

Investigation of the Genotoxic Effect of Acetamiprid in *Cyprinus carpio* Using the Micronucleus Analysis and the Comet Assay

Acetamiprid'in *Cyprinus carpio* da Genotoksik Etkisinin Mikronükleus Analizi ve Comet Testi ile Araştırılması

Türk Denizcilik ve Deniz Bilimleri Dergisi

Cilt: 8 Sayı: 2 (2022) 80-89

Funda TURAN¹ , **Ayşegül ERGENLER^{1,*}** 

¹ *Iskenderun Technical University, Faculty of Marine Science and Technology, Hatay, Turkey*

ABSTRACT

Pesticides are considered to be one of the biggest economic and ecological problems in the aquatic ecosystem. Monitoring for toxic effects and screening for different insecticides is vital and crucial for reducing adverse effects on aquatic organisms and public health. Therefore, in this study, we aimed to determine genotoxic effect of acetamipridine in a model fish species, *Cyprinus carpio*, using the micronucleus test and Comet assay. Common carp (average weight of 1.35 ±0.11g) were exposed to three different concentrations of acetamipridine (0.2, 0.4, and 0.8 g/L) based on previously detected aquatic environmental concentrations, constituting an acute test for a week. At the end of study, the Damage frequency (%), Arbitrary unit and Genetic damage index (%) were evaluated in gill and liver cells of carp by Comet assay. Also, micronucleus frequencies and erythrocyte abnormalities were determined in erythrocytes cells of carp by micronucleus test. Our results revealed significant increases in the frequencies of micronuclei and DNA strand breaks in *C. carpio*, following exposure to acetamipridine and thus demonstrated the genotoxic potential of this pesticide on fish. Our findings also indicated the suitability of the fish micronucleus test and comet assay in assessment of aquatic genotoxicity of insecticides.

Keywords: DNA damage, Acetamipridine, Micronucleus test, Comet assay, Pesticide, *Cyprinus carpio*

Article Info

Received: 17 December 2021

Revised: 24 March 2022

Accepted: 28 March 2022

* (corresponding author)

E-mail: aergenler@gmail.com

To cite this article: Turan, F. and Ergenler, A., (2022). Investigation of the Genotoxic Effect of Acetamiprid in *Cyprinus carpio* Using the Micronucleus Analysis and the Comet Assay, *Turkish Journal of Maritime and Marine Science* 8(2): 80-89. doi: 10.52998/trjmms.1037906

ÖZET

Pestisitler, sucul ekosistemlerdeki en büyük ekonomik ve ekolojik sorunlardan biri olarak kabul edilmektedir. Suda yaşayan organizmalar üzerinde farklı insektisitlerin verdiği toksik etki izlenerek zararlı etkilerin azaltılması halk sağlığı açısından önemlidir. Bu çalışmada Asetamiprid'nin model organizma olan *Cyprinus carpio*'da genotoksik etkilerini Mikronükleus testi ve Comet testi ile belirlenmiştir. Sazan balıkları (ortalama ağırlık $1,35 \pm 0,11$ g) ortamdaki konsantrasyona bağlı olarak üç farklı asetamipridin konsantrasyonuna (0,2, 0,4 ve 0,8 g/L) maruz bırakılmıştır. Uygulama bir hafta uygulanarak akut test değerlendirilmesi yapılmıştır. Çalışmanın sonunda, Sazanların solungaç ve karaciğer dokularına Comet testi uygulanarak Hasar sıklığı (%), Arbitrary unit ve Genetik hasar indeksi (%) değerlendirilmiştir. Ayrıca mikronükleus test tekniği ile sazan balıklarının kırmızı kan hücrelerinde mikronükleus frekansı hesaplanarak eritrosit anormallikleri saptanmıştır. Sonuç olarak; Asetamiprid maruz bırakılan *C. carpio*'da çekirdek anomaliliği ve DNA yapısında önemli farklılıklar gözlemlenmiştir. Elde edilen bulgular ayrıca; pestisitlerin sucul sistemdeki genotoksik etkilerinin değerlendirilmesinde comet testi ve mikronükleus test tekniğinin uygunluğunu da göstermiştir.

Anahtar sözcükler: DNA hasarı, Acetamiprid, Mikronükleus test, Comet test, Pestisit, *Cyprinus carpio*

1. INTRODUCTION

The extensive pesticide applications in agriculture and urban areas possesses the risk for aquatic environments, due to the contamination and persistency potencial of themselves or their metabolites (Turgut Meriç and Keskin, 2017). They can reach the food chain by seriously affecting non-target organisms and threatening biodiversity and ecological balance (Abd El Megid *et al.*, 2020). Consumption of fish, which constitutes an important part of the aquatic ecosystem, poses a risk to human health (Ghayyur *et al.*, 2021). Pesticides enter into aquatic ecosystems by agricultural run-off and may cause in physiological abnormalities, in aquatic organisms (Wanule and Siddique, 2010). Neonicotinoids are a relatively new class of pesticides, whose large scale application began around 1990 (Berheim *et al.*, 2019). These compounds have been indicated as organophosphate substitutes, as they display reduced effects on ecosystems, due to their specific mechanism of action (of inhibiting nerve impulse transmissions in insects due to their structural similarity to nicotine (Yamamoto *et al.*, 2012; Wang *et al.*, 2015). Today, they are used against a wide range of insects due to their high efficacy and versatility of use. The acetamiprid (ACE) insecticide class contains at

least seven major compounds with a market share of more than 25% of total global pesticide sales and replaces older worldwide groups such as organophosphate and carbamate insecticides. They are considered highly selective neurotoxins for insects and likely affect many more taxa, with far broader ecological effects than expected since the introduction of these third-generation insecticides (Vehovszky *et al.*, 2018).

Acetamiprid is a fairly new member of the neonicotinoid group of insecticides to control insects and mites that damage plants. Intense and unconscious use of acetamiprid, which has the property of accumulating in water, adversely affects animals and environmental health. Acetamiprid has cytotoxic and genotoxic properties in mammals and aquatic organisms. It has been reported that it causes sister chromatid exchanges in cultures, micronuclei formation in blood lymphocytes and chromosomal anomalies (Hladik *et al.*, 2018; Ma *et al.*, 2019). Due to its physical and chemical properties, Acetamiprid is highly soluble in water and other organic solvents, stable to hydrolysis and photolysis (Guedegba *et al.*, 2019). Considering the studies, it caused toxicity that led to behavioral changes in African catfish fry (Houndji *et al.*, 2020). Acetamiprid was found to be risky on change in metabolites of zebrafish (Zhang and Zhao, 2017). It also severely affects health. Antioxidant

biomarkers of aquatic invertebrates such as *Cirrhinus mrigala*, *Biomphalaria straminea* (Cossi *et al.*, 2020) and freshwater fish (Ghayyur *et al.*, 2021). Furthermore, subchronic exposure of Acetamiprid induced oxidative stress in worms through reactive oxygen species (ROS) accumulation and altered catalase (CAT) and glutathione S transferase (GST) activities, in addition to elevation of lipid peroxidation (LPO) and DNA damage. (Li *et al.*, 2018). Acetamiprid caused increased oxidative stress and neurotoxicity in mammals, rats (Dhouib *et al.*, 2017; Doltade *et al.*, 2019), and mice (Zhang *et al.*, 2011).

Amongst various aquatic organisms, fish is a valuable bio monitor of aquatic ecosystem. Fish are the top consumers and play an important role in aquatic food chain by maintaining a balance in aquatic ecosystem pollution. Fish is an ideal indexical organism for assessment and documentation of water pollution, due to their potential to be directly exposed to different xenobiotics. Xenobiotics or carcinogenicity when come in contact with fish, different reactions are initiated among chemical and biological systems in body, that ultimately result into biochemical disturbances. Hence, it is necessary to determine the contaminant action mechanism and potential means to mitigate their impacts. For this reason, fish may be used as bio indicators of aquatic pollution for the quality assessment of the aquatic system (Bonomo *et al.*, 2021). Fish is the best suitable to estimate potential risks due to their ability to metabolize and bio-accumulate contaminants in their bodies (Turan and Ergenler, 2019). Amongst various aquatic organisms, fish is a valuable bio monitor of water. Fish are the top consumers and play an important role in aquatic food chain by maintaining a balance in aquatic ecosystem pollution. Fish is an ideal indexical organism for assessment and documentation of water pollution, due to their potential to be directly exposed to different xenobiotics. Xenobiotics or carcinogenicity when come in contact with fish, different reactions are initiated among chemical and biological systems in body, that ultimately result into biochemical disturbances. Hence, it is necessary to determine the contaminant action mechanism and potential means to mitigate their

impacts. For this reason, fish may be used as bio indicators of aquatic pollution for the quality assessment of the aquatic system (Bonomo *et al.*, 2021). Common carp is also introduced as one of the most suitable fish models for toxicological studies (OECD, 1992). The dominance of common carp in the aquatic systems and having a better capacity for resistance against pollutants rather than other laboratory fish such as zebrafish and Japanese medaka are common reason for choosing this species for toxic test (Li *et al.*, 2018).

Advances in technology and frequent use of pesticides have led to pollution of the environment and aquatic ecosystems (Gibbons *et al.*, 2015). Pesticides are known to be the biggest problem for economically and ecologically important non-target aquatic species, including fish living in water bodies (Prusty and Patro, 2015; Rejczak and Tuzimski, 2015). Monitoring for toxic effects and screening for different insecticides is vital and crucial for reducing adverse effects on non-target organisms and public health. Therefore, in this study was aimed to determine genotoxic effect of acetamipridine in a model fish species, *Cyprinus carpio*, using the micronucleus analysis and Comet assay.

2. MATERIAL AND METHOD

2.1. Experimental Design

The experiment was carried out with 180 common carp (*C. carpio* L.) (with an average weight of 1.35 ± 0.11 g) at the Iskenderun Technical University, Faculty of Marine Sciences and Technology, Aquaculture Research and Development Center, Turkey. The carps were acclimated for 15 days in a well-aerated 30 L glass aquarium containing dechlorinated water, at room temperature (± 23 °C) with a constant photoperiod (12:12 light / dark cycle). The specimens were fed with commercial carp feed of 3% of their body weight and feeding was stopped 24 h prior to exposure of the insecticide. After acclimation the fishes were randomly divided into four groups (experimental and control groups with $n = 15$ fish per group). Three different concentrations of acetamipridine (0.2, 0.4, and 0.8 g/L) were selected based on previously detected aquatic environmental

concentrations, constituting an acute test for a week. Each treatment group consisted of triplicates of 45 fish. At the end of the experiment, fish were anaesthetized with 5 mg /L quinaldine sulphate (Sigma Chemical Company, Germany) (Yanar and Genç, 2004). The specimens were manipulated only once they were unresponsive to physical stimuli (approximately 1 – 2 min), for the removal of tissue (gill and liver) for Comet assay and blood sampling for micronucleus assay.

2.2. Micronucleus (MN) Assay

Blood sampling was performed via cardiac puncture using a heparinized syringe and whole blood was used for subsequent analysis. Blood samples were taken from 15 individuals and the micronucleus test was applied to the erythrocytes and the formation frequencies were calculated. Three blood smears from each individual were prepared immediately after sampling as described in Mitkovska *et al.* (2020). After the prepared preparations are dried in air, they are mixed in 95% ethanol for 20 minutes. They are stained with 5% Giemsa solution for 20 minutes. Micronucleus evaluation was made by counting 1000 cells from each preparation. Morphological nucleus irregularities by peripheral smear Carrasco *et al.* (1990); They were evaluated under four main groups: notched nucleus, kidney nucleus, budded nucleus, lobed nucleus and binucleus.

2.3. Comet Assay

Comet assay was done according to cellular dissociation technique improved from Cavalcante *et al.* (2008). Firstly, gill

cell suspension, and then the cell pellet was retained. Singh *et al.* (1988) was followed for performing the single-cell gel electrophoresis. The slides were neutralized with ice-cold 0.4 M Tris buffer (pH 7.5), stained with 80 ml ethidium bromide (20 mg mL⁻¹). The slides were then examined at X40 magnification using a fluorescence microscope Image2M Zeiss). Images of 100 cells from each sample (gill and liver cell) were visually scored as proposed by classifying the nucleoids, which were assigned to one of five classes (0–4; with 0 signifying no visible tail and 4 almost all DNA in the tail) according to intensity of the comet tail. For comparison of the data from the comet assay, the damage percentage (%DF), the arbitrary units values (AU) and genetic damage index (GDI) were calculated as defined by Pitarque *et al.* (1999) and Collins (2004).

2.4. Statistical Analysis

Before statistical treatment, all data were tested for normality (Shapiro–Wilk test) and homogeneity (Levene analyze test). One-way ANOVA was performed in order to assess significant difference among treatment groups. Duncan's multiple range (DMR) test was used to compare means. Differences were regarded as statistically significant at P < 0.05 (Norusis, 1993).

3. RESULTS

Means and standard deviations of micronuclei and means of different classes of nuclear abnormalities counted in *C. carpio* from control and three different concentrations of acetamipridine are given in Table 1 and Figure 1.

Table 1. Means (%) and standard deviations of micronuclei and means of different classes of erythrocyte abnormalities counted in *C. carpio* obtained from control and three different concentrations of Acetamipridine (n=15).

Group	Micronucleus	Kidney	Binucleus	Notched	Lobed	Budded
Control	3.267±0.252 ^a	5.167±0.153 ^a	5.200±0.100 ^a	7.933±0.666 ^a	5.233±0.208 ^a	4.167±0.153 ^a
0.2 g/L	5.300±0.082 ^b	6.067±0.368 ^b	8.233±0.205 ^b	8.067±0.090 ^a	11.067±0.450 ^b	19.467±0.094 ^b
0.4 g/L	7.500±0.500 ^c	8.200±0.557 ^c	11.933±0.987 ^c	12.333±0.152 ^b	14.500±0.500 ^c	20.866±0.152 ^c
0.8 g/L	18.467±0.351 ^d	12.233±0.208 ^d	14.300±0.264 ^d	13.367±0.153 ^c	17.433±0.208 ^d	22.767 ±0.153 ^d
P	***	***	***	***	***	***

The data are shown as arithmetic mean \pm standard deviation. *Values with different superscripts in each column indicate significant differences. Indicate significance level between micronucleus frequencies and erythrocyte

abnormalities in peripheral erythrocytes of carps obtained from control and three different concentrations of acetamipridine (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

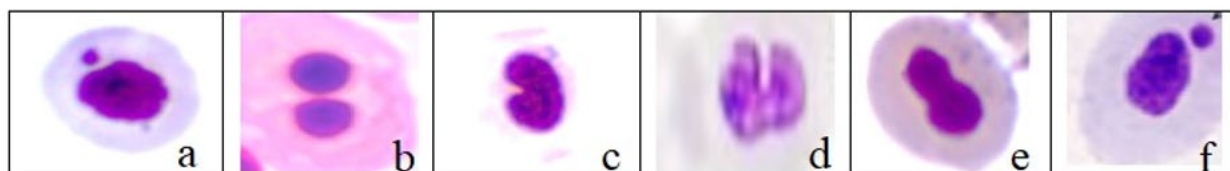


Figure 1. Nuclear anomalies in erythrocyte of *Cyprinus carpio* (a: Micronucleus, b: Binucleus, c: Kidney micronucleus, d: Notched micronucleus, e: Lobbed micronucleus, f: Budded micronucleus).

No fish mortality was observed at Acetamipridine treatment groups and the control during the experiment. In the erythrocytes of the carp, various nuclear abnormalities (micronucleus, binucleus, kidney nucleus, notched nucleus, lobbed nucleus and bud nucleus) were detected at treatment groups. As shown in the table 1, significant differences were observed ($P < 0.001$) in the frequency of micronucleus and other nuclear irregularities (kidney nucleus, binucleus, notched nucleus, lobbed nucleus and budded nucleus) compared with the control group and Acetamipridine treatment groups during a week (Table 1). As result of the study, it is determined that the highest micronucleus frequency and erythrocyte abnormalities is significantly observed in 0.8 g L^{-1}

group ($p < 0.001$). Besides, it is observed that the other nuclear abnormalities (kidney nucleus, binucleus, notched nucleus, lobbed nucleus and budded nucleus) in peripheral erythrocytes of carps at all treatment groups are significantly higher ($p < 0.001$) compared to the control group (Table 1). As can be seen in our results, Acetamipridine treatment significantly increased the frequencies of nuclear abnormalities ($P < 0.001$).

Means and standard deviations of the damage frequency (DF %), arbitrary units values (AU) and genetic damage index (GDI %) in the gill and liver cells of *C. carpio* obtained from the control and three different concentrations of Acetamipridine are summarized in Table 2 and Figure 2.

Table 2. Means and standard deviations of DNA damage in the gill and liver cells of carp obtained from the control and three different concentrations of Acetamidridine (n=15).

Groups (g L ⁻¹)	Damage Frequency (%)	Arbitrary Unit (AU)	Genetic Damage Index (DI) (%)
GILL			
Control	25.667±3.055 ^a	48.667±2.051 ^a	0.486±0.021 ^a
0.2	54.667±3.055 ^b	133.333±9.018 ^b	1.333±0.09 ^b
0.4	69.333±1.154 ^c	187.000±2.645 ^c	1.870±0.02 ^c
0.8	78.667±5.131 ^d	188.333±6.506 ^c	1.883±0.065 ^c
P	***	****	***
LIVER			
Control	36.333±2.309 ^a	36.333±2.309 ^a	0.363±0.023 ^a
0.2	38.666±4.509 ^a	73.333±6.658 ^b	0.733±0.066 ^b
0.4	58.000±0.001 ^b	108.666±5.507 ^c	1.086±0.055 ^c
0.8	68.000±1.732 ^c	184.666±7.371 ^d	1.846±0.073 ^d
P	***	****	***

The data are shown as arithmetic mean ± standard deviation. *Values with different superscripts in each column indicate significant differences. Indicate significance level between DNA damage in gill tissues of carps obtained from control and three different concentrations of acetamidridine (*, P<0.05; **, P<0.01; ***, P<0.001).

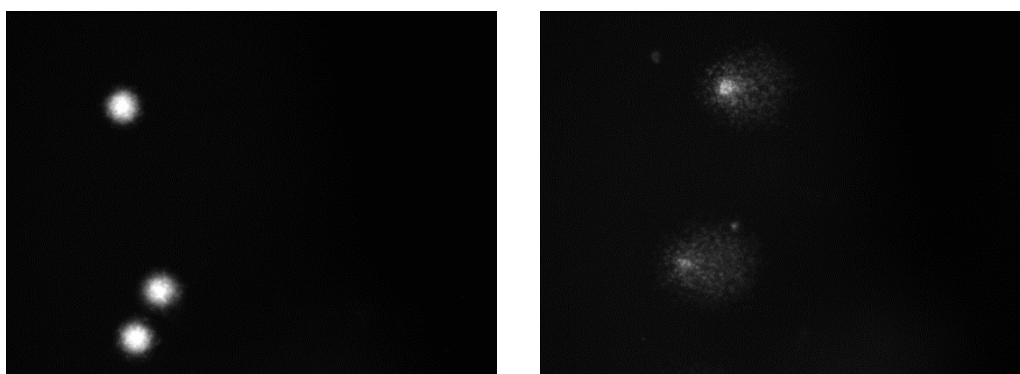


Figure 2. DNA damage in the tissues of *C. carpio* (undamaged (left picture) and damaged (right picture) cells)

As shown in the table 2, significant differences were observed (P<0.001) in the damage frequency and other parameters (AU and GDI) compared with the control and Acetamidridine treatment groups during the experiment. Acetamidridine treatment significantly increased the percentage of DNA damage in gill and liver cells of *C. carpio* (P<0.001). Similarly, Arbitrary Unit and Genetic Damage Index values are affected by Acetamidridine treatment (P<0.001). As a result of the study, it is determined that the highest damage frequencies (%) as 78.667±5.131 and 68.000±1.732 were significantly observed in 0.8 g L⁻¹ group at gill and liver cells respectively

(P<0.001). The lowest damage frequencies (%) as 25.667±3.055 and 36.333±2.309 were obtained in the liver and gill cells of control group in this study. Besides, it is observed that other damage parameters (Arbitrary unit and genetic damage index) in the gill and liver samples of 0.2 and 0.4 g L⁻¹ group were significantly higher (P<0.001) compared to the control group (Table 2, Figure 2). The lowest AU and GD were significantly obtained in control group in this research. In this study, the DNA damage increased due to the increase in the concentrations of acetamidridine.

4. DISCUSSION

Acetamiprid is a relatively new member of the neonicotinoid group of pesticides used to control insects and mites that damage plants. Intensive and unknowing use of acetamiprid, which has the property of accumulating in water, adversely affects the health and environment of animals (Ma *et al.*, 2019). Our findings revealed significant damage to the cells of the *C. carpio* following exposure to acetamipridine at different concentrations by the micronucleus test and comet assay. Our results also showed that blood, gill and liver cells of *C. carpio* can respond differently to DNA damage, reinforcing the importance of using different tissues as complementary tools for detecting genotoxicity in fish.

The acute toxicity of acetamipridine has been studied earlier in African catfish and the toxicity was found to be moderate to very high in terms of the 96-h LC50 value (Houndji *et al.*, 2020). Houndji *et al.* (2020) suggested that ecological risk assessment of acetamipridine (neonicotinoid) and lambda-cyhalothrin (pyrethroid), in aquatic environments should consider their contamination levels, and also recommended to pay special attention to behavioral changes related to their neurotoxicity for additional monitoring of the adverse effects of these insecticides. Yao *et al.* (2006) reported that the acetamipridine increases the SOD and CAT enzyme levels in three bacteria species for a short time. The presence of SOD and CAT enzyme activities is important to indicate the presence of superoxide radicals (Turan *et al.*, 2020). In physiological conditions, superoxide anions (O_2^-) are reduced by SOD to hydrogen peroxide (H_2O_2). CAT enzymes prevent the formation of hydroxyl radicals by converting hydrogen peroxide into H_2O and O_2 . However, when the production of ROS and RNR is too high, an imbalance occurs between the antioxidant system and free radicals, which is called oxidative stress. This leads to the formation of hydroxyl free radicals which can cause DNA strand breakage by increasing superoxide and hydrogen peroxide anions (Paravani *et al.*, 2019). ACE-induced cytotoxicity has been reported to

be caused by superoxide anions (Gökalp Muranlı *et al.*, 2015).

Some investigations have reported the genotoxic effect of acetamipridine. Sandayuk and Kılıçlı (2020) investigated genotoxic effect of acetamiprid in mouse bone marrow cells by CA (chromosomal aberration) and MN (micronucleus) test methods, reported that acetamiprid at 15 mg/kg dose was genotoxic-cytotoxic in mouse. Gokalp Muranlı *et al.* (2015) studied the genotoxic effects of single and combined uses of acetamiprid and propineb insecticides in human peripheral blood lymphocytes using micronucleus test technique. In their study, lymphocytes were exposed to acetamiprid (0.625, 1.25, 2.5 $\mu\text{g/mL}$), propineb (12.5, 25, 50 $\mu\text{g mL}$) and cetamiprid- propineb mixture (0.625 + 12.5, 1.25 + 25, 2.5 + 50 $\mu\text{g/mL}$) for 1 and 2 days). They found that exposure to a 48-hour acetamiprid- propineb mixture produced a significant increase in MN rates. Guedegba *et al.* (2019) reported that acetamipridine (neonicotinoid) and lambda-cyhalothrin (pyrethroid) demonstrated an antagonistic effect for lethal concentrations of 5% to 15% lethal at 96 h (96 h-LC 5-15 in on Nile tilapia The results suggest that ecological risk assessment of these molecule (acetamipridine (neonicotinoid) and lambda-cyhalothrin (pyrethroid) in aquatic environments should consider their contamination levels. Cavas (2011) reported that acetamipridine has cytotoxic and genotoxic potential on small intestine cells using MN, comet and γH2AX test methods on CaCo-2 cells. Similarly, Hathout *et al.* (2021) investigated the protective potential of ascorbic acid (Asc) against oxidative stress and genotoxicity induced by sub-lethal concentrations (10, 20 and 50 mg kg^{-1}) of acetamiprid (Aceta) in *Oreochromis niloticus*. The results determined that acetamiprid (10 and 20 ppm) concentrations induced oxidative stress by changing antioxidant enzyme activities and transcripts. They observed that exposure to acetamiprid had genotoxic effects in DNA-damaged cells and ascorbic acid combined exposure could be an effective treatment against acetamiprid-induced oxidative stress in Tilapia. At this point our results are in agreement with

those reported genotoxic potential of commercial formulations of acetamipride.

5. CONCLUSIONS

The current findings reveal that the acetamipride is a genotoxic insecticide inducing micronucleus frequency, erythrocyte abnormalities and DNA damage frequencies in *C. carpio*. Our findings also indicated the suitability of the fish micronucleus test and comet assay in assessment of aquatic genotoxicity of insecticides.

ACKNOWLEDGEMENTS

Thanks to The Scientific & Technological Research Council of Turkey (TUBITAK-2211/C National PhD Scholarship Program for Priority Areas) and The Council of Higher Education for 100/2000 PhD scholarship program for A. ERGENLER.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

ETHICS COMMITTEE PERMISSION

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

FUNDING

No funding was received from institutions or agencies for the execution of this research.

ORCID IDs

Funda TURAN:

 <https://orcid.org/0000-0002-0257-6009>

Ayşegül ERGENLER:

 <https://orcid.org/0000-0001-9186-3909>

6. REFERENCES

Abd El Megid, A., Abd Al Fatah, M.E., El Asely, A., El Senosi, Y., Moustafa, M.M., Dawood, M.A., (2020). Impact of pyrethroids and organochlorine pesticides residue on IGF-1 and CYP1A genes expression and muscle protein patterns of cultured Mugil capito. *Ecotoxicology and Environmental Safety* 188: 109876.

Berheim, E.H., Jenks, J.A., Lundgren, J.G., Michel, E.S., Grove, D., Jensen, W.F., (2019). Effects of neonicotinoid insecticides on physiology and reproductive characteristics of captive female and fawn white-tailed deer. *Scientific Reports* 9(1): 1-10.

Bonomo, M.M., de Castro Sachi, I.T., Paulino, M.G., Fernandes, J.B., Carlos, R.M., Fernandes, M.N., (2021). Multi-biomarkers approach to assess the impact of novel metal-insecticide based on flavonoid hesperidin on fish. *Environmental Pollution* 268: 115758.

Carrasco, K.R., Tilbury, K.L., Myers, M.S., (1990). Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. *Canadian Journal of Fisheries and Aquatic Sciences* 47(11): 2123-2136.

Cavalcante, D.G.S.M., Martinez, C.B.R., Sofia, S.H., (2008). Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 655(1-2): 41-46.

Cavas, T., (2011). In vivo genotoxicity evaluation of atrazine and atrazine-based herbicide on fish *Carassius auratus* using the micronucleus test and the comet assay. *Food and Chemical Toxicology* 49(6): 1431-1435.

Collins, A.R., (2004). The comet assay for DNA damage and repair. *Molecular Biotechnology* 26(3): 249-261.

Cossi, P.F., Herbert, L.T., Yusseppone, M.S., Pérez, A.F., Kristoff, G., (2020). Toxicity evaluation of the active ingredient acetamiprid and a commercial formulation (Assail® 70) on the non-target gastropod *Biomphalaria straminea* (Mollusca: Planorbidae). *Ecotoxicology and Environmental Safety* 192: 110248.

Dhouib, I.B., Annabi, A., Doghri, R., Rejeb, I., Dallagi, Y., Bdiri, Y., Gati, A., (2017). Neuroprotective effects of curcumin against acetamiprid-induced neurotoxicity and oxidative stress in the developing male rat cerebellum: biochemical, histological, and behavioral changes. *Environmental Science and Pollution Research* 24(35): 27515-27524.

- Doltade, S., Lonare, M., Raut, S., Telang, A., (2019).** Evaluation of acetamiprid mediated oxidative stress and pathological changes in male rats: ameliorative effect of curcumin. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 89(1): 191-199.
- Ghayyur, S., Khan, M.F., Tabassum, S., Ahmad, M.S., Sajid, M., Badshah, K., ... Qamer, S., (2021).** A comparative study on the effects of selected pesticides on hemato-biochemistry and tissue histology of freshwater fish *Cirrhinus mrigala* (Hamilton, 1822). *Saudi Journal of Biological Sciences* 28(1): 603-611.
- Gibbons, D., Morrissey, C., Mineau, P., (2015).** A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. *Environmental Science and Pollution Research* 22(1): 103-118.
- Gokalp-Muranli, F.D., Göç Rasgele, P., Kekecoglu, M., Kanev M., Ozdemir, K., (2015).** Potential genotoxicity of acetamiprid and propineb singly or in combination in cultured human peripheral blood lymphocytes by using mn assay. *Fresenius Environmental Bulletin* 24: 3947-3955.
- Guedegba, N.L., Imorou Toko, I., Agbohessi, P.T., Zoumenou, B.S., Douny, C., Mandiki, S.N., Kestemont, P., (2019).** Comparative acute toxicity of two phytosanitary molecules, lambda-cyhalothrin and acetamiprid, on Nile Tilapia (*Oreochromis niloticus*) juveniles. *Journal of Environmental Science and Health* 54(7): 580-589.
- Hathout, H.M., Sobhy, H.M., Abou-Ghanima, S., El-Garawani, I.M., (2021).** Ameliorative role of ascorbic acid on the oxidative stress and genotoxicity induced by acetamiprid in Nile tilapia (*Oreochromis niloticus*). *Environmental Science and Pollution Research* 1-13.
- Hladik, M.L. Main, A.R. Goulson, D., (2018).** Environmental risks and challenges associated with 418 neonicotinoid insecticides. *Environmental Science and Technology* (6): 3329-3335.
- Houndji, M.A., Imorou Toko, I., Guedegba, L., Yacouto, E., Agbohessi, P.T., Mandiki, S.N., ... Kestemont, P., (2020).** Joint toxicity of two phytosanitary molecules, lambda-cyhalothrin and acetamiprid, on African catfish (*Clarias gariepinus*) juveniles. *Journal of Environmental Science and Health* 55(7): 669-676.
- Li, B., Xia, X., Wang, J., Zhu, L., Wang, J., Wang, G., (2018).** Evaluation of acetamiprid-induced genotoxic and oxidative responses in *Eisenia fetida*. *Ecotoxicology and Environmental Safety* 161: 610-615.
- Ma, X., Li, H., Xiong, J., Mehler, W.T., You, J., (2019).** Developmental toxicity of a neonicotinoid insecticide acetamiprid to zebrafish embryos. *Journal of Agricultural And Food Chemistry* 67(9): 2429-2436.
- Norusis M.J., (1993).** Advanced Statistics, SPSS for Windows, Release 6.0, p. 578.
- Mitkovska, V.I., Dimitrov, H.A., Kunchev, A.I., Chassovnikarova, T.G., (2020).** Micronucleus Frequency in Rodents with Blood Parasites. *Acta Zoologica Bulgarica* 15: 33-41.
- OECD, (1992).** Organisation for Economic Co-operation and Development. OECD's guidelines for the testing of chemicals: 203 acute toxicity test for fish. p.12, Paris, OECD Publishing.
- Paravani, E.V., Simoniello, M.F., Poletta, G.L., Casco, V.H., (2019).** Cypermethrin induction of DNA damage and oxidative stress in zebrafish gill cells. *Ecotoxicology and Environmental Safety* 173: 1-7.
- Pitarque, M., Creus, A., Marcos, R., Hughes, J.A., Anderson, D., (1999).** Examination of various biomarkers measuring genotoxic endpoints from Barcelona airport personnel. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 440(1): 195-204.
- Prusty, J. K., Patro, S. K., (2015).** Properties of fresh and hardened concrete using agro-waste as partial replacement of coarse aggregate—A review. *Construction and Building Materials* 82: 101-113.
- Rejczak, T., Tuzimski, T., (2015).** A review of recent developments and trends in the QuEChERS sample preparation approach. *Open Chemistry* 13.
- Sandayuk, Ş., Kiliçle, P.A., (2020).** Investigation of the genotoxic effect of acetamiprid in mouse bone marrow cells by CA (chromosomal aberration) and MN (micronucleus) test methods. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi* 15(2): 130-137.
- Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., (1988).** A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research* 175(1): 184-191.
- Turan, F., Eken, M., Ozyilmaz, G., Karan, S., Uluca, H., (2020).** Heavy metal bioaccumulation, oxidative stress and genotoxicity in African catfish *Clarias gariepinus* from Orontes river. *Ecotoxicology* 29(9): 1522-1537.
- Turan, F., Ergenler, A., (2019).** Assessment of DNA damage by comet assay in *Trachinotus ovatus* cells from Mersin Bay in the Northeastern Mediterranean. *Nature and Engineering Sciences* 4(3): 25-31.

Turgut Meriç, İ., Keskin, E., (2017). Risk assessment of a formamidine pesticide, Amitraz, focusing on thyroid hormone receptors (TRs) in rainbow trout, *Oncorhynchus mykiss*. *Cellular and Molecular Biology* 63(9): 29-34.

Vehovszky, Á., Farkas, A., Csikós, V., Székács, A., Mörtl, M., Györi, J., (2018). Neonicotinoid insecticides are potential substrates of the multixenobiotic resistance (MXR) mechanism in the non-target invertebrate, *Dreissena* sp. *Aquatic Toxicology* 205: 148-155.

Wang, K., Pang, S., Mu, X., Qi, S., Li, D., Cui, F., Wang, C., (2015). Biological response of earthworm, *Eisenia fetida*, to insecticides. *Chemosphere* 132(1): 120-126.

Wanule, D., Siddique, M.S., (2010). Effect of acetamiprid on behavior of fish *Channa punctatus*. *BIOINFOLET - A Quarterly Journal of Life Sciences* 7(2): 188.

Yamamoto, A., Terao, T., Hisatomi, H., Kawasaki, H., Arakawa, R., (2012). Evaluation of river pollution of neonicotinoids in Osaka city (Japan) by LC/MS with dopant-assisted photoionisation. *Journal of Environmental Monitoring* 14(8): 2189-2194.

Yanar, M., Genç, E., (2004). Farklı sıcaklıklarda kinaldin sülfatı n diazepam ile birlikte kullanılmasının *Oreochromis niloticus* L. 1758 (Cichlidae) üzerindeki anestezik etkileri. *Turk Journal Veterinary Animal Science* 28: 1001-1005.

Yao X.H., Min, H., Lv, Z.M., (2006). Response of superoxide dismutase, catalase, and ATPase activity in bacteria exposed to acetamiprid. *Biomedical Environmental Science* 19: 309-314.

Zhang, H., Zhao, L., (2017). Influence of sublethal doses of acetamiprid and halosulfuron-methyl on metabolites of zebra fish (*Brachydanio rerio*). *Aquatic Toxicology* 191: 85-94.

Zhang, Z., Yuan, B., Bao, M., Lu, N., Kim, T., Liu, Y.J., (2011). The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nature immunology* 12(10): 959-965.