* | 4.58* | 24.33* |
| 12 | 50% for 24h | 11.8 | 16.52* | 6.22* | 22.74* |
| 13 | 100% for 3h | 15.3 | 27.72* | 10.39* | 28.11* |
| 14 | 100% for 6h | 14.5 | 22.24* | 8.58* | 30.82* |
| 15 | 100% for 12h | 13.9 | 21.21* | 6.97* | 28.18* |
| 16 | 100% for 24h | 11.4 | 19.72* | 3.89* | 23.61* |

*Values significant at $p<0.05$ by post hoc least significant (LSD). MI= Mitotic index, CA= Chromosomal aberrations, NA= Nuclear aberrations.

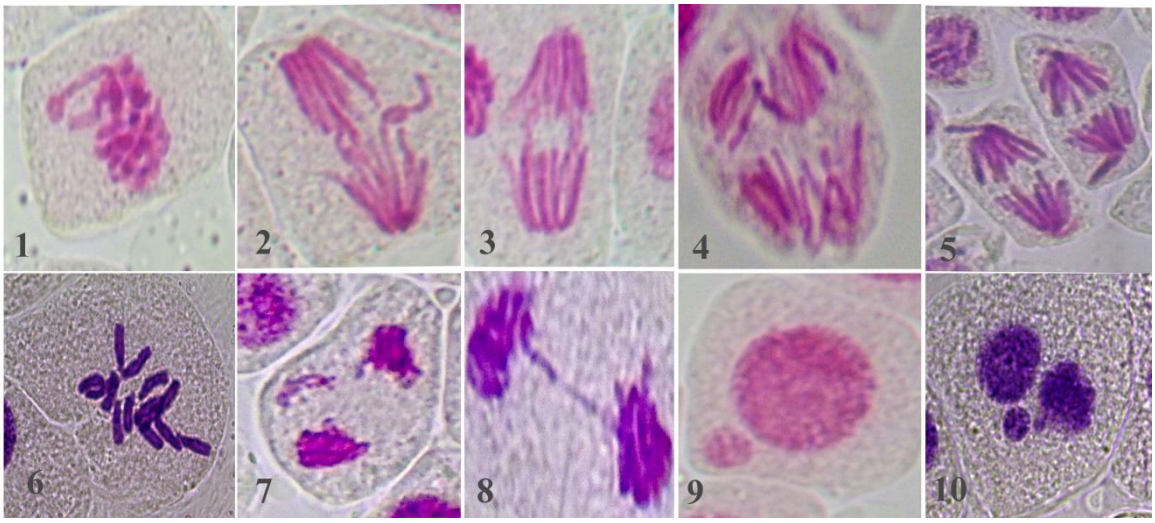


Fig. 1. Chromosomal aberrations induced in V. faba root tips by water extract of P. harmala seeds: (1) sticky chromosomes at metaphase, (2) Anaphase bridge with laggard, (3) Anaphase bridges, (4) multipolar anaphase (5) Vagrant chromosomes at anaphase, (6) C- metaphase, (7) Telophase with lagging chromosome, (8) Telophase bridge, (9) Micronucleus, (10) multinucleate cell.

**Protein polymorphism:**

Table 3 and figure 2 illustrate the SDS-PAGE electrophoretic banding patterns of *V. faba* M2 plants seed proteins following treatment of parent plants with water extract of *P. harmala* seeds. A total of 27 protein bands were observed in all examined M2 plants; 14 bands were polymorphic (51.85%) and 13 are monomorphic. Protein profiles showed great variations in bands number, thickness, intensity and relative mobility depending on the treatments compared to the control (Fig. 2). Eight new bands were induced which were not found in control plants. These new bands have molecular weights of 127, 46, 38, 31, 29, 28, 25, and 24 kDa.

Figure 2 illustrates that bands with molecular weights of 127 and 29 KDa were

expressed following all treatments but a band of 46 KDa was expressed in the treatment 9 (50% extract concentration for 3 hr.) Bands with a molecular weight of 38 kDa was expressed following all treatments except in the treatment with 12.5% concentration for 3 hr and 6 hr., 25% for 24 hr, 50% for 6 hr and 100% for 6, 12, and 24 hr, respectively. Bands of 28 kDa and 24 kDa were initiated following all treatments except 25% *P. harmala* seed extract for 3 hr., 50% for 3 hr and 6 hr while the band 24 kDa disappeared following the 12.5% extract for 12 hr. Bands of 31 kDa and 25 kDa were present following 12.5% *P. harmala* seed extract for 3, 6, and 24 hr, 25% extract treatment for 6 and 24 hr while a 25 kDa band was found also following 12.5% extract for 12 hr and 25% for 12 hr. A band with a molecular weight of 80 kDa was expressed obviously with more thickness and intensity following treatment with 50% concentration of seeds extract for 12 hr, that band was absent following the treatments 25% for 3h, 50% for 6h and 100% for 12 hr and observed but very faint in control and the other samples.

Table 3. The analysis seed protein electrophoretic profiles; number of polymorphic bands including unique bands and the percentage of polymorphism for the control and M2 V. faba plants following exposure to different treatments with P. harmala seed extract (u = unique).

| Treat. Code | Treatment | No of bands | polymorphic bands No | % of polymorphism |
|---|---|---|---|---|
| C | Control | 19 | 6 | 31.6 |
| 1 | 12.5% for 3 hr. | 24 | 11 | 45.8 |
| 2 | 12.5% for 6 hr. | 24 | 11 | 45.8 |
| 3 | 12.5% for 12 hr. | 24 | 11 | 45.8 |
| 4 | 12.5% for 24 hr. | 25 | 12 | 48.0 |
| 5 | 25% for 3 hr. | 20 | 7 | 35.0 |
| 6 | 25% for 6 hr. | 26 | 13 | 50.0 |
| 7 | 25% for 12 hr. | 25 | 12 | 48.0 |
| 8 | 25% for 24 hr. | 25 | 12 | 48.0 |
| 9 | 50% for 3 hr. | 21 | 8 + 1u | 38.1 |
| 10 | 50% for 6 hr. | 19 | 6 | 31.6 |
| 11 | 50% for 12 hr. | 24 | 11 | 45.8 |
| 12 | 50% for 24 hr. | 23 | 10 | 43.5 |
| 13 | 100% for 3 hr. | 24 | 11 | 45.8 |
| 14 | 100% for 6 hr. | 22 | 9 | 40.9 |
| 15 | 100% for 12 hr. | 21 | 8 | 38.1 |
| 16 | 100% for 24 hr. | 21 | 8 | 38.1 |

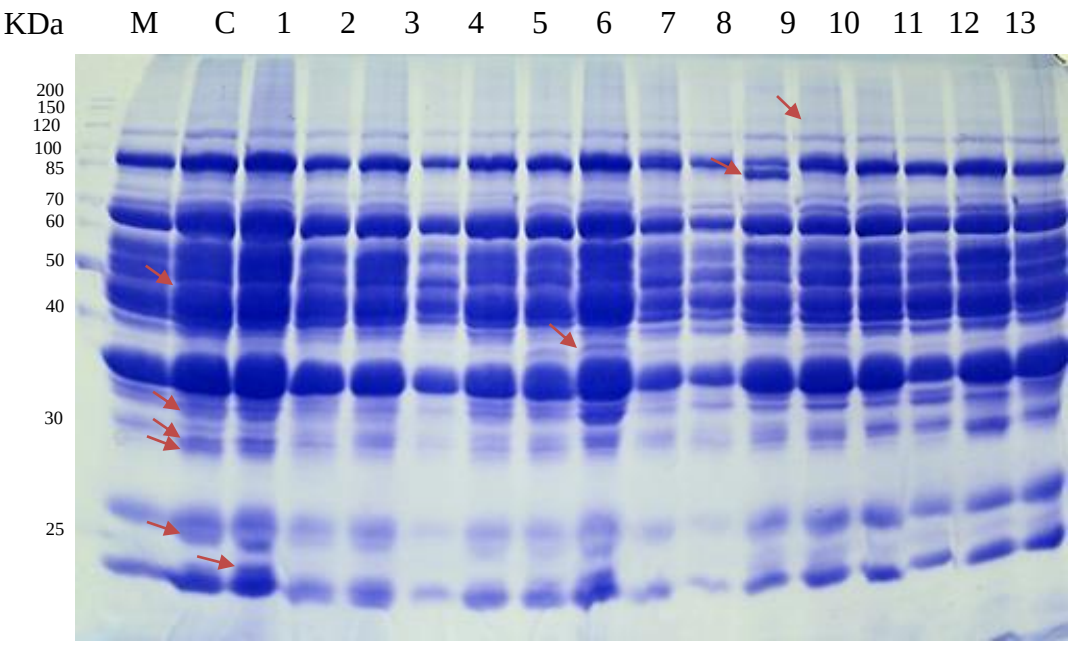


Fig. 2. Electrophorogram produced by SDS-PAGE analysis of seed proteins of *V. faba* M2 plants following exposure to different treatments with water extract of *P. harmala* seeds. C = control and 1-16 = *V. faba* M2 plants following treatments as given in table 2.

**SSR fingerprinting:**

Twenty-five SSR alleles were detected in the control and the *V. faba* M2 plants following exposure to treatments with water extract of *P. harmala* seeds using 11 VfG SSR primer pairs including three monomorphic alleles and three unique alleles. The number of alleles in the control plants was only seven that was much lower than the number of alleles in the M2 plants produced following exposure to the *P. harmala* extracts, which ranged between 10 and 17 (Fig. 3).

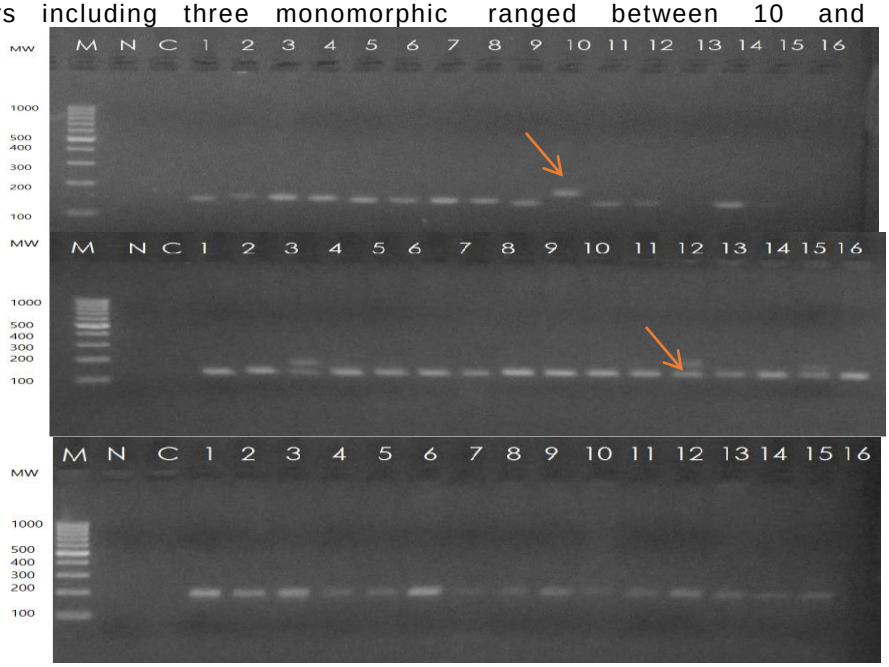


Fig. 3. SSR fingerprinting produced by 3 of the used primers (A; VfG 93 VfG 313, and VfG 344) in of *V. faba* M2 plants following exposure to treatments with different concentrations of water extracts of *P. harmala* seeds (N = negative control, C = control and (1-16) = M2 *V. faba* plants following treatments as in Table 2. The arrow indicates unique allele.

Table 4. Number and molecular size of SSR bands, and band type produced by the used SSR primers for the control and in M2 *V. faba* plants following exposure to treatments with *P. harmala* seeds extract (p = polymorphic, m = monomorphic, u = unique). Treatment codes are as given in table 2.

| Brand No. | Pr. Code | BP | C | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Band Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | VfG13 | 295 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | P |
| 2 | | 256 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | P |
| 3 | | 255 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | P |
| 4 | VfG 33 | 210 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | P |
| 5 | | 190 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 6 | VfG 93 | 160 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 7 | | 140 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | P |
| 8 | VfG 193 | 225 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | P |
| 9 | | 205 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | P |
| 10 | | 300 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | P |
| 11 | VfG 283 | 252 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | P |
| 12 | | 211 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | P |
| 13 | VfG 313 | 171 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | -u |
| 14 | | 182 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | P |
| 15 | VfG 344 | 224 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | P |
| 16 | | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | P |
| 17 | VfG 413 | 185 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | P |
| 18 | | 155 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | P |
| 19 | VfG 444 | 267 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | P |
| 20 | | 240 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | M |
| 21 | VfG 693 | 205 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | P |
| 22 | | 165 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | M |
| 23 | | 240 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | P |
| 24 | VfG 873 | 215 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | P |
| 25 | | 60 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | M |
| Polym | | | 4 | 7 | 8 | 9 | 11 | 11 | 9 | 9 | 10 | 13 | 9 | 9 | 10 | 11 | 14 | 7 | 7 | |
| Total | | | 7 | 10 | 11 | 12 | 14 | 14 | 12 | 12 | 13 | 16 | 12 | 12 | 13 | 14 | 17 | 10 | 70 | |
| %Polym | | | 57.1 | 70 | 70 | 75 | 78.6 | 78.6 | 75 | 75 | 76.9 | 81.25 | 75 | 75 | 76.9 | 78.6 | 82.35 | 70 | 70 | |

The number of polymorphic bands (4) was also much lower than the numbers of alleles in the M2 plants which ranged between 7 and 14 (Table 4). Consequently, the percentage of polymorphism was low in the control plants (57.1%) and much higher following treatments. The polymorphism in the M2 plants following treatments ranged between 70% and 82.35%. The highest proportion of polymorphism was scored in plants exposed to 100% for 6 hr. (Table 4). Careful scoring of SSR alleles showed that 18 new bands were induced following treatments which were not detected in the control plants. Two unique alleles with a size of 190 and 160 bp were produced by primers VfG 33 and VfG 93 following treatment coded 4 (12.5% *P. harmala* extract for 24 hr.) and treatment coded 10 (50% for 6 hr.).

Careful scoring of the SSR alleles showed that two alleles, with size 171 and 224 bp produced by primers VfG 313 and VfG 344, were absent in the control plants and present in M2 plants. The profiles produced by primer VfG 33 showed three alleles of molecular sizes 225 bp, 210 bp and 190 bp; all were absent in the control plants; the 225 bp allele was induced in M2 plants after treatment for longer durations with 12.5, 25 and 50 % concentrations of *P. harmala* seed extract (treatments coded 3, 4, 7, 10, 11, and 12); the 190 bp allele was unique to treatment 4 alone (12.5% for 24 hr.). Two alleles were expressed by primer VfG 93; an allele of a molecular size 140 bp was produced following all treatments except treatments 10, 13, and 16 only); the other allele was 160 bp and was unique. Primer VfG 193 produced two alleles (205 and 225 bp), however, three alleles (211 bp, 252 bp and 300 bp) were produced by primer VfG 283, all of them were polymorphic; the first one and the third one are present in two treatments each, in 3 treatments coded 4 and 9, 14 respectively. The primer VfG 313 produced two alleles (171 bp and 182 bp) the former allele (171 bp) was present in plants following all treatments and absent in the control. On the other hand, primer VfG 413 produced 3 alleles (200 bp, 185 bp and155 bp); all of them were polymorphic. Primer VfG 444 produced two alleles one of them (240 bp) was monomorphic. Also primer VfG 693 produced two alleles (165 bp and 205 bp) both of them were present in the control and the second one (165 bp) was monomorphic. The profile produced by the primer 873 revealed three alleles, one of them (60 bp) is monomorphic, the second allele (215 bp) was present only in treatments coded 5, 9, 12 and 13 and the third (240 bp) was present mostly following exposure to higher concentration 100% (treatments coded 14, 15, and 16) and also in treatments coded 6, 10 and 11.

## DISCUSSION:

Mitotic index in *V. faba* root meristems was slightly increased with increasing concentration of *P. harmala* seeds aqueous extract and decreased with increased duration compared to the control. This result agrees with that of a previous study on M1 generation of the examined plants (Mekki, 2014). The most chromosomal abnormalities observed in the present study samples were, sticky chromosomes, anaphase and/or telophase bridges, laggard chromosomes, disordered anaphase, C metaphase and vagrant chromosomes. All of these abnormalities were also detected in the M1 generation but at higher frequency (Mekki, 2014) and were also frequently reported in plant and animal test systems by extracts of other medicinal plants (El-Shazly, 1990; Shehab and Adam, 1983; Gadano *et al*., 2006).

The abnormalities may be of three types; one due to an action on the spindle formation and thus resulted in cell division disturbances such as c-metaphase, lagging chromosomes, vagrant chromosomes and multipolar mitosis (Badr, 1983; Grant, 1999; Badr *et al*., 2013). Their presence may be attributed to the failure of the spindle apparatus to organize and function in a normal way. C-metaphase was regarded as indicative of a weak toxic effect which may be reversible (Fiskesjö, 1985). The second type is stickiness that may result from improper folding of chromosome fibers which makes the chromatids connected by means of sub-chromatid bridges (McGill *et al*., 1974; Klasterska *et al*., 1976) or may be interpreted as a result of depolymerization of DNA, partial dissolution of nucleoproteins or even breakage and exchanges of the basic folded fiber units of chromatids and the stripping of the protein associated with DNA in chromosomes (Mercykutty and Stephen, 1980). Stickiness may indicate irreversible highly toxic effect, which may probably lead to cell death (Fiskesjö, 1985).

The third type of chromosome abnormalities includes breakage and bridges; bridges were commonly observed during anaphase and telophase indicating clastogenic effect caused by breakage and fusion of chromatids or sub-chromatids (Badr, 1983; Grant, 1999) indicating stable structural aberrations that are transmissible such as inversions, translocations and some small deletions. Bridges reported here like bridges produced by other mutagenic agents might have arisen through breaks followed by reunion of chromosomes (Badr, 1988; Shreekrishna, 2006) or due to stickiness of chromosome at metaphase and their failure to separate at anaphase (Grant, 1999; Dhanavel *et al.,* 2012). Besides the types of chromosome abnormalities, the formation of

micronucleus in interphase cells was obviously higher than the control group ($p < 0.05$) at all tested treatments. Stable structural aberrations that are transmissible may also be the result of severe stickiness of chromosomes at metaphase and their failure to separate at anaphase (Badr, 1988; Grant, 1994).

The induction of micronuclei was regarded as the manifestation of fragments or vagrant chromosomes (Yi and Meng, 2003; Caritá and Marin-Morales, 2008; Hoshina and Marin-Morales, 2009). Micronuclei also arise if laggards or non-oriented chromosomes fail to reach the poles in time to be in main telophase nucleus (Badr, 1988; Utsunomiya *et al*., 2002). Micronuclei derived from a whole chromosome, due to lagging chromosomes, have higher probability to survive and undergo condensation in synchrony with the main nuclei than micronuclei derived from a chromosome fragment (Gustavino *et al*., 1987). The chromosomal and nuclear abnormalities that have been observed in second generation plants indicate genotoxic potential of their inducers (Badr *et al*., 1987; Grant, 1999; Gadano *et al*., 2006) and confirm their mutagenic potential.

The SDS-PAGE analysis revealed distinctive qualitative and quantitative alterations in electrophoretic SDS-protein banding styles of treated and non-treated M2 *V. faba* plants. These alterations are based on variations in number of polypeptide bands, molecular weights, and intensities of polypeptide bands, as well as gain or loss of protein bands. Each polypeptide band represents the final products of transcriptional and translational events occurring due to active structural genes (Sadia *et al*., 2009). The appearance of new bands may be mutational events at the regulatory gene(s) or result from different DNA structural changes (breaks, transpositions, deletion, etc.) which led to change in amino acids of which the proteins are formed (Shehab *et. al.*, 2004). On the other hand, the disappearance of some protein bands could be attributed to the loss of genetic material due to the breaking of a small number of peptide bonds to form polypeptides of shorter length than the original protein (Abdel-Hameid *et al*., 2011). The changes in band intensity on the other hand could be the result of gene duplication or point mutation that leads to production of shorter and longer polypeptide chains or alteration in the structural genes which may be due to the changes in regulator gene(s) expression (Shehab *et al*., 2004; Abdel-Hameid *et al*., 2011). The distinction protein polymorphisms in all treatments and control samples in the present study may be the result of insertions or deletions between mutated sites and could be used as biomarkers for identification of mutagenic potential (Mondini *et al*., 2009). The production of different proteins entails a vast array of DNA binding proteins that act in various combinations to either activate or repress gene expression (Freeman *et al.*, 2003).

Twenty-five SSR alleles were detected in the control and M2 plants of *V. faba* following treatment of parent plants seeds with water extract of *P. harmala* seeds extract using 11 SSR primer pairs. The number of alleles in the control plants was much lower than the number of alleles in the M2 plants following exposure to the *P. harmala* water extracts. Consequently, the percentage of polymorphism was much higher following treatments compared to the control. No correlation was evident between the concentrations of the *P. harmala* seed extract or the duration of the exposure as the highest proportion of polymorphism was scored in plants exposed to 100% for 6 hr. Careful scoring of SSR alleles showed 18 bands following treatments which were not detected in the control plants indicating that *P. harmala* seed extract induced changes in the nucleotide sequence of the DNA in the SSR regions reflecting a mutational potential of the applied *P. harmala* seed extract.

The application of SSR marker analysis was used as in a previous study on M1 plants by Mekki *et al*. (2015) who reported similar high proportions of SSR polymorphism (100%). However, the variations in the SSR fingerprinting in the M2 plants indicate the possibility of transmitting the mutational events to the next generations. The changes in the SSR profiles in the M2 generation of *V. faba* plants indicate the induction of mutations on almost all the tandem repeat region. These results agree with the result of Roychowdhury *et al*. (2012) who concluded that chemical mutagenesis was higher in M1 *Dainthus* plants than M2 plants. In the M2 generation, observed mutations are considered more stable and are more likely to be transferred to next generations (Parry *et al*., 2009). The changes in SSR finger printing might be connected to structural rearrangements in DNA caused by different types of DNA damages.

In conclusion, *P. harmala* seeds aqueous extract showed slight effects on mitotic activity in the M2 plants of *V. faba* and produced three types of chromosomal abnormalities; due to an action on the spindle formation and thus resulted in c-metaphase, lagging and vagrant chromosomes and stickiness that may cause an irreversible a highly toxic effect, that may probably lead to cell death and chromosomal aberrations such as breakage and bridges; bridges were commonly observed during anaphase and telophase indicating clastogenic effect caused by breakage and fusion of chromatids or sub-chromatids; these structural modifications that

are transmissible mutations. The SDS-PAGE analysis illustrated distinctive qualitative and quantitative alterations based on variations in number of polypeptide bands, molecular weights, and intensities of polypeptides bands. The production of different proteins entails a vast array of DNA binding proteins that act in various combinations to either activate or repress gene expression. In addition, the number of alleles in the control plants was much lower than the number of alleles in the M2 *V. faba* plants following of parent plants seeds with exposure to the *P. harmala* extracts. The changes in SSR finger printing might be connected to structural rearrangements in the tandem DNA caused by different types of DNA damages.

## REFERENCES:


Abdel-Hameid NR, Elanany MAM, Haiba AAA, Abd El-Hady EAA. 2011. Relative mutagenicity of some food preservatives on plant cells. Aust. J. Basic Appl. Sci., 5(12): 2817–2826.

Aghili MH. 2009. Makhzan-Al-Advia. Tehran University of Medical Sciences, Tehran, Iran. http://aqlibrary.org/UserFiles/File/makhzan.pdf .

Al-Mizrakchi A. 1998. Adherence of Mutans *Streptococci* on Teeth Surfaces. Microbiological and Biochemical Studies. Ph.D. Thesis, Al-Mustansiriya University, Baghdad.

Badr A, Hamoud MA, Haroun SA. 1987. Effect of the herbicide turbutryn on meiosis, yield and chromosomes in $C_2$ plants of *Vicia faba* L. Biol. Plantarum, 29(1): 70-72.

Badr A, Zaki H, Germoush MO, Tawfeek AQ, El-Tayeb MA. 2013. Cyto-physiological impacts of Metosulam herbicide on *Vicia faba* plants. Acta Physiol. Plantarum, 35(6): 1933-1941.

Badr A. 1983. Mitodepressive and chromotoxic activities of two herbicides in *Allium cepa.* Cytologia, 48: 451-457.

Badr A. 1988. Cytogenetic activities of some fungicides. Cytologia, (53): 635-640.

Batanouny KH. 1999. Wild medicinal plants in Egypt. Palm Press, Cairo, Egypt. 166-167.

Burstin J, Deniot G, Potier J, Winachter C, Aubert G, Baranger A. 2001. Microsatellite polymorphism in *Pisum sativum.* Plant Breed., 120(4): 311—317.

Cao R, Chen H, Peng W, Ma Y, Hou X, Guan H, Liu X, Xu A. 2005. Design, Synthesis and in vitro and in vivo antitumor activities of novel beta-carboline derivatives. Eur. J. Med. Chem., 40(10): 991–1001.

Cao R, Peng W, Wang Z, Xu A. 2007. Beta-Carboline Alkaloids: Biochemical and Pharmacological Functions. Curr. Med. Chem., 14(4): 479-500.

Caritá R, Marin-Morales MA. 2008. Induction of chromosome aberrations in the Allium *cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. Chemosphere, 72(5): 722–725.

Dhanavel D, Gnanamurthy S, Girija M. 2012. Effect of gamma rays on induced chromosomal variation in cowpea (*Vigna unguiculata* L. Walp.). Int. J. Curr. Sci., 2012: 245 – 250.

Efferth T, Kaina B. 2011. Toxicities by herbal medicines with emphasis to traditional Chinese medicine. Curr. Drug Metab., 12(10): 989-996.

El-Bahri L, Chemli R. 1991. *Peganum harmala* L. a poisonous plant of North Africa. Vet. Hum. Toxicol., 3(3): 276-277.

El-Shazly HH. 1990. Cytogenetic effects of some medicinal plants on *Allium cepa* roots. MSc Thesis. Girls College, Ain Shams University, Egypt.

Fiskesjö G. 1985. The Allium test as a standard in environmental monitoring. Hereditas, 102(1): 99–112.

Freeman WJ, Holmes MD, Burke BC, Vanhatalo S. 2003. Spatial spectra of scalp EEG and EMG from awake humans. Clin. Neurophysiol., 114(6): 1053–1068.

Gadano AB, Gurni AA, Carballo MA. 2006. Argentine folk medicine: Genotoxic effects of Chenopodiaceae family. J. Ethnopharmacol., 103(2): 246–251.

Grant WF. 1994. The present status of higher plant bioassays for the detection of environmental mutagens. Mutation Research, (310): 175-185.

Grant WF. 1999. Higher plant assays for the detection of chromosomal aberrations and gene mutations - a brief historical background on their use for screening and monitoring environmental chemicals. Mutat. Res., 426(2): 107–112.

Gustavino B, Vitagliano E, Sottili A, Rizzoni M. 1987. A comparison between short-tem evolution of micronuclei induced by X-ray and colchicine in root tips of *Vicia faba*. Mutat. Res., 192(2): 109-119.

Hoshina MM, Marin-Morales MA. 2009. Micronucleus and chromosome aberrations induced in onion (*Allium cepa*) by a petroleum refinery effluent and by river water that receives this effluent. Ecotoxicol. Environ. Saf., 72(8): 2090-2095.

Kapelan B. 2003. Bio-Imaging Gmb H, www.labimage.cominfo@ labimage.com.

Klasterska I, Natarajan AT, Ramel C. 1976. An interpretation of the origin of subchromatid aberrations and chromosome stickiness as a category of chromatid aberrations. Hereditas, 83(2): 153–169.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227(5259): 680-685.

Mahmoudian M, Jalilpour H, Salehian P. 2002. Toxicity of *Peganum harmala* review and a case report. IJPT, 1(1): 1–4.

McGill M, Pathak S, Hsu TC. 1974. Effects of ethidium bromide on mitosis and chromosomes: A possible material basis for chromosome stickiness. Chromosoma, 47(2): 157–167.

Mekki L, Hussein A Mansour H. 2015. Genotoxic effect of *Peganum harmala* extracts on the growth of *Vicia faba* L. and DNA using nuclear microsatellite. Pak. J. Bot., 47(3): 995-1006.

Mekki L. 2014. Cytogenetic effects of crude extracts of *Peganum harmala* seeds and their effects on *Vicia faba* plants. Cytologia, 79(2): 1-12

Mercykutty VC, Stephen J. 1980. Adriamycin induced genetic toxicity as demonstrated by the *Allium* test. Cytologia, 45(4): 769–777.

Mondini L, Noorani A, Pagnotta MA. 2009. Assessing plant genetic diversity by molecular tools. Diversity, 1(1): 19–35.

Parry MA, Madgwick PJ, Bayon C, Tearall K, Hernandez-Lopez A, Baudo M, Rakszegi M, Hamada W, Al-Yassin A, Ouabbou H, Labhilili M, Phillips AL. 2009. Mutation discovery for crop improvement. J. Exp. Bot., 60(10): 2817–2825.

Roychowdhury R, Alam MJF, Bishnu S, Dalal T, Tah J. 2012. Comparative study for effects of chemical mutagenesis on seed germination, survivability and pollensterility in M1 and M2 generations of *Dianthus*. Plant Breed. Seed Sci., 65(1): 29–38.

Sadia M, Malik SA, Rabbani MA, Pearce SR. 2009. Electrophoretic characterization and the relationship between some *Brassica* species. Electr. J. Biol., 5(1): 1–4.

Shehab AS, Adam ZM. 1983. Cytological effects of medicinal plants in Qatar III. Mitotic effect of water extract of *Anastatica hierochuntico* L. on *Allium cepa.* Cytologia., 48(2): 343–348.

Shehab AS, Tawab SAF, Morci MM. 2004. Stimulation of cell division and gene expression in *Vicia faba* L. using leaf powder of *Azadirachte indica*. Egypt. J. Biotech., 17: 499- 514.

Shreekrishna V. 2006. Cytological Abnormalities in *Amaranthus paniculatus* treated with Ethyl Methyl sulphonate. J. Cytol. Genet., 7: 101-104.

Simaan JA. 2009. Herbal medicine, what physicians need to know. J. Med. Liban., 57(4): 215-217.

Tar'an B, Zhang C, Warkentin T, Tullu A, Vandenberg A. 2005. Genetic diversity among varieties and wild species accessions of pea (*Pisum sativum* L.) based on molecular markers, and morphological and physiological characters. Genome, 48(2): 257-272.

Utsunomiya KS, Bione NCP, Pagliarini MS. 2002. How many different kinds of abnormalities could be found in unique endogamous maize plant? Cytologia, 67(2): 69-176.

WHO. 2013. World Health Organization 2014-2023 traditional medicine strategy. WHO Library Cataloguing-in-Publication Data., pp. 16.

Yi H, Meng Z. 2003. Genotoxicity of hydrated sulfur dioxide on root tips of *Allium sativum* and *Vicia faba.* Mutation Research. Genet. Toxicol. Environ. Mutagen., 537(1): 109–114.

Zhou J, Ouedraogo M, Qu F, Duez P. 2013. Potential genotoxicity of traditional chinese medicinal plants and phytochemicals: an overview. Phytother. Res., 27(12): 1745-1755.

## تقدير السمية الوراثية والخلوية للمستخلص المائي لبذور نبات الحرمل علي الجيل الثاني لنباتات الفول

**ليلي مكي[1]، عزيزة سليمان الخولي[2]، حنان سيد احمد[3]، عبد الفتاح بدر[4]**

[1] قسم النبات، كلية العلوم، جامعة قناة السويس، الاسماعيلية، مصر

[2] قسم النبات، كلية العلوم، جامعة كفر الشيخ، كفر الشيخ، مصر

[3] قسم النبات، كلية العلوم، جامعة طنطا، طنطا، مصر

[4] قسم النبات والميكروبيولجي، كلية العلوم، جامعة حلوان، حلوان، مصر

تم تقييم مدى السمية الوراثية والخلوية للمستخلص المائي لبذور نبات الحرمل علي الجيل الثاني من نباتات الفول، بعد تعريض بذور الآباء إلى التركيزات 12,5، 25، 50 و 100٪ من المستخلص المائي لمدة 3 و 6 و 12 و 24 ساعة، وذلك باستخدام ثلاث أنظمة حيوية وهي التشوهات الوراثية الخلوية وبروتينات البذور والبصمات الوراثية للتتابعات القصيرة المتكررة (SSR) فى الدنا. وقد بينت النتائج ظهور تغيرات كروموسومية بسبب التأثير على خيوط المغزل مما يؤدي إلى ظهور الطور الإستوائى الكولشيسينى والكروموسومات غير الموجهه ولزوجة الكروموسومات والجسور الكروماتينية في الطورين الانفصالي والنهائي بالاضاقة الي الطور الانفصالي المختل، وكذلك الأنوبة الدقيقة وتعدد الأنوية في خلايا الطور البيني. بعض هذه التغيرات قد تؤدي إلى تحورات شكلية دائمة للكروموسومات قد تنتقل إلى الأجيال التالية. أظهر تحليل التفريد الكهربى لبروتينات البذور باستخدام SDS-PAGE تغيرات نوعية وكمية مميزة في الحزم البروتينية من حيث العدد والأوزان الجزيئية والكثافة. وتشير هذه التغيرات إلى تغيرات للحمض النووي المرتبط بانتاج هذه البروتينات التي تعمل في توليفات مختلفة إما لتنشيط أو تثبيط التعبير الجيني. تم أيضا تسجيل 25 من أليلات التكرارات البسيطة المتكررة SSR في نباتات الجيل الثانى من الفول المعاملة بالمستخلص المائي لنبات الحرمل باستخدام 11 زوج من بوادئ التكرارات البسيطة المتكررة ، وكان عدد الأليلات في النباتات الغير معاملة أقل بكثير من عدد الأليلات في نباتات الجيل الثاني المعاملة بالمستخلص المائى لبذور الحرمل. وهذه التغييرات في بصمات التكرارات البسيطة المتكررة قد تكون مرتبطة بالتغيرات الهيكلية في الكروموسومات أو الحمض النووي مما يؤكد القدره علي احداث التغيرات التي سبقت الإشاره اليها.