Plant Archives Volume 20 No. 2, 2020 pp. 7451-7456 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

A PHENOTYPIC, QUANTITATIVE AND MOLECULAR STUDY OF PEA PLANT AFTER TREATMENT WITH PENDIMETHALIN HERBICIDE IN DIFFERENT WAYS

Nahla Salim Hammok[1], Mohammed Abdelilah Al-Shakarchi[2*] and Fathi Abdullah AL-Mandeel[3]

[1,3]Environmental Researches center, University of Mosul, Iraq.
[2*]Biology Department, College of Education For Pure Sciences, University of Mosul, Iraq.

Abstract

Pendimethalin pesticide (PM)is a powerful pesticide so be careful when dealing with it. The research was carried out in the wired house of the Faculty of Education, Life Science Department to study the effect of the pesticide (PM) on the morphological and quantitative traits and changes at the molecular level of the DNA in the pea plant when applied field in two ways (pre and post emergence). The results of the morphological study showed a significant decrease in the germination percentage when applying a pesticide (pm) in the pre- emergence. The length of the plant also decreased after 30 days have passed and the decrease in the above characteristics has started from the lower concentrations and the decrease has increased with increasing concentration. The overlap between (methods of applying the pesticide × concentrations) It was also significant in the percentage of germination, plant height (cm), the amount of chlorophyll and the global treatment (post emergence × 4.1 ml) recorded the highest rate of the value of the amount of chlorophyll. (32.51) mg/cm^2 (morphology was observed) where the pea leaves were colored in dark green at Treated in high concentrations 4.1 ml/Seen. Through the comparison between pea plants treated with the pesticide using the RAPD-PCR technique and electrophoresis of its products and determining the genetic distance (UPGMA Analyzing) between the genotypes of the plants treated by the pesticide pre and post-emergence and comparison plants, a difference was observed between the plants treated pre-emergence the emergence of the pesticide at a high concentration of 4.1 ml/liter while the effect was Less when using the concentration of 2.8 and 1.4 ml/l. This indicates a change in the genotype of the plants treated before emergence, as well as plants treated after emergence, but to a lesser degree. From the study, it was found that the highest contrast was when using the OPA-11 and OPC-12 primer. This is shown by the study of the phenotypic pattern of treated plants and the negative effect of treatment of plants with pesticide by the occurrence of a genetic mutation by removing or adding nucleotides, which is the reason for the emergence of a number of packages not present in the genetic material of untreated plants when studying the genetic appearance of them.

Key words: pendimethalin, pea, pre-emergence, RAPD-PCR, post-emergence, mutation.

Introduction

The increasing use of pesticides that infect plants such as dithane, malathion and many other pesticides in agriculture causes genetic mutations in the neighborhoods where they are treated. However, there is a lack of research on the genetic toxicity of these pesticides, which causes genetic changes within the plant cells of the treated crops, either at the chromosome or genetic level, especially on economically important crops (Maity, 2014). Pesticide Pendimethalin (PM) is a meiotic inhibitor that inhibits cell division in the root meristem. It also discourages the construction of the microtubule that is important for cell wall formation and spindle thread and thus chromosome separation (Singh and Srivastor, 2014). Some studies have indicated that the Pesticide (PM) causes cellular, genetic and morphological toxicity in unintended organisms of the treatment. When evaluating the toxicological effects of the pesticide, on the genetic material and some morphological characteristics of the *Vicia faba* L., they found abnormal mitosis percentage reached to 63.28%, with a reduction in plant height, root ,wood vessel diameter, phloem thickness and vascular bundles in Pesticide treated plants. As a Pesticide (PM) caused changes in the bean protein bundles, three packages disappeared after treatment with the pesticide, while some packages appeared after the addition of

***Author for correspondence :** E-mail : dr.mohammedsh@uomosul.edu.iq

Pseudomonas resinovorans E20 (Aboulila *et al.,* 2016). He also studied the toxic effect of (PM) exogenous *in vivo* on Chinese hamster cells (CHO) by applying a single cell gel electrophoresis (comet) test and observed a significant increase in DNA damage to CHO cells after treatment with different concentrations 1,100,1000, and 10000mM for (PM) (Demir *et al.,* 2017). And when assessing the effect of (PM) on human lymphocytes in humans and mice, they found that the PM (pesticide) binds to DNA by forming covalent bonds with the nitrogenous bases G and C in addition to ribose sugar. It also urges the formation of micronucleus (mn) nuclei in human lymphocytes (Ansari *et al.,* 2018) Recently, it was found that the pesticide (PM) causes toxic effects on the seed and growth of maize *Zea mayas* L. With the presence of UV-C ultraviolet radiation, distortions appeared in the vegetative and root groups when treating the seedlings at the following concentrations of the pesticide 0.0, 1.4, 2.8, 4.1 ml/L. Researchers (Demir *et al.,* 2017; Ansari *et al.,* 2018; Hammok, 2019) have found where its inhibitory effect starts from low concentrations.

The aim of this study, to detect the mutagenic effect of pendimethalin herbicide on pea plant *Pisum sativum* L. using different concentrations of (PM) when applied pre and post-emergence by identifying the genetic mutations using genetic isolation and migration technique as well as the use of RAPD-PCR.

Materials and Methods

Pea (Pisum sativum L.) local class was cultivated during the winter season 2018-2019 and the use of the pesticides pendimethalin (PM) (supplied by Agrichem company-Australia) in two methods pre and post-emergence (as shown in research 1(unpublished results). After that, the growth of the pea plant in the field was monitored and the following studies were conducted on it.

Morphological studies

The germination percentage was measured using the following law. Percentage = number of seeds grown / total number of seeds × 100 (Hamid, *et al.,* 2013). After 30 days of planting, the height of the plant (cm) was measured using a ruler.

Quantitative study

The amount of chlorophyll was measured in leaves (micrograms cm^2) using the SPAD-502 chlorophyll meter and she took 10 readings of ten plants per treatment and then took the rate and then the reading was converted to micrograms cm^2 through the following equation: 99* spad)/(144-spad) = (Chl) $mg.cm^2$ (Cerovic, *et al.,* 2012). Morphological and quantitative characteristics were analyzed using a program Gen stat v12 (welham *et al.,* 2015).

Molecular study

Extraction genomic DNA (mini Kit) protocol used to extract DNA from the plant leaves were treated bt the pesticides 0.0, 1.4, 2.8, 4.1 ml/Land measuring to purity and concentration of DNA by the Nanodrop, Fig. 1 of on pea plants that treated by the two methods mentioned previously by conducting using a random amplification Polymorfic Reaction (RAPD) using five random Primers table 1 under codition reaction (Denaturation 94/1min, Annealing 37/2 min, Extension 72/2min) in 44 cycle than electrophoresis concentration 2% agarose gel device to detect the DNA bundles generated by RAPD and then the data obtained using the UPGMA program was analyzed to see the degree of difference and similarity between plants. Treatment to reveal the extent of treatment plants affected by the pesticide study Alopecia changes to the genetic material by creating a dendogram.

Table 1: Sequence of Primer used in the RAPD-PCR.

No.	Primer	Sequence
1	UBC-13	5'CCTGGGTGGA3'
2	OPC-12	5'TGTCATCCCC3'
3	OPA-11	5'CAATCGCCGT3'
4	OPJ-05	5'CTCCATGGGG3'
5	RPI-1	5'AAAGCTGCGG3'

Results and Discussion

Morphological study

It is clear from table 2 that there is a significant difference in the germination percentage, as the germination rat decreased significantly when applying pesticide (PM) in pre-emergence compared to post-emergence. Where the pesticide (PM) obstructs the meristematic tissue (Growth apical) (Verma *et al.,* 2018; Singh and Srivastor, 2014) where it was observed that the application of the pesticide field pre-emergence is more influencing the germination percentage due to the possibility of contact the pesticide with emerging seeds

Table 2: The effect of apply methods, Concentrations and interaction between them on germination percentage.

Apply Methods	Concentrations				Apply Methods Means
	0	1.4	2.8	4.1	
Pre-emergency	100 a	73.33 b	56.67 b	33.33 c	65.80 b
Post-emergency	100 a	100 a	96.67 a	73.33 b	92.50 a
Con. Means	100 a	86.70 ab	76.70 b	53.30 c	

The numbers with same letter within each comparison mean "No significant difference" at 5%.

and germination ceased when the pesticide touched the meristematic tissue. Where as applying the pesticide (PM) to seedlings was less effective for the spraying is on the leaves and does not come into contact with the roots. The same table also recorded a significant decrease in the germination percentage between the concentration, the global treatment was at a high concentration (4.1m/L) is the lowest among the treatments, where the germination percentage has reached half (53.33c). The decrease has started from the lower concentration and increased with increasing concentration. Thus we agree with the researcher karaye, 2014 that the high concentration are inhibitory for germination, as we agree with Verma *et al.,* 2018; Ansari *et al.,* indicates that the pesticide (PM) possesses strong toxicity even in low concentration, where the effect on the pea plant started from low concentration. The table also shows that there is significant overlap between the methods of application of the pesticide and the concentration of the pesticide where the global treatment (pre-emergence)×(4.1ml/L) was recorded. Less than half the germination percentage, whereas the post- emergence did not affect the germination percentage except when using high concentration 4.1ml/L and this is a positive result of the method post- emergence.

Table 3 also show that there were no significant difference in the characteristic of the height of the plant after 30 days of planting when applying the pesticide in the tow methods pre and post-emergence ,whereas the effect of the pesticide concentration was evident in the characteristic of plant height as the height of the plant decreased significantly with increasing concentration and that the decreased in the height of the plant has started from the lower concentration. The decreased in the height of the plant may be attributed to the role of the pesticide (PM) in its inhibition of the rate of normal division and its reflection on the germination and growth process (Verma *et al.,* 2018). The overlap between (method of application of pesticide × concentration) was significant in the characteristic of plant height and was recorded high concentration (4.1ml/L) for tow methods pre and post-emergence the highest decrease in the plant height after 30 days, we agree with this result with wagner, 2006, who indicated the reduction of the vegetative group of pea plants when applying the pesticide (PM) at high concentration pre-emergency.

Table 3: The effect of apply methods, Concentrations and interaction between them on Plant height.

Apply Methods	Concentrations				Apply Methods Means
	0	1.4	2.8	4.1	
Pre-emergency	25.47 a	14.91 c	13.89 c	7.42 d	15.42 a
Post-emergency	20.70 b	12.52 c	13.87 c	5.90 d	13.25 b
Con. Means	23.08 a	13.71 b	13.88 b	6.66 c	

The numbers with same letter within each comparison mean "No significant difference" at 5%.

Table 4: The effect of apply methods, Concentrations and interaction between them on chlorophyll.

Apply Methods	Concentrations				Apply Methods Means
	0	1.4	2.8	4.1	
Pre-emergency	21.40ab	25.99ab	24.39ab	21.52ab	23.30 a
Post-emergency	19.07b	23.83ab	22.26ab	32.51a	24.40 a
Con. Means	20.20a	24.90a	23.30a	27.00a	

The numbers with same letter within each comparison mean "No significant difference" at 5%.

Quantitative study:

Table 4 shows the effect of the application method :pre and post- emergency, concentration (con): (0, 1.4, 2.8, 4.1ml/L) as well as the overlap between them. Since it is clear from the table that there were no significant differences between the application method and also no significant differences between the concentration, except that there were significant differences in the overlap of the application method with the concentration ,the global treatment recorded (post- emergency×4.1ml/L) the highest average attribute rat was (32.51mg/cm^2). The increase in chlorophyll when applying high concentration post- emergency may first be due to the water tension caused by the pesticide. It was found (Wasfi and samia, 2016) that spraying the pesticide (PM) on cotton seedling *Gossypium hirsutum* L. and *Zea mays* L. three weeks at a concentration (5ppm) resulted in higher chlorophyll in cotton compared to maize the Reason was attributed to the influence of the amount of chlorophyll, due to the growth and development of plants and other factors, the intensity of light, the availability of water, nutrients, and pesticide tension. Secondly, it may be due to the mutant action of pesticide (PM), especially since the pesticide was in direct contact with the leaves and apical of the leaf and affected the chloroplast DNA of the leaves. Some research has indicated to the generation of reactive oxygen species (ROS) after treatment with (PM), which leads to mutation in cell (Aboulila *et al.,* 2016; Demir *et al.,* 2017; Ansari *et al.,* 2018).

Molecular study

By studying the results obtained from the electrophoresis of the DNA isolated from the pea plants treated with pesticide using the electrophoresis device in order to detect the purified genetic material and

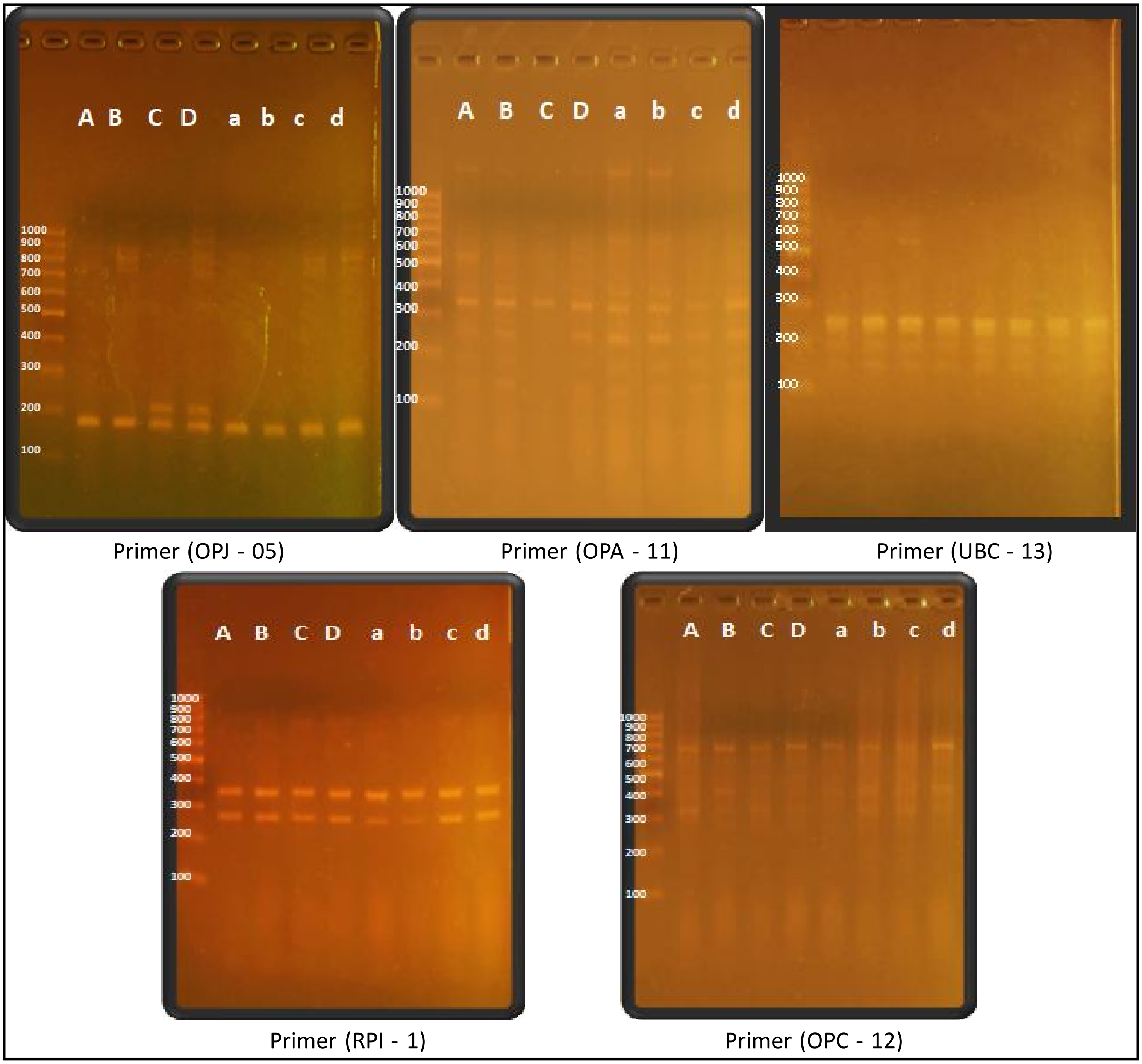

Fig. 1: Shows the random multiplication interaction of plants treated with pesticide (PM)(A-D) post-emergence and (a-d) pre-emergence.

perform the random multiplication reaction (RAPD). It was found that the use of the Primer (RPI-1) showed a difference in the size of the generated DNA Bands (290 and 360) bp and for all parameters, while it was found that there was a variation of the bands generated using the initiator (OPC-12) by the lack of bands disappearance at (400) bp For plants treated in the second method (post-emergence) of the two concentrations (2.8 and 4.1), while the packages were not generated at (500) bp for all isolates treated with concentrations (1.4, 2.8 and 4.1), this shows the great effect of this hand and directly on the genetic material, especially when the treatment is performed before the emergence of seedling, It was also noted that there are packages generated at (500 and 600) bp for concentration 4.1 and (600) bp at concentration (2.8) for plants treated in the post- emergence that appeared when using the initiator (UBC-13). On the contrary, toxic toxicity was observed on the genetic material of the seedlings. Treatment with pesticide, such as after germination, where bundles were generated when using the starter (OPC-05) at size (700, 800, 900 and 1000) for plants treated after germination with concentrations (2.8 and 4.1), as well as the appearance of bundles at (200) and for the same concentrations of samples treated (pre-emergence) Also, when performing the random multiplication reaction using the initiator (OPA-11) a number of beams (330 and 500) bp appeared for the developing growing plants. Pesticide (4.1) and packaged within (500 and 600) bp when treating seeds pre-emergence with a pesticide with a concentration of (1.4) was found that the primer OPA-11 was better than the rest of the other primers efficient 28.65, while the efficiency of the PR-1 was the lowest, and the best Discriminatory was for the Primer OPA-11, table 5.

Researchers reported abnormalities like chromosomes with inactivated centromeres, isochromosome, picnosis, vagrant, stickiness, bridges, precocious separation and lagging chromosomes, reduction in mitotic index, micronuclei, multipolar cells, sister chromatid exchanges (Nag *et al.,* 2013) The genotoxic effects of insecticide Telliton and fungicide Dithane M-45 in the meiotic cell divisions and changes in the seed and plant of *Vicia faba*. The percentage of abnormal pollen mother cells, (PMCs) increased as the concentration of both pesticides increased, and showed that Telliton has more mutagenic effects than Dithane M-45 (Haiba *et al*., 2011), Another study to Ahmad *et al*., (2018) were found the effect of herbicides, pendimethaline (PND) on male mice by different oral doses of 62.5, 125 and 250 mg/kg of body weight. For 14 days. Notice the presence of toxic effects in terms of oxidative stress, DNA damage, tissue changes, Significant DNA damage was recorded through comet assay in liver and kidney cells of treated animals as compared to control.

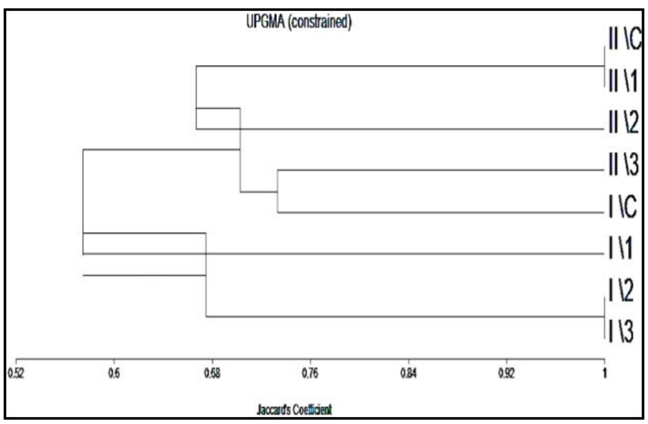

Fig. 2: Phylogenetic tree Show the degree of similarity and difference between plants treated by pesticide (PM).C: control; I: pre-emergence and II: post- emergence.

Table 5: The number of bands and the sequence, efficient and Discriminatory of the primers were used.

Discrim -inatory	efficient	Number of Polymorphic	Number of bands	The sequence	Primer	No.
0.0	14.63	0	24	5'AAAGCTGCGG3'	PR-1	1
21.25	18.29	17	30	5'TGTCATCCCC3'	OPC-12	2
26.25	17.68	21	29	5'CTCCATGGGG3'	OPJ-05	3
2.5	20.73	2	34	5'CCTGGGTGGA3'	UBC-13	4
50.00	28.65	40	47	5'CAATCGCCGT3'	OPA-11	5
		80	164	Total of bands		

Table 6: The form of a matrix showing the degree of similarity between treated plant pesticide (PM). C: control; I: pre-emergence and II: post- emergence.

UPGMA
Jaccard's Coefficient
Constrained clustering strategy

Similarity matrix

	II \C	II \1	II \2	II \3	I \C	I \1	I \2	I \3
II \C	1.000							
II \1	1.000	1.000						
II \2	0.667	0.667	1.000					
II \3	0.688	0.688	0.375	1.000				
I \C	0.933	0.933	0.600	0.733	1.000			
I \1	0.588	0.588	0.833	0.333	0.529	1.000		
I \2	0.800	0.800	0.467	0.714	0.857	0.412	1.000	
I \3	0.800	0.800	0.467	0.714	0.857	0.412	1.000	1.000
	II \C	II \1	II \2	II \3	I \C	I \1	I \2	I \3

Analyze the results of the RAPD-PCR reaction

The UPGMA statistical analysis program was used in analyzing the results of the PCR reaction to find the extent of the genetic convergence between the treated samples and the extent of the effect of the pesticide concentrations on the DNA of the treated plants and for both methods compared with the control sample. There was no significant difference between control and treatment samples at the low concentation in both methods, while a large genetic separation of the treated sample at a concentration 2.8 ml/L and 4.1ml/L of the pesticide for tow methods pre and post- emergence and this indicates the significant effect of the pesticide on the plant when separated the leaves, which indicates the occurrence of a genetic change between the treated plants, it was also found that there are varying degrees of difference between plants treated with Pendimethalin, From the matrix a significant difference is observed between plant treatments, especially in the high concentration of the pesticide.

This is what was found by researchers Al-zahrani *et al*., 2012 By studying the effect of the cytogenotoxicity of creatine on monohydrate concentrations on *Vicia faba* plants The highest concentration of creatine showed a polymorphic number of genetic bands by using RAPD-PCR product comparing with control. Results strongly suggested that creatine monohydrate is clastogenic. This is what Cenkci *et al.,* (2009) found when studying the toxic effect of chemicals in the soil on the genetic material of bean plants. That was also noticed Al-Nuaimi, 2019 revealed genetic changes on the DNA of the treated wheat plants (*Triticum aestivum* L.) with heavy metals by using four Random Amplifications of Polymorphic DNA (RAPD) which was represented in the loss or development of new bundles compared to the controlling treatment, indicating a change in the sequence of the nitrogen bases, This change at the molecular level has led to changes on the phenotype of plants.

Conclusions

We conclude from our current study that a pesticide (PM) has a mutagenic effect on pea plant, where the effect of (PM) started from the low concentrations in both methods pre and post-emergence. Its effect was more pronounced in the high concentrations, where significant differences were recorded in the phenotypic and molecular studies.

Acknowledgements

The research was supported by the Environmental Researches center, University of Mosul.

Conflict of Interest: No conflict of interest was declared by the authors

References

Aboulila, A., E.B. Belal, M.M. Metwaly and H.R. El-Ramady (2016). Degenotoxicity of pendimethalin Contaminated Clay Soil By *Pseudomonas resinovarans* Using Anatomical Cytogenetic and Biochemical Analysis in *Vicia faba* plants. *International Journal of Current Research in Biosciences and plant Biology,* **3(2):** 38-53.

Ansari, S., S.M. Quaiser, M.A. Sabry and M.A. Eslam (2018). Pendimethalin induces oxidative stress DNA damage and mitochondrial dysfunction to tigger apoptosis in human lymphocytes and rat bone-narrow cells. *J. of. Histochemistry and cell Biology,* **149(2):** 127-141.

Ahmid, M.I., F.Z. Mohd, J. Mehjbeen and M. Ahmad (2018). Pendimethalin-induced oxidative stress, DNA damage and activation of anti-inflammatory and apoptotic markers in male rats.https://doi.org/10.1038/s41598-018-35484-3.

Al-zahrani, N. H., K.H. Alamoudi and S.M. Al-shamrani (2012). Cytogenetic and molecular variation on *Vicia faba* treated with creatine monohydrate. *J. Life Science,* **9(3):** pp. 584-590.

Al-Nuaimi, D.A. Fatthi (2019). The effect of the Heavy Metals pollution In Wheat Plant (*Triticum aestivum* L.) on some physiological and anatomical characteristics and molecular Level. Thesis Ph.D. College of Environmental University of Mosul, Iraq.

Celik, M., D. Yusbasioglu, F. Unal, O. Arslan and S. Kashyap (2005). Effects of Dinocap on the mitosis of *Allium cepa* L., *Cytol,* **70(1):** pp. 13-22.

Cenkci, S., M. Yýldýz, Y.H. Ciðerci, M. Konuk and A. Bozdað (2009). Toxic chemicals-induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris* L.) seedlings. *J. Chemosphere,* **76:** pp. 900-906.

Cerovic, Z.G., G. Masdoumier, N.B. Ghozlen and G. Latouche (2012). A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. *Physiol. Plant.,* **146:** 251-260.

Demir, N., A. Sevtap and U.B. ÜlKÜ (2017). Assessment of genotoxic effects of pendimethalin in Chinese hamster over cells by single cell gel electrophoresis (comet) assay. *turk. J. Pharm. Sci.,* **14(2):** 185-190.

El-Awadi, M.E. and Esmat A. Hassan (2011). Improving growth and productivity of fennel plant exposed to pendimethalin herbicide: stress-recovery treatments. *Nature and science,* **9(2):** 97-108.

Haiba, A. Atef, H. Nagwa, R. Abd El-Hamid, A. AElhamAbd El-Hady and M.F. Al-AnsaryAbd El-Rahman (2011). Cytogenetic effect of Insecticide Telliton and Fungicide Dithane M-45 on Meiotic Cells and Seed Storage Proteins of *Viciafaba*. *J. Ameri. Sci.,* **7(1):** pp.19-25.

Hammok, N.S. (2019). Toxic Effect Of Pendimethalin and UV-C Radiation On Germination and Corn Growth *Zea mays* L. Seedling. Proceeding of 6th International Conference of Biotechnology, *Environment and Engineering Sciences (ICBEI),* Alexandria-Egypt, 28-29 December, 130-139.

Hamid, S.H., D.A. Mohammed and A.A. Obaid (2013). Effect of Irrigation water salinity, magnetic and soaking by the ascorbic acid and seaweed (olIgo-x) on Germination and seedling grouth of hybreds cucumber (Dalia) in protected environment. *Diyaia Journal of Agricultural sciences,* **5(2):** 222-213.

Karaye, I.U., A.A. Aliero and I.S. Adili (2014). Effects of butachlor and pendimethalin herbicides on seed germination and early seedling growth of species of cowpea. *Annals of Biological Sciences*, **2(4):** 11-15.

Nag, S., A.K. Jain and M.S. Dhanya (2013). Mutagenic effect in vegetables by pesticides. In: Environmental Sustainability: Concepts, Principles, Evidences and Innovations. pp.338-343.

Maity, S.K. (2014). Effects of Dithane m-45 (a fungicide) on root meristem of *Vigna mungo* L. hepper. *International J. of adv. Res. in engineering and applied sciences,* **3(4):** pp. 1-6.

Singh, N. and A. Srivastva (2014). Biomonitoring of Genotoxic Effect of Glyphosate and pendimethalin in *Vigna mungo* populations. *Cytologia*, **79(2):** 173-180.

Verma, S. (2018). Morphotoxicity and cxtogenotoxicity of pendimethalin in the test plant *Allium cepa* L. -A biomarker based study. *Journal Chemosphere,* **206:** September, p: 248-254.

Wasfi, M.A. and Samia M. Ali (2016). Effect of the herbicide pendimethalin on the chlorophyll content in *Zea mays* L. and *Gosspium hirsutum* L. seedling. *J. plant production,* **7(7):** 759-761.

Wagner, G. and E. Nadasy (2006). Effect of pre- emergence herbicides on growth parameters of green pea. *Common Agric. Appl. Biol. Sci.,* **71(3PtA):** 809-813

Welham, S.J., S.A. Gezan, S.J. Clark and A. Mead (2015). Statistical methods in biology Design and Analysis of Experiments and Regression. Taylor and Francis Group, USA.