

Research Article

Genotoxicity and genomic instability in oral epithelial cells of agricultural workers exposed to pesticides using micronucleus and comet assay in Nineveh, Iraq

Alaa Y. M. Alhamadany*

Department of Desertification Control Techniques, Technical Agricultural College, Northern Technical University, Mosul, Iraq

Sarab D. Khalaf

Department of biology Sciences, Collage of Sciences, Tikrit University, Tikrit, Iraq

Yahya N. M. Alkateb

Department of Animal Production, Technical Agricultural College, Northern Technical University, Mosul, Iraq

*Corresponding author. E-mail: AlaaAlhamadany1985@gmail.com

Article Info

<https://doi.org/10.31018/jans.v15i2.4329>

Received: December 28, 2022

Revised: April, 8, 2023

Accepted: April 17, 2023

How to Cite

Alhamadany, A. Y. M. *et al.* (2023). Genotoxicity and genomic instability in oral epithelial cells of agricultural workers exposed to pesticides using micronucleus and comet assay in Nineveh, Iraq. *Journal of Applied and Natural Science*, 15(2), 473 - 479. <https://doi.org/10.31018/jans.v15i2.4329>

Abstract

In agriculture, pesticides are used to preserve plants, but they might be dangerous for farmers and the environment. The present study aimed to use the comet assay and the micronucleus (MN) test to assess the genotoxic effects on lymphocytes and buccal exfoliation in pesticide-exposed male agricultural workers. The samples were collected from 102 workers having exposure to pesticides (Roundup SL, Weed waster, and paraquat 20% SL) and 100 control individuals (without pesticide exposure) from different Mosul, Iraq, neighbourhoods. With the help of the comet assay and the MN test, exfoliated buccal cells from the individuals were analyzed for DNA damage. Each individual's lymphocytes and epithelial baseline cells had their comet tail length assessed, along with any other nuclear abnormalities such as nuclear buds, karyolysis, karyorrhexis, and binucleate cells. The results showed that the frequency of MN considerably rose in the exposed group, and that group also revealed nuclear anomalies linked to cytotoxic or genotoxic effects. There were significant disparities in the amount of DNA damage between recently exposed employees and controls and recently exposed and followed-up cases. In comparison to controls, there was a considerable increase in the and frequency of cells that migrated in exposed workers. However, it was shown that confounding factors, such as age and the varying length of pesticide exposure, substantially impacted DNA damage. Educational programs for agricultural workers are critical to limit the use of chemicals in agriculture, given the evidence of a genetic risk associated with exposure brought on by the extensive use of pesticides.

Keyword: Agricultural workers, Comet assay, DNA damage, Genotoxicity, Micronucleus

INTRODUCTION

Pesticides are routinely employed worldwide to increase agricultural output and prevent illnesses spread by insects, and in recent years, their usage has drastically expanded. Unfortunately, these substances are emitted in large concentrations into the environment, and many have an adverse effect on creatures other than their intended targets, posing a risk to human health (Rajmohan *et al.*, 2020). Pesticides are used to ward against, eliminate, or control pests, such as plants and animals. Such chemical substances may be categorized into three main groups: fungicides, which are

applied to eliminate fungus and their spores in order to prevent them from harming plants, herbicides, which are used to eliminate undesired vegetation (Tudi, 2021). Pesticides are widely used around the world, and recently, their usage has risen further. Pests that harm crops are produced more frequently due to widespread pesticide usage in agriculture, but pests that are resistant to pesticides have also arisen. Increasing agricultural productivity necessitates using more insecticides (Mostafalou and Abdollahi, 2013). Numerous potentially hazardous compounds have been discharged into the environment in large quantities due to the increasing use of agricultural pesticides in the fields.

of food production and public health. Most of these substances are unspecific, so they could potentially target the human body (Rajmohan *et al.*, 2020). People are always exposed to pesticides, including when food is contaminated in the manufacturing process, at the home, at work, in hospitals, and in schools (Aprea *et al.*, 2012). Pesticide exposure can lead to both acute and persistent toxic effects. Headache, nausea, and/or other more severe symptoms, including even death, are examples of the acute effects that are present immediately. Chronic health impacts develop when people are exposed to foreign chemicals repeatedly or continuously. The consequences of acute exposure are well characterized in scientific literature. However, more research is still needed to fully understand the impacts of long-term exposure, particularly how they are initiated (Pathak *et al.*, 2022).

Health risk analyses can measure the level of harm related to chemical exposure. A single ingredient or intricate combinations can be taken into consideration when evaluating chemical exposure (OECD, 2018). Combinations of toxins may have an impact on and perhaps increase the toxicity of certain components through synergistic, potentiating, antagonistic, inhibiting, or additive activities. Understanding of the underlying intoxication processes should be improved by the study of chronic exposure to pesticide combinations (Vaou *et al.*, 2022). In fact, there are a significant number of findings in the scientific literature on the effects of health consequences of persistent pesticide exposure (Mostafalou and Abdollahi, 2013). Therefore, people working in the agricultural or freight industries or regularly exposed to low quantities of pesticides (such as agrochemicals) may offer an excellent chance to examine the harmful consequences of chronic pesticide exposure to human health (Damalas and Koutroubas, 2016). The genetic risk resulting from cumulative exposure to intricate pesticide mixtures may thus be determined via geno toxicological assessment in human populations (Moshou *et al.*, 2020). Several cytogenetic tests have been created to assess the potential genotoxicity of pesticide exposures in people exposed to them at work (Sánchez-Alarcón *et al.*, 2021). However, data on both harmful and beneficial genotoxic effects in populations exposed to pesticides exist (Kaur and Kaur, 2018).

The ability of pesticide compounds to alkylate causes DNA breaks, affecting DNA's capacity for information storage and replication (Islas-Gonzalez *et al.*, 2005). Genomic instability may be established by DNA damage and cellular response through a variety of pathways, and this process can be used to assess risk. Pesticide exposure at work poses a significant genotoxic risk and measuring that risk in exposed persons can serve as a reasonably accurate biomarker of early biological abnormalities (Sánchez-Alarcón *et al.*, 2021).

The biomarkers comet test, sister chromatid exchanges, micronuclei production, and chromosomal abnormality are often employed to evaluate the genotoxic effects of pesticides. So, the present study aimed to assess the potential extent of DNA damage in agricultural workers in Mosul city, Iraq, who had exposure to different pesticides such as Roundup SL, Weed waster, and paraquat 20% SL at work for varying periods of time.

MATERIALS AND METHODS

Study design

The individuals involved in the study were workers who used pesticides to grow agriculture in Mosul, Iraq. The approval was taken from the people from whom the samples were taken. They were divided into two groups: 100 unexposed control individuals who were engaged in other activities and had no prior history of occupational exposure to pesticides; and 102 farmers who had exposure while working in the fields (Mosul 36.2296° N, 42.2362° E, Tel Afar 36° 22' 59.99" N 42° 26' 59.99" E, and Rabia 36.8063579, 42.092199, districts) of Nineveh Governorate and using pesticide (Roundup SL, Weed waster, and paraquat 20% SL) dispersions. To establish if pesticide applicators' lifestyle choices may impact the results of genotoxicity and aberrant nuclear comet assays. Both groups were matched for age, years of experience applying pesticides (Roundup SL, weed waster, and Paraquat 20% SL), and lifestyle habits, including smoking. All subjects gave their consent before the intervention, and the Northern Technical University Ethics Committee/Mosul city authorized this study.

Sampling

Between the dates of 15 September 2022 and 15 December 2022, buccal mucosal cells were collected using a wooden spatula, and fresh peripheral blood samples were acquired from 100 healthy controls, and 102 workers were exposed individuals. The range of their ages was 15 to 57 years.

Methods

Micronucleus test in oral epithelial cells

In the private clinics in Mosul city and Tikrit University, Collage of Sciences, tests on the patients were performed to make sure that the oral tissues were in good health under the prescribed lighting circumstances. According to Bortoluzzi *et al.* (2014), the exfoliated cells of oral mucosa scraping gather were made as follows: To remove dust and debris, distilled water was utilized for a gentle mouthwash. The inner cheeks on the right and left were then lightly scraped with a wooden spatula that had been soaked in water. Then, two glass slides were used to hold the samples, which were then

allowed to air dry at room temperature. After the slides had air dried, May Granwald-Giemsa stain was applied. The fixation phase was done with 100% methanol.

Scoring

Under a light microscope with a 40x objective magnification, the selection and counting of the MN form were compiled following the guidelines of Thomas *et al.* (2009). The MN was counted in 100 cells.

Evaluation of DNA damage in peripheral lymphocytes

Sample preparation

Freshly drawn blood was centrifuged to extract peripheral blood lymphocytes, which were then thrice washed in phosphate-buffered saline.

Cell viability test

The trypan blue exclusion technique was used to determine cell viability beforehand (Altman, Randers and Rao, 1993). The nucleus of dead cells is stained by trypan blue after it has penetrated the damaged membrane. 10 μ L of cell pellet and 10 μ L of trypan blue were combined and incubated for 3 min. Then, 100 consecutive cells were counted again to determine the number of dead cells.

Alkaline comet assay

The Trevigen protocol instructions (Trevigen, Inc., Gaithersburg, US) were followed to conduct this test. After making the Lysis Solution, it was cooled for at least 20 minutes at 4°C. Then, pipette 50 μ L onto CometSlide™ while combining cells with molten low melting point (LMA) agarose at a ratio of 1 x 10⁵/ml. The agarose/cells were distributed equally around the sample area using the side of the pipette tip. The slides were then kept flat and chilled for 10 minutes at 4°C with a dark field. The gelling time can be extended to 30 minutes in environments with high humidity levels to improve sample adherence. After 30 to 60 min in 4°C Lysis Solution, slides were removed from the solution and any remaining buffer was discarded from CometSlide™. For one hour, at a temperature of 4°C, newly made Alkaline Unwinding Solution with a pH greater than 13 was submerged in. After adding the alkaline electrophoresis solution, the slides were placed in the electrophoresis slide tray, with the slide labels towards the black cathode. The power source was then set to 21 volts, and voltage was administered for 30 minutes. The samples were gently submerged twice for five min., each in distilled water, twice in extra electrophoresis solution, and finally once for five minutes in 70% alcohol (Ethanol). The samples were dried at 37 °C for 10 to 15 min. and then evaluated. The CometSlide™ was stained with 1X Ethidium Bromide and rinsed with

cold distilled water to remove any leftover stain. Then, the slides are quickly graded. The extent of the DNA damage was assessed using a Fluorescence microscope with a 40X lens and a digital camera to read the slides. The analytical procedure was used on four comet pictures, with 25 cells on each plate represented by 50 randomly selected cells. The amount of migration between damaged cells, the quantity of migrated cells, and the amount of migration per cell were compared.

Statistical analysis

The data was analyzed using a T-test for independent samples. The initial multiple range test developed by Duncan was used to reduce the difference between means as means SD. All statistical analysis was performed using the statistics software SPSS 28.0. (UK's SPSS Ltd., Surrey).

RESULTS

Micronucleus test on oral epithelial cells

The outcomes of MN in oral epithelial cells for the groups of pesticide-exposed workers and healthy controls are summarized in Table 1. The findings showed that employees exposed to pesticides had higher MN and KR of 4.12±0.058 and 18.83±0.275, respectively, compared to the healthy control group with values of 0.32±0.097 and 3.11±0.899. Whereas, the results of BN, and KL showed that workers exposed to pesticides group were lower (0.392± 0.076, 1.95± 0.986) respectively than the individuals of the healthy controls group (0.49± 0.069, 3.93± 0.934) respectively (Table 1).

Table 2 shows the results of MN in oral epithelial cells in pesticide-exposed workers and healthy control age groups. The results showed that the mean of MN (5.58±0.044) was substantially higher in the group of exposed workers in the age range of 50 to 60 years, followed by those in the range of 40 to 50 years (4.89±0.065) and 30 to 40 years (3.73±0.062). While the younger age group (10–20 years) had a lower mean of MN (2.87±0.072).

As the individual cell DNA migration patterns produced by this experiment resemble cometary stars when viewed via a fluorescence microscope, they are therefore known as "comets" (Fig. 1). Each biological sample normally receives an evaluation of 50–100 comets. Table 3 shows the distribution in the levels of DNA damage frequencies evaluated through comet assay among study groups. It was found that level 2 of DNA damage was higher (31.84%) in workers exposed to pesticides, followed by level 3 of DNA damage (29.18%). While the lower level of DNA damage in workers exposed to pesticides was level 0 (13.72%). The distribution of DNA damage frequencies showed that level 0 of DNA damage was higher (48.96%) in

Table 1. Total MN in the oral epithelial cells of the study groups

Parameters		Total MN	BN	PN	KR	KL	DIF
Study groups							
Healthy controls	Mean±SD	0.32±0.097	0.490±0.069	0.091±0.121	3.11±0.899	3.93±0.934	93.46±2.961
	No.	102	102	102	102	102	102
Workers exposed	Mean±SD	4.12± 0.058 *	0.392±0.076	0.17±0.094	18.83± 0.275*	1.95± 0.986*	82.42± 1.863
	No.	100	100	100	100	100	100

* Mean difference is significant at 0.05 level (t-Test); Total MN: Total Micronucleus; BN: Binucleated; PN: Pyknotic nucleus; KR: Karyorrhexis; KL: Karyolytic cell; DIF: Normal differentiated cell

Table 2. Total MN in the study groups' oral epithelial cells in different age groups

Study groups	Healthy controls	Workers exposed		
Age groups (Years)	Mean± SD	No.	Mean± SD	No.
10-20	0.39±0.059	21	2.87±0.072	24
20-30	0.28±0.192	28	3.52± 0.032*	26
30-40	0.25±0.367	20	3.73± 0.062*	22
40-50	0.17±0.083	18	4.89± 0.065*	19
50-60	0.51±0.041	13	5.58± 0.044*	11
Total	0.32±0.097	100	4.12± 0.058*	102

* Mean difference is significant at 0.05 level (t-Test)

healthy control, followed by level 1 (29.18%). The lower level of DNA damage in healthy control was level 3 (7.68%).

The comet test findings for the total number of peripheral cells with DNA damage among pesticide-exposed workers and healthy controls, broken down by age groups are shown in Table 4. The results showed that the mean TCWD was substantially higher in the group of pesticide-exposed workers between the ages of 50 and 60 (11.00±0.800), followed by those between the ages of 40 and 50 (8.50±0.578, 7.85±0.449), respectively. The TCWD mean (7.09±0.413) was lower for the age group of 20 to 30 year (Table 4).

Table 5 shows the results of the comet assay for the total DNA damage in peripheral lymphocytes in pesticide-exposed workers and healthy controls of different age groups. The results showed that those who had been exposed to pesticides were significantly higher susceptible to have had mean total DNA damage in the age group of 50-60 (19.00 ±1.522), followed by those between the age of 40-50 years (14.63 ±1.099) and 30-40 years (14.15 ±0.855), respectively. The mean total DNA damage was lower for the age group of 10-20 years, at 11.67 ±0.894.

DISCUSSION

According to a thorough clinical assessment, several pesticide-exposed workers showed signs of severe intoxication, nausea, headaches, skin irritations, and irritations of the nasal mucosa on occasion. The comet assay in exfoliated buccal cells was used to examine the effects of a different pesticide (Roundup SL, Weed waster, and paraquat 20% SL) on agricultural workers, and the results show that DNA damage increased in the workers in the study regions among study compared to the non-exposed workers. The study observed that DNA damage increased significantly at ≤ 0.05 after being exposed to a pesticide combination at work.

The buccal mucosa epithelial cells' micronucleus test concluded that pesticide exposure significantly enhanced genetic damage. This indicates that the tissue had mitotic spindle alterations, chromosome breakage, or with various nuclear anomalies such nuclear buds, pycnosis, karyolysis, and karyorrhexis. The MN has been utilized as a marker for genotoxicity, although other nuclear degenerative alterations have also been proposed. These alterations consisted of pycnosis, chromatin condensation, and karyorrhexis associated to

Table 3. Distribution of DNA damage frequencies observed in comet assay of study groups according to 4 levels

Study groups	Level of damage			
	0	1	2	3
Healthy control	48.96%	29.19%	13.77%	7.68%
Workers exposed	13.72%	25.26%	31.84%	29.18%

0 indicating: DNA without damage; 1, 2, and 3 indicating damage levels

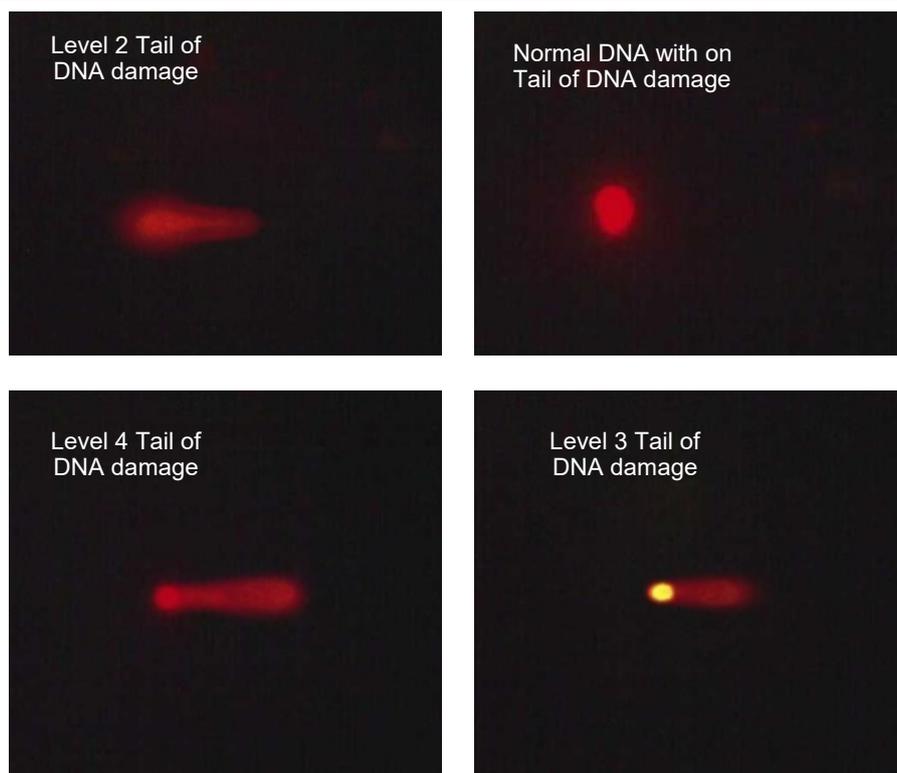


Fig. 1. Showing tails of DNA damage through comet assay

cytotoxicity (keratinization and necrosis); karyolysis, which is connected to cell toxicity, was also employed. To reduce the potential harm to health associated with exposure to these compounds, significant modifications in the appropriate use of pesticides are needed in Nineveh as well as in other agricultural areas of the city such as Al-Hamdaniya District.

Baseline genetic damage is affected by a variety of intrinsic and extrinsic circumstances, but it is not yet known how a person's innate genetic makeup may affect how much harm they produce. Due to this, the level of DNA damage in 102 occupationally exposed Nineveh farmers and 100 matched control subjects were assessed. According to the results, the levels of DNA damage in exposed employees were significantly ≤ 0.05 higher than in controls (Table 3). The previous *in vivo* and *in vitro* research is compatible with the current findings and appears to be more prevalent in the researchers (Bhalli *et al.*, 2006; Liu *et al.*, 2006). This may result from these workers using pesticides carelessly and without purpose. Additionally, most of the time, these workers did not take any preventive measures and instead applied a combination of two or more pesticides, such as Roundup SL and paraquat 20% SL) from several chemical families, likely having some sort of antagonistic or synergistic impact.

Some researchers have investigated DNA damage in farmers who had frequent contact with pesticides, while others have looked at DNA damage in pesticide production employees who had constant contact with pes-

ticides (Rajmohan *et al.*, 2020). Many of the toxicological effects of pesticides are known to be mediated by enhanced redox signaling. The symptoms of oxidative stress brought on by exposure to various pesticides include reactive oxygen species (ROS), lipid peroxidation, and DNA damage. The mechanisms leading to changes in cellular redox homeostasis in response to certain pesticides are still incompletely understood. The enzymatic conversion of pesticides into secondary reactive products (including ROS), the depletion of cellular antioxidant defences, and/or the degradation of antioxidant enzyme capabilities are some of the processes by which pesticides might change cellular redox equilibrium (Sule *et al.*, 2022).

Another factor that could affect the degree of DNA damage is age and gender. According to research by Fenech and Bonassi (2011), DNA damage rises with age and is likely caused by various causes, including poor diet, exposure to genotoxins at work or in the environment, and a wide range of other bad lifestyle choices. Chromosome aberrations (CA) (Jonnalagadda *et al.*, 2012), sister chromatid exchange (SCE), micronuclei (MN) (Benedetti *et al.*, 2013), and Comet cells (Benedetti *et al.*, 2013) are some of the effects of pesticide exposure that have been reported. In general, considerably higher amounts of these biomarkers were discovered, pointing to the pesticides' severe genotoxic effects. Significant cytogenetic damage has been shown in several investigations on agricultural labourers in cotton fields, vineyards, and other agricultural

Table 4. Total peripheral lymphocytes with DNA damage in study groups according to age groups

Study groups	Healthy controls		Workers exposed	
	Statistical parameters			
Age groups (Years)	Mean \pm SD	No.	Mean \pm SD	No.
10-20	1.18 \pm 0.751	21	7.19 \pm 0.452*	24
20-30	1.33 \pm 0.485	28	7.09 \pm 0.413*	26
30-40	1.80 \pm 0.447	20	7.85 \pm 0.449*	22
40-50	1.50 \pm 0.707	18	8.50 \pm 0.578*	19
50-60	2.33 \pm 0.577	13	11.00 \pm 0.800*	11
Total	1.42 \pm 0.636	100	8.11 \pm 0.362*	102

* Mean difference is significant at 0.05 level (t-Test)

Table 5. Total DNA damage of peripheral lymphocytes in study groups according to age groups

Study groups	Healthy controls		Workers exposed	
	Statistical parameters			
Age groups (Years)	Mean \pm SD	No.	Mean \pm SD	No.
10-20	1.55 \pm 1.128	21	11.67 \pm *0.894	24
20-30	1.72 \pm 1.018	28	12.45 \pm 0.786*	26
30-40	2.40 \pm 1.140	20	14.15 \pm 0.855*	22
40-50	2.00 \pm 1.414	18	14.63 \pm 1.099*	19
50-60	4.00 \pm 1.732	13	19.00 \pm 1.522*	11
Total	1.93 \pm 1.248	100	14.26 \pm 0.688*	102

* Mean difference is significant at 0.05 level (t-Test)

fields, among others (Damalas and Koutroubas, 2016). Studies using biomarkers of exposure are frequently performed to measure occupational exposure, that is, to link exposure to chemical reagents with health consequences. Several biomarkers relating to exposure, effect, or sensitivity to xenobiotics are utilised to quantify the degree to which a particular biological system interacts with a certain genotoxin (Aprea, 2012). In a few studies, exposed workers' DNA damage did not significantly increase compared to controls. This may be because of the different work settings, such as various levels of protective equipment and varying exposure times (Leite *et al.*, 2019).

Conclusion

Pesticides did damage DNA, regardless of the period of exposure. The effects of pesticide exposure vary significantly within and between groups of workers, which suggests that genetic, environmental, and lifestyle variables may have an impact. The present findings and the investigated biomarkers are useful in determining the level of pesticide exposure in the workplace and the environment and in assessing the risk of long-term harmful health impacts.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

- Altman, S. A., Randers, L. & Rao, G. (1993). Comparison of trypan blue dye exclusion and fluorometric assays for mammalian cell viability determinations. *Biotechnology Progress*, 9(6), 671-674. doi: <https://doi.org/10.1021/bp00024a017>
- Aprea, C. M. (2012). Mini review: Environmental and biological monitoring in the estimation of absorbed doses of pesticides. *Toxicology Letters*. 210, 110-118. <https://doi.org/10.1016/j.toxlet.2011.08.008>
- Benedetti, D., Nunes, E., Sarmento, MS., Porto, C., Santos, CEI., Dias, J F. & Da Silva, J. (2013). Genetic damage in soybean workers exposed to pesticides: Evaluation with the comet and buccal micronucleus cytome assays. Mutation research. *Genetic Toxicology and Environmental Mutagenesis*. 752, 28-33. <https://doi.org/10.1016/j.mrgen.tox.2013.01.001>
- Bhalli, JA., Khan, QM. & Nasim, A. (2006). DNA damage in Pakistani pesticide manufacturing workers assayed using the Comet assay. *Environ Mol Mutagen*. 47(Suppl 8):587-93. <https://doi.org/10.1002/em.20232>
- Bortoluzzi, M. C., Campagnoli, E. B., Milan, J. R., Reinheimer, A., Masson, M. & Capella, D. L. (2014). Frequency of micronucleus in oral epithelial cells after exposure to mate-tea in healthy humans. *Medicina Oral, Patologia Oral y Cirugia Bucal*, 19(4), e345. <https://doi.org/10.4317/medoral.19570>
- Damalas, C. A., & Koutroubas, S. D. (2016). Farmers' exposure to pesticides: toxicity types and ways of prevention. *Toxics*, 4(1), 1. <https://doi.org/10.3390/toxics4010001>
- Fenech, M. & Bonassi, S. (2011). The effect of age, gen-

- der, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes. *Mutagenesis*, 26(1), 43-49. <https://doi.org/10.1093/mutage/geq050>
8. Islas-González, K., González-Horta, C., Sánchez-Ramírez, B., Reyes-Aragón, E. & Levario-Carrillo, M. (2005). In vitro assessment of the genotoxicity of ethyl paraoxon in newborns and adults. *Human & experimental toxicology*, 24(6), 319-324. <https://doi.org/10.1191/0960327105ht534oa>
 9. Jonnalagadda, P. R., Jahan, P., Venkatasubramanian, S., Khan, I. A., Prasad, A. Y. E., Reddy, K. A., ... & Hasan, Q. (2012). Genotoxicity in agricultural farmers from Guntur district of South India—a case study. *Human & Experimental Toxicology*, 31(7), 741-747. doi:10.1177/0960327111408151
 10. Kaur, K. & Kaur, R. (2018). Occupational pesticide exposure, impaired DNA repair, and diseases. *Indian journal of occupational and environmental medicine*, 22(2), 74. https://doi.org/10.4103/ijoem.IJOEM_45_18
 11. Leite, S. B., de Diana, D. M. F., Abreu, J. A. S., Avalos, D. S., Denis, M. A., Ovelar, C. C., ... & Corvalan, R. (2019). DNA damage induced by exposure to pesticides in children of rural areas in Paraguay. *The Indian Journal of Medical Research*, 150(3), 290. https://doi.org/10.4103/ijmr.IJMR_1497_17
 12. Liu, Y. J., Huang, P. L., Chang, Y. F., Chen, Y. H., Chiou, Y. H., Xu, Z. L. & Wong, R. H. (2006). GSTP1 genetic polymorphism is associated with a higher risk of DNA damage in pesticide-exposed fruit growers. *Cancer Epidemiology Biomarkers & Prevention*, 15(4), 659-666. <https://doi.org/10.1158/1055-9965.EPI-05-0617>
 13. Moshou, H., Karakitsou, A., Yfanti, F., Hela, D., Vlastos, D., Paschalidou, A. K., ... & Petrou, I. (2020). Assessment of genetic effects and pesticide exposure of farmers in NW Greece. *Environmental Research*, 186, 109558. <https://doi.org/10.1016/j.envres.2020.109558>
 14. Mostafalou, S. & Abdollahi, M. (2013). Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. *Toxicology and applied pharmacology*, 268(2), 157-177. <https://doi.org/10.1016/j.taap.2013.01.025>
 15. Organisation for Economic Co-operation and Development. (2018). *Considerations for assessing the risks of combined exposure to multiple chemicals*. OECD Publishing. <https://doi.org/10.1787/ceca15a9-en>.
 16. Pathak, V. M., Verma, V. K., Rawat, B. S., Kaur, B., Babu, N., Sharma, A., ... & Cunill, J. M. (2022). Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. *Frontiers in Microbiology*, 2833. <https://doi.org/10.3389/fmicb.2022.962619>
 17. Rajmohan, K. S., Chandrasekaran, R. & Varjani, S. (2020). A review on occurrence of pesticides in environment and current technologies for their remediation and management. *Indian journal of microbiology*, 60, 125-138. <https://doi.org/10.1007/s12088-019-00841-x>
 18. Sánchez-Alarcón, J., Millić, M., Kašuba, V., Tenorio-Arvide, M. G., Montiel-González, J. M. R., Bonassi, S. & Valencia-Quintana, R. (2021). A systematic review of studies on genotoxicity and related biomarkers in populations exposed to pesticides in Mexico. *Toxics*, 9(11), 272. <https://doi.org/10.3390/toxics9110272>
 19. Sule, R. O., Condon, L., & Gomes, A. V. (2022). A Common Feature of Pesticides: Oxidative Stress-The Role of Oxidative Stress in Pesticide-Induced Toxicity. *Oxidative medicine and cellular longevity*, 2022, 5563759. <https://doi.org/10.1155/2022/5563759>
 20. Thomas, P., Holland, N., Bolognesi, C., Kirsch-Volders, M., Bonassi, S., Zeiger, E., ... & Fenech, M. (2009). Buccal micronucleus cytome assay. *Nature protocols*, 4(6), 825-837. <https://doi.org/10.1038/nprot.2009.53>
 21. Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., ... & Phung, D. T. (2021). Agriculture development, pesticide application and its impact on the environment. *International journal of environmental research and public health*, 18(3), 1112. <https://doi.org/10.3390/ijerph18031112>
 22. Vaou, N., Stavropoulou, E., Voidarou, C., Tsakris, Z., Rozos, G., Tsigalou, C. & Bezirtzoglou, E. (2022). Interactions between medical plant-derived bioactive compounds: focus on antimicrobial combination effects. *Antibiotics*, 11(8), 1014. <https://doi.org/10.3390/antibiotics11081014>