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In vitro genotoxicity study of the lambda-cyhalothrin insecticide on Sf9 insect cells line using Comet assay

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Abstract

The Synthetic Pyrethroid Lambda-cyhalothrin is one of the most common and used pesticides worldwide for pest control. Its application has resulted in serious environmental hazards and health concerns and has led to the development of resistant pest populations. There are few studies of insecticide toxicity and genotoxicity on insects; therefore, in this study, we evaluated the potential genotoxic activity of Lambda-cyhalothrin using the single-cell microgel electrophoresis or comet assay (the alkaline comet assay) on Sf9 insect cell line. Four different concentrations of Lambda-cyhalothrin were used (0.5, 5, 25, and 100 µm) to treat Sf9 cells for 24 h. UVC (for 45 min) was used as a positive control. The results showed that Lambda-cyhalothrin induced a statistically significant increase in DNA damage in Sf9 insect cell line compared with negative control ($p < 0.05$), except at the 5 µm concentration. UVC induces oxidative stress as Lambda-cyhalothrin insecticide. Lambda-cyhalothrin was more genotoxic than UVC on the Sf9 cell line. This may suppose that Lambda-cyhalothrin insecticide has other genotoxic effects on the Sf9 insect cell line than what is known.

Keywords: Comet assay, DNA damage, Pyrethroid, lambda-cyhalothrin, Sf9 cells.

1. Introduction

The excessive use of synthetic pesticides in agriculture has resulted in serious environmental hazards (Packiam *et al*., 2015), health concerns, and a spike in resistant pest populations (Giner *et al*., 2012; Nagy *et al*., 2014). Therefore, several assays were recently developed to evaluate the genotoxic effects of chemicals and other potent environmental toxicants in living organisms, for example, the structural chromosomal aberrations assay (SCA), micronucleus test (MN), and sister chromatid exchanges and comet assay (Mohanty *et al*., 2011). Among these assays, single-cell gel electrophoresis or comet assay has been widely used in the detection and evaluation of genotoxic compounds in several test systems (Singh *et al*., 1988; Collins, 2004) both *in vitro* and *in vivo* (Sasaki *et al*., 2007).

Lambda-cyhalothrin is a synthetic pyrethroid type II insecticide that contains a cyano group widely used to control agricultural and domestic insect pests of cotton, cereals, hops, ornamentals, and vegetables. It is also used in public health applications to control insects, ticks, and flies which may act as disease vectors (Abdel Aziz and Abdel Rahem, 2010). Lambda-cyhalothrin is classified as a class D carcinogen by US EPA (US Environmental Protection Agency, 2012). It is moderately persistent in the soil environment (Saleem *et al*., 2014).

Lambda-cyhalothrin penetrates the insect cuticle, disrupting nerve conduction within minutes by interacting with sodium channels on nerve membranes (Chakravarthi *et al*., 2007; Shaurub and Abd El-Aziz, 2015). Upon application, the insect suffers loss of muscular control, which results in paralysis and eventually death. There are several reports of lambda-cyhalothrin toxicity to mammals and its ability to induce oxidative stress *in vivo* and *in vitro* (Tukhtaev *et al*., 2012). Lambda-cyhalothrin is highly toxic to fish, aquatic arthropods, and honeybees (Muranli, 2013; Aldehamee, 2015).

Some studies have reported lambda-cyhalothrin genotoxicity using structural chromosomal aberrations assay (SCA), micronucleus test (MN), and comet assay (Çelik *et al.*, 2005a, b; Naravaneni and Jamil, 2005). Most studies focused on lambda-cyhalothrin toxicity to vertebrates, including cytotoxicity (Çelik *et al.*, 2005 b), endocrine disruption (Al-Sarar,2015; Kim *et al.*,2015), genotoxicity like induction of micronucleus (MN), nuclear abnormalities formation on mosquitofish (Muranli and Güner, 2011) and chromosomal aberrations to *Mystus gulio fish* (Velmurugan *et al.*,2006). Some assays also indicated immunotoxicity *in vitro* models, such as human lymphocytes (Naravaneni and Jamil, 2005) and rat bone marrow (Çelik *et al.*, 2005 a, b; Zhang *et al.*, 2010). Whereas the studies of insecticide toxicity and genotoxicity on insects were few, some studies were carried out using the *in vivo* model as the effect of lambda-cyhalothrin on desert locust, *Schistocerca gregaria Forsk* (Al Hariri and Suhail, 2001) that caused an increase in the total counts and abnormal haemocytes.

The genotoxicity of anther pyrethroid insecticide" Deltamethrin "was investigated on cell-mediated immune of *Galleria mellonella* (Lepidoptera: Pyralidae) that induced genotoxic damage by micronucleus formation (Kurt and Kayiş, 2015). The majority of studies focused on

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the genotoxic effect of radiation on DNA damage *in vivo* model on *Ephestia kuehniella* insect (Tuncbilek *et al.*, 2011), and *in vitro* model on Sf9 cell line resistance to DNA-damaged treatments, by radiation and hydrogen peroxide(Chandna *et al.*,2004; Cheng *et al.*,2009).

Comet assay was first described by Ostling & Johanson (1984), and numerous modifications have been reported to date to allow the detection of various types of DNA damage (Gaivão and Sierra, 2014). Different types of the comet assay for different purposes have been described by Collins (2004); the neutral comet assay to detect Double-strand breaks (DSB) and the alkaline comet assay to detect DNA single-strand breaks (SSB).

The most widely used method for the assessment of DNA damage is the alkaline comet assay (Nandhakumar *et al.*, 2011).

The comet assay is a micro electrophoretic technique for the direct visualization of DNA damage at the level of the individual cell (Hamdani *et al.*, 2014). DNA damage is evaluated by the proportion of DNA which migrates out of the nuclei toward the anode when individual cells or isolated nuclei, embedded in a thin agarose layer, are subjected to electrophoresis that results in a "comet-like" shape of nuclei. The Comet is examined after staining with an appropriate fluorochrome stain like ethidium bromide using a fluorescence microscope or with silver staining (Nadin *et al.*, 2001; Garcia *et al.*, 2007*).* The comets can be classified either by visual scoring or by using image analysis software packages (Collins , 2004; Collins *et al.*, 2008). According to visual scoring, the comets are classified into five different classes, from 0 (no tail) to 4 (almost all DNA in tail), based on the tail length and the amount of DNA present in the tail. If 100 comets are scored, and each comet assigned a value of 0 to 4 according to its class, the total score for the sample gel will be between 0 and 400 "arbitrary units or damage index" (Garcia *et al.*,2004; 2011; Collins *et al.*, 2008; Collins *et al.*,2014). Due to the lack of *in vitro* data on the effect of lambda-cyhalothrin on insect cells, results necessitate more genotoxicity studies of pesticides using different assays with different test materials (Bhoopendra and Nitesh, 2015).

This study aimed to investigate the *in vitro* genotoxic effects induced by analytical grade Lambda-cyhalothrin on Sf9 insect cells line as a model for lepidopteran insect cells by using the comet assay.

2. Materials and methods

2.1. Chemicals

Lambda-cyhalothrin (RS)-α-cyano-3-phenoxybenzyl (1R)-cis-3-(Z)-(2-chloro-3, 3, 3-trifluorop1-enyl)-2, 2-dimethylcyclopropanecarboxylate, purity of (98.7%) were obtained from Syngenta.

A stock solution of 10 mM of lambda-cyhalothrin was prepared using DMSO (Dimethyl sulfoxide) freshly made before cell treatment.

2.2. Cell culture

The Sf9 cells derived from pupal ovarian tissue of *Spodoptera frugiperda* (Vaughn *et al.,* 1977) were purchased from Gentaur-Belgium. The culture was routinely maintained at 27°C using the incubator (Selecta) in 25-cm2 culture flasks (TPP)by adding 4ml of EX-cell medium (serum-free medium), (Sigma-Aldrich). Cells formed a monolayer and were sub-cultured every 3–4 days using (TPP) scraper.

2.3. Cell treatment

Cells were seeded into a 6 well tissue culture plate with a density of $1x10^6$cells/ml and allowed to grow for 24 h. The cultures were then treated for 24 h with four concentrations (0.5, 5, 25 and 100 µM) of lambda-cyhalothrin that induced inhibition of cells growth (6, 24, 39, 51%) respectively, based on previous studies, then the culture media were removed and the cells were washed with cold (PBS) Phosphate Buffer Saline (Ca^{++} and Mg^{++}) free, and scraped, centrifuged and resuspended in 200 µl PBS for the comet assay.

2.4. Comet assay

The comet assay was performed using the Comet Assay® Silver Staining Kit Catalog #4251-050-K (Trevigen). Alkaline CometAssay®following the manufacturer's instructions with slight modification. Briefly, cells (1×10^5 cells /ml) were mixed with molten LM Agarose (at 37°C) at a ratio of 1: 10 (v/v).Then, a 50 µL of mixing was pipetted onto the Comet Slide™ area immediately. Cells exposed to UVC (257.3 nm) for 45 min were used as positive controls, and cells treated with DMSO alone were used as a negative control. To prevent additional damage, all the steps described above were conducted under dim light.

The slides were incubated at 4°C for 1 h to accelerate the gelling of the agarose disc and then transferred to prechilled lysis solution (cat# 4250-050-01) 40 ml with 10% DMSO incubate overnight at 4°C. Comet Slide™ was immersed in alkali unwinding solution (pH=13, 300 mM NaOH, 1 mM EDTA) at room temperature, in the dark for 30 minutes.

Slides placed in an electrophoresis slide tray, and then covered with 500 ml alkaline electrophoresis solution pH=13 (300 mM NaOH, 1 mM EDTA). Electrophoresis was performed for 30 minutes (1 Volt/cm / 300 mA). Then slides were immersed twice in dH2O for 5 minutes then in 70% ethanol for 5 minutes. Samples were dried at 37°C for 10-15 minutes and stained with silver staining.

2.4.1. Silver Staining:

The sample area was covered with 100 µl of prepared fixation solution :10X Fixation Additive (cat# 4254-200-05), dH2O, methanol, glacial acetic acid, incubated for 20 minutes at room temperature, and then rinsed in dH2O for 30 minutes. The samples were then covered with 100 µl of prepared staining solution : dH2O, 20X Staining Reagent #1 (cat# 4254-200-01), 20X Staining Reagent #2 (cat# 4254-200-02), 20X Staining Reagent #3 (cat# 4254-200-03), and incubated at room temperature for 5 - 20 minutes. The intensity of staining was visualized under the microscope using a 100X objective, and the reaction stopped when comets were easily visible by covering samples with 100 µl of 5% acetic acid for 15 minutes and rinsing in dH2O.

2.4.2. Comet analysis:

Comets (more than 50 per treatment) were captured, digitized, and copied to the computer.

Each comet was identified by a number from 0 to 4 with various degrees of DNA damage. Class 0 represents no damage with the head being large and intact and comet without a tail. Class 1 represents slight damage with the head being large and little affected and a short tail whose length is less or equal to one head diameter. Class 2 represents medium damage with the head being large and little affected, and a short tail. Class 3 represents extensive damage with the head being reduced, long and large tail. Class 4 represents severe damage with the head being greatly reduced; long and large tail whose contour is difficult to determine due to the dispersion of small DNA fragments (Collins, 2004). Then DNA damage was calculated in AU as using the formula:

$$AU = \frac{0 \times N0 + 1 \times N1 + 2 \times N2 + 3 \times N3 + 4 \times N4}{\#\text{comets analyzed}} \times \mathbf{100}$$

Where N0, N1, etc. are the numbers of comets in categories 0, 1, etc. (Garcia *et al*., 2011).

2.5. Statistical analysis

For each treatment, two slides were prepared, in two independent experiments performed. Statistical analyses were performed with the Mann-Whitney test using SPSS-17.0 Software. Error bars represent standard deviation. Results were considered statistically significant when $p < 0.05$.

3. Results

The results showed that lambda-cyhalothrin, significantly increased DNA damages in Sf9 insect cell line, compared with negative controls ($P > 0.05$), except at the 5 µM concentration, while no significant difference was observed between the all lambda -cyhalothrin concentrations and positive control, as shown in figure (1).DNA damage index was: 125,100,125,158, using concentrations (0.5, 5, 25 and 100 µM) of Lambda-cyhalothrin that induced inhibition percentage of cell growth, (6, 24, 39, 51%) respectively .

It was observed that lambda-cyhalothrin insecticide induced DNA-damage of 125 AU at the lowest concentration of 0.5 µM compared to 25 AU of untreated cells. The lowest DNA damage was measured in 100 AU at 5 µM concentration, while the higher value of damage was 158 AU at 100 µM, which is the highest concentration of lambda -cyhalothrin insecticides tested here.

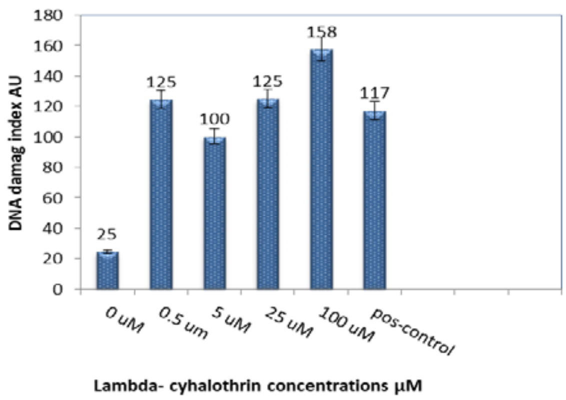

Figure 1. DNA damage induced by lambda-cyhalothrin in Sf9 insect cell line expressed in arbitrary units (AU) in the comet assay. Data are means of values of repeated experiments ± standard deviation. A statistically significant increase ($p < 0.05$) as determined by comparing the values of DNA damage induced by various concentrations of lambda-cyhalothrin with the negative control (with 10% DMSO).

Figure 2 shows the Images of the silver-stained comet of Sf9 insect cell line, with various degrees of DNA damage. Class 0 represents undamaged cells and class 4 represents the most heavily damaged.

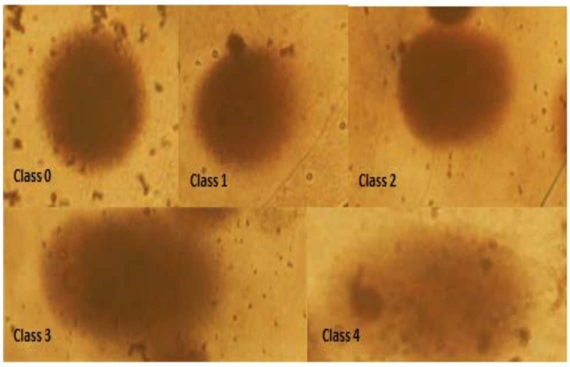

Figure 2. Visual scoring of DNA damage from 0 to 4 according to comet appearance, Study sample: SF9 insect cells, Stain: Silver nitrate, Magnification: 200X

4. Discussion

Long-term extensive use of insecticides has been a major cause for insecticides-resistance development in insects, which creates an important problem and is a major threat to agriculture, human, and animal health.

Measuring DNA damage is a key step in a broad range of biomedical and toxicological research studies. Among several methods of detecting DNA damage, the comet assay, being very simple, cheap, and not requiring sophisticated high-cost equipment, has been most widely adopted and used.

Resistance to Pyrethroids insecticides refers to the genetic change in the insect pest genome. This genetic change can occur by two main mechanisms: 1) increased levels of detoxification enzymes resulting in metabolic resistance, and 2) target-site mutations in the voltage-gated sodium, known as knock-down resistance (kdr) (Shen *et al.*, 2016).

Lambda-cyhalothrin belongs to Pyrethroids insecticides and is used extensively in pest control. It is essential to study and analyze the cytotoxic and genotoxic effects of Lambda-cyhalothrin on both environment and human health. These studies will enable better adoptions for measures that can protect humans from the potential mutagenic, carcinogenic effects of these insecticides, and halt the development of insecticide resistance in the insect pest (Nagy *et al*., 2014).

Most toxicity studies of the Lambda-cyhalothrin insecticide effect were performed on vertebrate's *in vivo*/*in vitro* model, whereas a few data are available for insects; therefore, this current research is applied on Sf9 insect cell line model using comet assay to investigate the genotoxicity of Lambda-cyhalothrin insecticide. The results indicate that Lambda-cyhalothrin insecticide is genotoxic to Sf9 insect cell line by causing DNA damage in all concentrations, which corresponds with previous studies applied on other organisms (Çelik *et al*., 2005a,b; Naravaneni and Jamil, 2005; Zhang *et al*., 2010; Muranli and Güner, 2011; Muranli,2013). The DNA damage index was measured as (125,100,125,158) AU, using concentrations (0.5, 5, 25, and 100 µM) of Lambda-cyhalothrin that induced inhibition of cells growth at (6, 24, 39, 51%), respectively. It was observed that DNA damage index is related to the inhibition rate except for the 5 µM concentration, and that can be explained by that the Sf9 cell's response to DNA damage is induced by Lambda-cyhalothrin insecticide. This response includes cell cycle arrest to allow DNA repair (Remington, 2010), or cell death via apoptosis if the damage encountered is great (Chandna *et al*., 2004).

A possible mechanism for lambda-cyhalothrin cytotoxicity could be the induction of oxidative stress by the increase in reactive oxygen species (ROS or free radicals). ROS will impair the balance between the ROS generation and antioxidant defense capability, and this will cause damage to the cell membrane lipids and proteins (Tukhtaev *et al*., 2012), in addition to single-strand DNA breaks, (Zhang *et al*., 2010; Fetoui *et al*., 2015; Deeba *et al*., 2017).

In this present study, it was found that Sf9 insect cell line is resistant to UVC effect, which explains the insignificant differences between all concentrations of Lambda-cyhalothrin and cells treated with UVC as a positive control (Cheng *et al.*, 2009). Chandna *et al* (2004; 2010) reported that Lepidopteran insect cells are known to exhibit very high radio-resistance, which is possibly caused by a stronger antioxidant system and active DNA repair mechanisms. Previously, we reported two different populations of Sf9 cells were identified, mononucleated and polynucleated according to their nuclear number (Saleh, 2011).The endopolyploid cells (polynucleated) possibly play a role in Sf9 cells metabolism and their ability to active DNA repair, as with Ivanov and others (2003) who also reported that endopolyploid cells produced after severe genotoxic damage, which might facilitate an alternative pathway of cell survival and therefore contribute to genotoxic resistance.
Although the mode of action of UVC and lambda-cyhalothrin insecticide is one, which is inducing oxidative stress, the genotoxic effect of lambda-cyhalothrin on Sf9 insect cell line was higher than UVC, which may indicate that Lambda-cyhalothrin insecticide has an additional genotoxic effect on Sf9 insect cell line.

In conclusion, the results from this present study indicate that Lambda-cyhalothrin insecticide is genotoxic to the Sf9 insect cell line, and can cause DNA damage in all tested concentrations. DNA damage index was: 125,100,125,158, using concentrations (0.5, 5, 25 and 100 µM) of lambda-cyhalothrin that induced inhibition percentage of cells growth :(6, 24, 39, 51%), respectively. The possible mechanism by which Lambda-cyhalothrin cytotoxicity occurs is by oxidative stress induction, and additional mechanisms await further characterization.

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References

Abdel Aziz KB and Abdel Rahem HM. 2010. Lambda, the pyrethroid insecticide as a mutagenic agent in both somatic and germ cells. *J Am Sci*, **6:**72-81.

Aldehamee MHM. 2015. Effect of Different Concentrations of Pesticide Colti 5 (Lambda-Cyhalothrin) On Water Flea *Daphnia pulex*. *Jubpas*, **23**: 680-685.

Al-Hariri MK and Suhail ANJUM. 2001. Effect of lambda-cyhalothrin and deltamethrin on the haemocytes of Desert Locust, Schistocerca gregaria Forsk. *Int J Agric Biol*, **3**:81-84.

Al-Sarar AS, Abobakr Y, Bayoumi AE, and Hussein HI. 2015. Cytotoxic and genotoxic effects of abamectin, chlorfenapyr, and imidacloprid on CHO K1 cells. *Environ Sci Pollut Res*, **22:** 17041-17052.

Bhoopendra K. and Nitesh K. 2014. Immunotoxicity of Lambda-Cyhalothrin in Wistar Albino Rats. *Int J Toxicol Pharmacol Res*, **6:** 47-56.

Çelik A, Mazmanci B, Çamlica Y, Çömelekoğlu U, and Aşkin A. 2005a. Evaluation of cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow by gavage administration. *Ecotoxicol Environ Sa*, **61:** 128–133.

Çelik A, Mazmanci B, Çamlica Y, Aşkin A, and Çömelekoğlu Ü. 2005b. Induction of micronuclei by lambda-cyhalothrin in Wistar rat bone marrow and gut epithelial cells. *Mutagenesis*, **20:** 125-129.

Chakravarthi K, Naravaneni R, and Philip GH. 2007. Study of cypermethrin cytogenesis effects on human lymphocytes using in-vitro techniques. *J Appl Sci Environ Manage*, **11:** 77 - 81.

Chandna S, Dwarakanath BS, Seth RK, Khaitan D, Adhikari JS, and Jain V. 2004. Radiation responses of Sf9, a highly radioresistant lepidopteran insect cell line. *Int J Radiat Biol*, **80:** 301-315.

Cheng IC, Lee HJ, and Wang TC. 2009. Multiple factors conferring high radioresistance in insect Sf9 cells. *Mutagenesis*, **24:** 259-269.

Collins AR. 2004. The comet assay for DNA damage and repair. *Mol. Biotechnol*, **26**: 249–261.

Collins AR, El Yamani N, Lorenzo Y, Shaposhnikov S, Brunborg G, and Azqueta A. 2014. Controlling variation in the comet assay. *Front Genet*, **5**:1-5.

Collins AR, Oscoz AA, Brunborg G, Gaivão I, Giovannelli L, Kruszewski M, Smith CC, and Štětina R. 2008. The comet assay: topical issues. *Mutagenesis*, **23**: 143-151.

Deeba F, Raza I, Muhammad N, Rahman H, ur Rehman Z, Azizullah A, Khattak B, Ullah F, and Daud MK. 2017. Chlorpyrifos and lambda cyhalothrin-induced oxidative stress in human erythrocytes: *in vitro* studies. *Toxicol Ind Health*, **33**: 297-307.

Fetoui H, Feki A, Salah GB, Kamoun H, Fakhfakh F, and Gdoura R. 2015. Exposure to lambda-cyhalothrin, a synthetic pyrethroid, increases reactive oxygen species production and induces genotoxicity in rat peripheral blood. *Toxicol Ind Health*, **31**: 433-441.

Gaivão I and Sierra LM. 2014. Drosophila comet assay: insights, uses, and future perspectives. *Front Genet*, **5**:1-8.

García O, Mandina T, Lamadrid AI, Diaz A, Remigio A, Gonzalez Y, Piloto J, Gonzalez JE and Alvarez A. 2004. Sensitivity and variability of visual scoring in the comet assay: results of an inter-laboratory scoring exercise with the use of silver staining. *Mutat Res Genet Toxicol Environ Mutagen*, **556**: 25-34.

Garcia O, Romero I, González JE and Mandina T. 2007. Measurements of DNA damage on silver-stained comets using free Internet software. *Mutat Res Genet Toxicol Environ Mutagen*, **627**: 186-190.

García O, Romero I, González JE, Moreno DL, Cuétara E, Rivero Y, Gutiérrez A, Pérez CL , Álvarez A, Carnesolta D and Guevara I. 2011. Visual estimation of the percentage of DNA in the tail in the comet assay: Evaluation of different approaches in an intercomparison exercise. *Mutat Res Genet Toxicol Environ Mutagen*, **720**: 14-21.

Giner M, Avilla J, Balcells M, Caccia S, and Smagghe G. 2012. Toxicity of allyl esters in insect cell lines and in *Spodoptera littoralis* larvae. *Arch Insect Biochem*, **79:** 18-30.

Hamdani DA, Javeed A, Ashraf M, Nazir J, Ghafoor A, and Altaf I. 2014. *In vitro* cytotoxic and genotoxic evaluation to ascertain toxicological potential of ketoprofen. *Afr J Pharm Pharmaco*, **8**: 386-391.

Ivanov A, Cragg MS, Erenpreisa J, Emzinsh D, Lukman H, and Illidge TM. 2003. Endopolyploid cells produced after severe genotoxic damage have the potential to repair DNA double-strand breaks. *J Cell Sci*, **116**: 4095-4106.

Kim CW, Go RE, and Choi KC. 2015. Treatment of BG-1 ovarian cancer cells expressing estrogen receptors with lambda-cyhalothrin and cypermethrin caused a partial estrogenicity via an estrogen receptor-dependent pathway. *Toxicol Res*, **31**:331-337.

Kurt D and Kayiş T. 2015. Effects of the pyrethroid insecticide deltamethrin on the hemocytes of *Galleria mellonella*. *Turk J Zool*, **39:** 452-457.

Mohanty G, Mohanty J, Dutta SK, and Jena SD. 2011. Genotoxicity testing in pesticide safety evaluation. , Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment. *Intech Open Access* Publisher, **614:** 403-426.

Muranli FDG and Güner U. 2011. Induction of micronuclei and nuclear abnormalities in erythrocytes of mosquitofish (*Gambusia affinis*) following exposure to the pyrethroid insecticide lambda-cyhalothrin. *Mutat Res Genet Toxicol Environ Mutagen*, **726:** 104-108.

Muranli FDG. 2013. Genotoxic and cytotoxic evaluation of pyrethroid insecticides λ-cyhalothrin and α-cypermethrin on human blood lymphocyte culture. *Bull Environ Contam Toxicol*, **90:** 357-363.

Nadin SB, Vargas–Roig LM and Ciocca DR. 2001. A silver staining method for single-cell gel assay. *J Histochem Cytochem*, **49:** 1183-1186.

Nagy K, Rácz G, Matsumoto T, Ádány R, and Ádám B. 2014. Evaluation of the genotoxicity of the pyrethroid insecticide phenothrin. *Mutat Res Genet Toxicol Environ Mutagen*, **770:** 1-5.

Nandhakumar S, Parasuraman S, Shanmugam MM, Rao KR, Chand P, and Bhat BV. 2011. Evaluation of DNA damage using single-cell gel electrophoresis (Comet Assay). *J Pharmacol Pharmacother*, **2**: 107-111.

Naravaneni R and Jamil K. 2005. Evaluation of cytogenetic effects of lambda-cyhalothrin on human lymphocytes. *J Biochem Mol Toxic*, **19:** 304–310.

Ostling 6 and Johanson KJ. 1984. Micro electrophoretic study of radiation-induced DNA damages in individual mammalian cells. *Biochem Biophys Res Commun*. **123:** 291-298.

Packiam SM, Emmanuel C, Baskar K, and Ignacimuthu S. 2015. Feeding deterrent and genotoxicity analysis of a novel phytopesticide by using comet assay against *Helicoverpa armigera* (Hübner)(Lepidoptera: Noctuidae). *Braz Arch Biol Techn*, **58:** 487-493.

Remington SE. 2010. Cellular response to DNA damage after exposure to organophosphates *in vitro* (Doctoral dissertation, Newcastle University).

Saleem U, Ejaz S, Ashraf M, Omer MO, Altaf I, Batool Z, Fatima R and Afzal M. 2014. Mutagenic and cytotoxic potential of Endosulfan and Lambda-cyhalothrin—*In vitro* study describing individual and combined effects of pesticides. *Int J Environ Sci*, **26:** 1471-1479.

Saleh M.2011. The effect of insecticides on insect cell culture *in vitro*. MSc dissertation, Damascus University, Syria.

Sasaki YF, Nakamura T, and Kawaguchi S. 2007. What is better experimental design for *in vitro* comet assay to detect chemical genotoxicity? *AATEX*, **14:** 499-504.

Shaurub ESH and El-Aziz NMA. 2015. Biochemical effects of lambda-cyhalothrin and lufenuron on *Culex pipiens* L. (Diptera: Culicidae). *Int J Mosq Res*, **2:** 122-126.

Shen XM, Liao CY, Lu XP, Wang Z, Wang JJ, and Dou W. 2016. Involvement of three esterase genes from *Panonychus citri* (McGregor) in fenpropathrin resistance. *Int J Mol Sci*, **17**:1-15.

Singh NP, McCoy MT, Tice RR, and Schneider EL. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res*, **175:** 184-191.

Tukhtaev K, Tulemetov S, Zokirova N, and Tukhtaev N. 2012. Effect of long term exposure of low doses of lambda-cyhalothrin on the level of lipid peroxidation and antioxidant enzymes of the pregnant rats and their offspring. *J Res Health Sci*, **13:** 93-99.

Tuncbilek AS, Kilicoglu H, Yazici N, Ozcan S, Erel Y, Canpolat U, Yay A and Bakir S. 2011. Detection of DNA Damage in *Ephestia kuehniell*a by single cell gel electrophoresis after exposure to gamma radiation. *Ann Univ Craiova Agr*, Montanology, Cadastre Series, **41:** 266-269.

U.S. EPA. **Environmental Protection Agency**. 2012. Office of Pesticide Programs: Chemicals Evaluated for Carcinogenic Potential, Annual Cancer Report.

Vaughn J L, Goodwin R H, Tompkins G J and Mccawley P. 1977.The Establishment Of Two Cell Lines From The Insect *Spodoptera Frugiperda* (Lepidoptera; Noctuidae). *In vitro*, **13:** 213- 217.

Velmurugan B, Ambrose T, and Selvanayagam M. 2006. Genotoxic evaluation of lambda-cyhalothrin in *Mystus gulio*. *J Environ Biol*, **27:** 247-250.

Zhang Q, Wang C, Sun L, Li L, and Zhao M. 2010. Cytotoxicity of lambda-cyhalothrin on the macrophage cell line RAW264.7. *J Environ Sci*, **22:** 428–432**.**