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RESEARCH ARTICLE

Investigation of DNA affinity levels of pesticides: docking analysis results

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ABSTRACT
Objective: According to studies conducted in recent years, pesticides can lead to the development of cancer, Parkinson's disease, Alzheimer's disease, reproductive system disorders, and birth defects. The aim of this study is to examine the DNA affinity levels of Alpha-cypermethrin, Malathion, Quinclorac, and Roundup (Glyphosate) plant protection products and to discuss them in the light of the literature. **Methods:** Docking results between ligand and receptor were detected using Hex 8.0.0 software. Preparation of the receptor and ligand for docking was done with UCSF Chimera 1.15 software. Docking visualizations were made with BIOVIA Discovery Studio and PyMol software. While the interaction images of pesticides with DNA were detected with BIOVIA Discovery Studio software, DNA binding images were detected with PyMol software. **Results:** In our study, the affinity levels of the plant protection products frequently used in the Çanakkale region were determined as Alpha Cypermethrin>Malathion>Quinclorac>Roundup /Glyphosate, respectively, according to the results of the docking analysis. The pesticide with the highest interaction with DNA was Alpha Cypermethrin (-248.24 KJ mol^{-1}) and the lowest binding energy was Roundup (-161.54 KJ mol^{-1}). **Conclusion:** In line with the literature, alpha-cypermethrin is the molecule with the highest toxicity and gene damage potential. Considering the variety of products, it is necessary to pay attention to the use of both single and multiple plant protection products. During the use of this molecule, plant protection products with lower DNA affinity and toxicity can be preferred as an alternative.

Keywords: molecular docking, alpha cypermethrin, malathion, quinclorac, roundup (glyphosate)

ÖZET
Pestisitlerin DNA affinite düzeylerinin incelenmesi: docking analiz sonuçlari
Amaç: Son yıllarda yapılan çalışmalara göre pestisitler kanser, Parkinson hastalığı, Alzheimer hastalığı, üreme sistemi bozuklukları ve doğum defektleri gelişimine yol açabilmektedir. Bu çalışmanın amacı Alfa-sipermetrin, Malathion, Quinclorac, Roundup (Glyphosate) bitki koruma ürünlerinin DNA afinite düzeylerini incelemek ve literatur eşliğinde tartışmaktır. **Yöntem:** Ligand ve reseptör arasındaki docking sonuçları Hex 8.0.0 yazılımı kullanılarak tespit edildi. Reseptör ve ligandın docking için hazırlanması UCSF Chimera 1.15 yazılımı ile yapıldı. Docking görselleştirmeleri BIOVIA Discovery Studio ve PyMol yazılımları ile yapıldı. Pestisitlerin DNA ile olan etkileşim görüntüleri BIOVIA Discovery Studio yazılımı ile tespit edilirken, DNA'ya bağlanma görüntüleri PyMol yazılımı ile tespit edildi. **Bulgular:** Çalışmamızda Çanakkale bölgesinde sık kullanılan bitki koruma ürünlerinin docking analiz sonuçlarına göre DNA molekülüne olan affinite düzeyleri sırasıyla Alpha Cypermethrin>Malathion>Quinclorac>Roundup/Glyphosate şeklinde tespit edildi. DNA ile en yüksek etkileşim içinde olan pestisit Alpha Cypermethrin (-248.24 KJ mol^{-1}) ve en düşük bağlanma enerjili ise Roundup (-161.54 KJ mol^{-1}) olarak tespit edildi. **Sonuç:** Alfa-sipermetrin literatürle de uyumlu olarak toksisistesi ve gen hasarı oluşturma potansiyeli en yüksek moleküldür. Ürün çeşitliliği göz önüne alındığında hem tekli hem de çoklu bitki koruma ürünleri kullanımına dikkat edilmesi gerekmektedir. Bu molekül kullanımı sırasında alternatif olarak yerine geçebilecek daha düşük DNA affinitesi ve toksisitesi olan bitki koruma ürünleri tercih edilebilir.

Anahtar kelimeler: moleküler docking, alfa-sipermetrin, malathion, quinclorac, roundup (glyphosate)

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INTRODUCTION

One of the principles of increasing crop production is to reduce the loss caused by harmful organisms, and the other is to develop varieties and lines with higher yields. Wheat, corn, paddy, cotton, etc., have been widely produced in breeding studies since the beginning of the 2000s. New findings that will cause a significant increase in yield in products cannot be obtained. In other words, the possibility of increasing the total agricultural production through breeding has decreased a lot, and the genetic capacity of the species has almost reached the limit. In addition, some negative effects of the agricultural production model made in the last two centuries on natural resources, especially soil, water, and biological diversity, have become visible [1].

Another important issue in pesticide use is the identification and management of health risks that may be reflected in the consumer. In this regard, "Codex Alimentarius" studies, known as International Food Standards, are carried out by the United Nations Food and Agriculture Organization (FAO) and the United Nations World Health Organization (WHO). With these studies, it is determined as a result of very detailed scientific research that pesticides used in agriculture will not pose a risk to human health in which product and in which ratio (Maximum Residue Limit -MRL). In this decision process, the amount of residue remaining in the plant because of the application, the amount of consumption of the product in question depending on the cultural conditions, and the amount of the same effective substance taken into the body with other foodstuffs, interaction, etc. Many aspects are considered. As a result of the studies, MRLs (Maximum Residue Limits) that are not harmful to human health have been determined for 4844 active substance/food product combinations for chemicals used in agriculture so far [2].

Plant protection products, which are widely used in agriculture, cause the formation of reactive oxygen species such as hydrogen peroxide (H2O2), superoxide (O2-•), and hydroxyl (•OH) radicals. These radicals can react with biological macromolecules, causing enzyme inactivation and DNA damage. Pesticides cause the peroxidation of polyunsaturated fatty acids (PUFA) by accumulating in fatty tissues. If these oxidants cannot be removed by the antioxidant defense system, they cause oxidative stress [3]. As a result of oxidative stress, pathological conditions such as DNA damage and cancer formations are observed. In some studies, a significantly increased risk of lung cancer, bladder cancer, and leukemia was observed in workers exposed to pesticides. In addition, organophosphate pesticides cause many neuropathological formations, including learning and memory functions, in the central nervous system (CNS) [4-6].

This study, it is aimed to examine the interactions of Alpha Cypermethrin, Malathion, Quinclorac, and Roundup plant protection products, which are frequently used in Çanakkale according to the product variety, with the DNA molecule and to compare the levels of this interaction.

MATERIALS and METHODS

Plant protection products selected for docking analysis and their areas of use

Alpha-cypermethrin (α-cypermethrin)

There are 525 licensed insecticides in use in Turkey containing 100 G/L ALPHA-CYPERMETHRIN as an active substance. All these products are used in the fight against organisms that harm especially olive, apple, tomato, sugar beet, corn, hazelnut, vineyard, cotton, and cereal products. The average time between the last spraying and harvest is 14 days (https://bku.tarimorman.gov.tr/AktifMadde/Details/221).

Malathion

There are 303 licensed insecticides in use in Turkey containing 650 G/L MALATHION as an active substance. All of these products are used in the fight against organisms that harm especially soybean, vineyard, cotton, corn, cereals, ornamental plants, sesame, poppy, oil rose, tomato, olive, bean, cherry, and apricot products. The time between the last spraying and harvest may vary according to the products and is generally 7 days on average (https://bku.tarimorman.gov.tr/AktifMadde/Details/77)

Quinclorac

Twenty licensed herbicides are in use in Turkey containing 250 G/L QUINCLORAC as an active ingredient. All of these products are used in the fight against weeds that damage the rice plant (https://bku.tarimorman.gov.tr/AktifMadde/Details/1492).

Roundup (Glyphosate)

There are 525 licensed insecticides in use in Turkey containing 100 G/L ALPHA-CYPERMETHRIN as an active substance. All these products are used in the fight against organisms that harm especially olive, apple, tomato, sugar beet, corn, hazelnut, vineyard, cotton, and cereal products. The average time between the last spraying and harvest is 14 days (https://bku.tarimorman.gov.tr/AktifMadde/Details/221).

Docking analysis

Docking results between ligand and receptor were detected using Hex 8.0.0 software. Preparation of the receptor and ligand for docking was done with UCSF Chimera 1.15 software. Docking visualizations made with BIOVIA Discovery Studio and PyMol software. Docking parameters in Hex 8.0.0 software Correlation type: shape+electrostatics+DARS, FFT mode: 3D, Post-processing: None, Grid dimension: 0.6, Receptor range: 180, Ligand range: 180, Twist range: 360, Distance range: 40 is set to be. In the preparation of the receptor for docking, water molecules were deleted, and polar hydrogen atoms were added in UCSF Chimera 1.15 software. For ligand preparation, 100 times conformation control was performed for each ligand in UCSF Chimera 1.15 software. While the interaction images of pesticides with DNA were detected with

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Table 1. Molecular docking results of pesticides with DNA.

Receptor name	Ligand names	E-value
DNA (PDB ID: 1BNA)	Alpha Cypermethrin (PubChem CID: 93357)	-248.24 KJ mol^{-1}
	Malathion (PubChem CID: 4004)	-242.9 KJ mol^{-1}
	Quinclorac (PubChem CID: 91739)	-205.23 KJ mol^{-1}
	Roundup (Glyphosate) (PubChem CID: 3496)	-161.54 KJ mol^{-1}

BIOVIA Discovery Studio software, DNA binding images were detected with PyMol software.

RESULTS

In Table 1, the interaction energies of Alpha Cypermethrin, Malathion, Quinclorac, and Roundup pesticides with DNA are given in order from the highest binding energy to the lowest binding energy. According to the results in Table 1, Alpha Cypermethrin (-248.24 KJ mol^{-1}) has the highest interaction with DNA, while Roundup (-161.54 KJ mol^{-1}) has the lowest binding energy.

Figure 1 shows the interactions of Alpha Cypermethrin pesticide with DNA. Accordingly, Alpha Cypermethrin DNA is DG(A:10), DC(A:11), DG(A:12), DC(A:9), DC(B:15), DG(B:14), DA (B:17), DA(B:18), DG(B:16) interact with nucleotides.

Figure 2 shows the interactions of Malathion pesticide with DNA. Accordingly, Malathion DNA has DT(A:7), DA(A:6), DA(A:5), DG(A:4), DC(B:21), and DC(B:23), DG(B:22) interacts with nucleotides.

Figure 3 shows the interactions of Quinclorac pesticide with DNA. Accordingly, Quinclorac DNA's DA(A:5), DT(A:7), DA(A:6), DT(A:8), DC(B:21), DT(B:20), DT(B:19) interacts with nucleotides.

Figure 4 shows the interactions of Roundup (Glyphosate) pesticides with DNA. Accordingly, Roundup (Glyphosate) DNA has DG(A:10), DC(A:11), DG(A:12), DG(B:14), and DG(B:16), and DC(B:15) DNA, interacts with DA(B:17), and DA(B:18) nucleotides.

DISCUSSION

Although studies examining the relationship between plant protection products and DNA damage are increasing rapidly, it is seen that docking analyzes are also carried out. In our study, the level of interaction of the most frequently used plant protection products (Alpha Cypermethrin, Malathion, Quinclorac, and Roundup/ Glyphosate) with DNA in terms of product diversity in our region was examined for the first time by docking

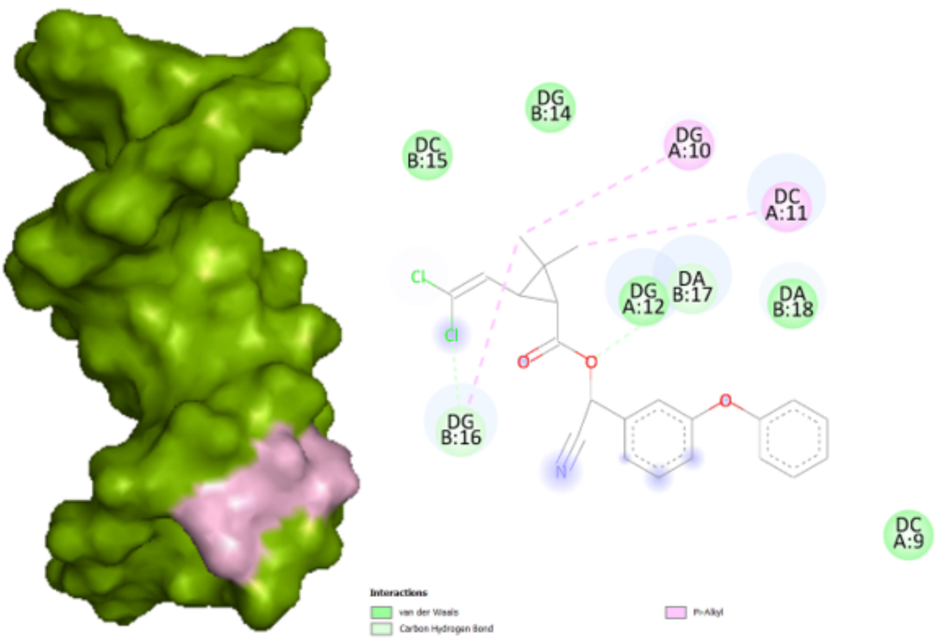

Figure 1. Interactions of Alpha Cypermethrin pesticide with DNA.

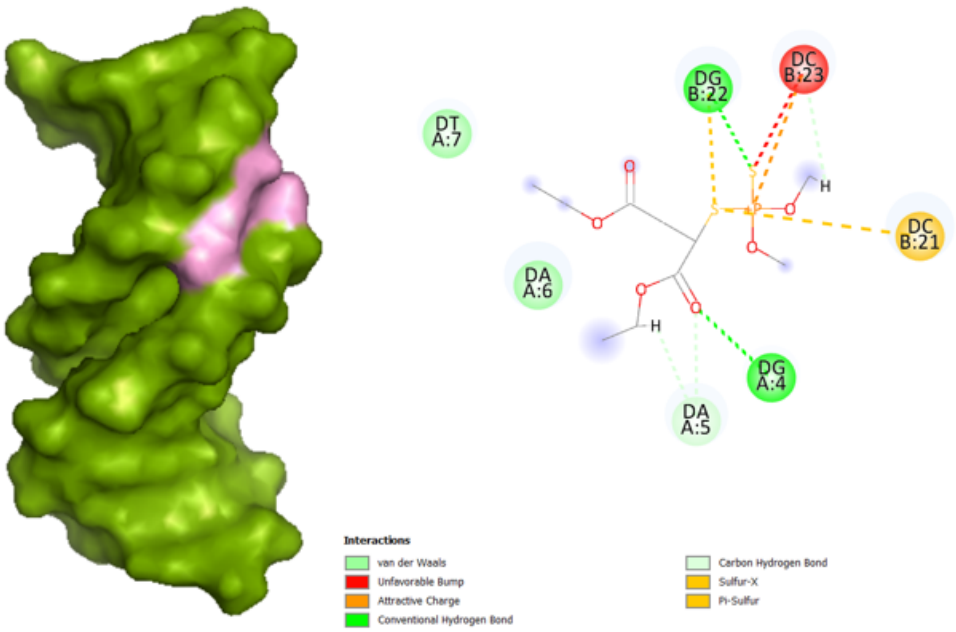

Figure 2. Interactions of Malathion pesticide with DNA.

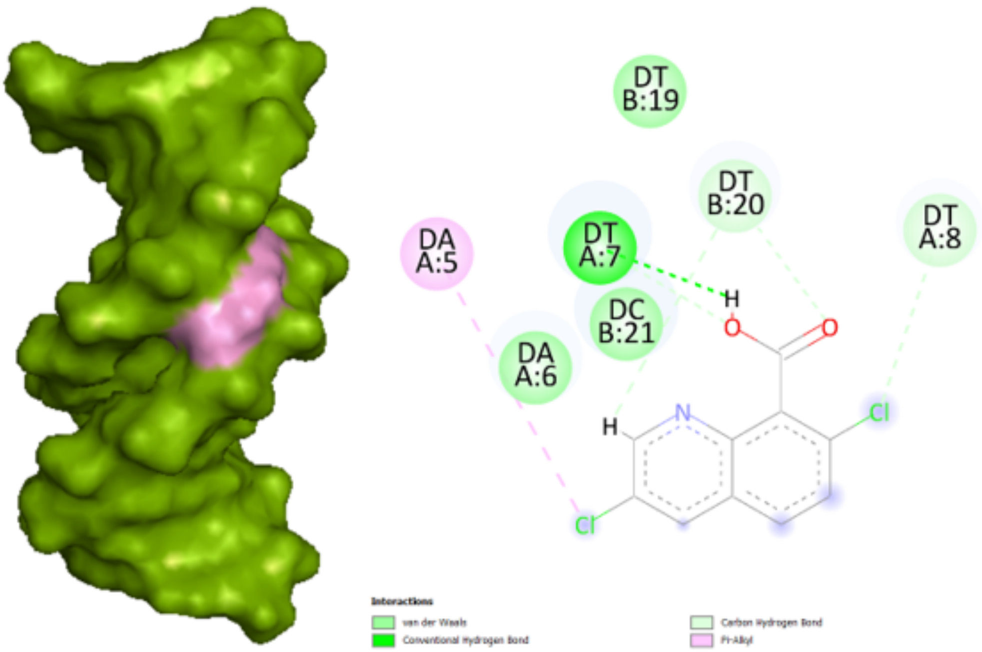

Figure 3. Interactions of Quinclorac pesticide with DNA.

analysis. According to the analysis results, α-cypermethrin pesticide binds to DNA with a higher affinity value (-248.24 KJ mol^{-1}) than Malathion, Quinclorac, and Roundup (Glyphosate) pesticides. In addition, when we look at the affinity values, it is seen that there is not a significant energy difference between Malathion (-242.9 KJ mol^{-1}) and α-cypermethrin (-248.24 KJ mol^{-1}) in terms of binding to DNA. According to this result, it can be said that Malathion pesticide has at least as harmful effects as α-cypermethrin. However, not as high affinity as α-cypermethrin and Malathion, Quinclorac (-205.23 KJ mol^{-1}) and Roundup

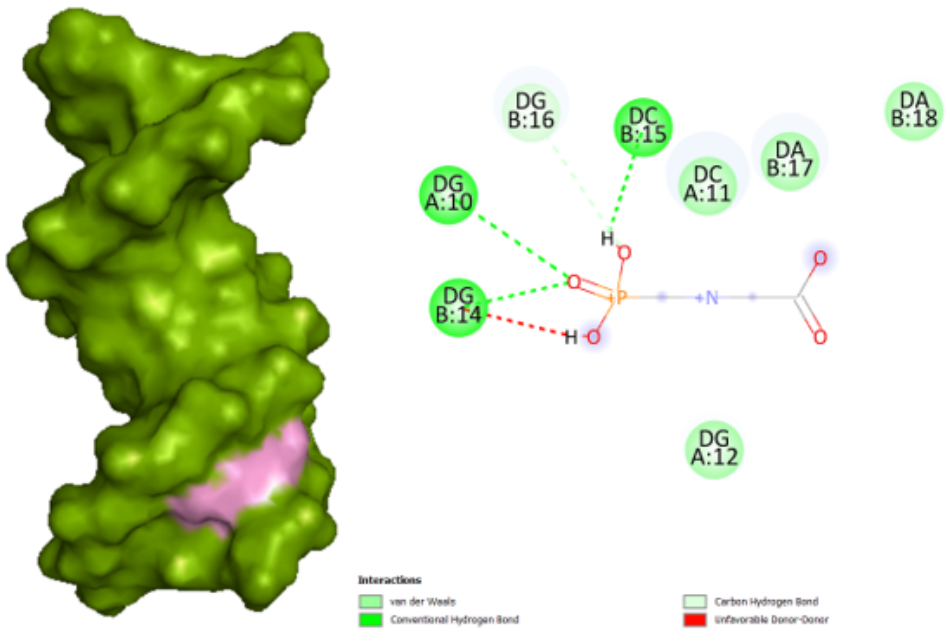

Figure 4. Interactions of Roundup (Glyphosate) pesticide with DNA.

(Glyphosate) (-161.54 KJ mol^{-1}) pesticides can also bind to DNA and have harmful effects.
Epidemiological assessment of cancer risk from pesticide exposure is difficult due to intermittent exposure to varying levels of pesticides and changes in pesticide use patterns over time [8]. To be able to make more precise evaluations, researchers ask about the usage status, duration, and frequency of each compound in these classes according to their functions, and main class chemical structure while examining the farmers' exposure levels to pesticides in their studies. Latifovic et al. showed an association between HL and insecticide exposure in their studies. The risk assessment illustrates the complexity of the relationships between pesticide exposures and the risk of HL [8].
A synergistic interaction is a situation in which drugs increase the effect of each other. However, if possible synergistic and additive interaction pesticide combinations can be identified in future studies, this may raise more concerns about the harm caused by pesticides. This information can assist pesticide users in making application and risk assessments and reduce exposure.
The prevalence of lymph and hematological cancers is high in farmers [8-12]. Although plant protection products are a significant need to meet the food supply, they can cause more severe health problems such as cancer, especially neurotoxic effects, as a result of exposure at both low and high doses and for a certain period [13-15]. Reports are showing that some chemicals used as plant protection products may be carcinogenic [16-18]. Pesticides can cause cell proliferation [19] and cytotoxicity [20-24]. Many pesticides, such as alpha-cypermethrin, may disrupt the endocrine system [25] and promote tumor growth by altering the immune response. When Zang et al. examined the potential toxicological effects of alpha-beta and tetra cypermethrin molecules, they showed that α-cypermethrin is the most potent endocrine-disrupting derivative [25]. In our study, by the literature, the interaction level of α-cypermethrin with DNA was found to be the highest compared to other molecules, and according to the results of docking analysis, it was determined as the molecule with the highest affinity for DNA. According to the literature, α-cypermethrin was the most investigated among the molecules whose docking analysis results presented in our study, and limited data obtained about other molecules.
Yao et al. (2019) showed enantioselective degradation of α-cypermethrin in tomato, cucumber, rapeseed, grape, pepper, and cabbage vegetables, and their results were found to be remarkable in terms of environment and food safety [26]. In the study of Okda et al., CD4/CD8 was statistically significantly lower in the group exposed to α-cypermethrin at a high rate compared to the control group. Regarding gene mutation, exons 5a and 6 were significantly more frequent in the high-exposure group to α-cypermethrin compared to the moderately exposed and control. Antioxidant levels were statistically significantly higher in the group not exposed to α-cypermethrin. In addition, in this study, it was found that there was a significant negative correlation between working time and antioxidant parameter levels [27]. Repeated exposure to α-CYP can lead to gene mutations, immunological disorders, and oxidative stress. Strict safety precautions are required not

only for those using plant protection products but also for biocidal uses in the community.

Studies have shown that cypermethrin has inhibitory effects on androgen transcription and new anti-androgenic mechanisms of cypermethrin with toxicological effects on the male reproductive system [28-29]. Permethrin damages the reproductive and immune systems and cardiovascular and hepatic metabolism. Deltamethrin induces inflammation, nephro- and hepatotoxicity and affects antioxidant enzyme activity in tissues, while α-cypermethrin impairs immunity; increases blood sugar and lipid levels [30].

Human studies have linked pesticides to hearing impairments but offer limited results due to multiple factors such as population exposure to noise. Frequencies of 8, 10, and 12 kHz in both ears (right p=0.003; 0.004; 0.008 and left 0.003; 0.016; 0.005) and right ear 4 and 6 (p=0.007 and 0.015, respectively) in the right ear resulted in reductions in animals exposed to cypermethrin. Subchronic inhalation exposure to cypermethrin produced ototoxicity in rats [31]. Another study investigated the protective effects of testosterone against reproductive toxicity caused by cypermethrin (50 mg/kg body weight) in rats. Significant reductions in circulating testosterone levels were also noted in rats exposed to cypermethrin [32].

Malathion is an organophosphate insecticide that acts as an acetylcholinesterase inhibitor. Studies of the effects of prolonged exposure to oral ingestion of malaoxone in rats have shown that malaoxone is 61 times more toxic than malathion. Malathion has been classified by the IARC as a probable carcinogen (group 2A). Malathion has been classified by the US EPA as having "evidence suggestive of carcinogenicity" [33]. This classification based on the occurrence of liver tumors in mice and female rats at overdose, and the presence of rare oral and nasal tumors in rats after exposure to large doses [34]. In one study, farmers using Malathion were significantly associated with poor performance in visual screening and processing tests [13].

Quinclorac (Table 1), which ranks third in terms of interaction level with DNA according to the results of docking analysis in our study, is a highly selective auxin-type herbicide and is widely used in the efficient control of barn grasses in paddy fields. Due to its widespread use, Quinclorac can be carried out of rice fields by drainage water, causing soil and water pollution and other environmental health problems [35].

The herbicide glyphosate, N-(phosphonomethyl)glycine, has been widely used in recent years as it has fewer side effects. The World Health Organization reclassified glyphosate as a possible human carcinogen in 2015. Studies examining the distribution and residues of glyphosate and its degradation product aminomethyl phosphonic acid (AMPA) and its effects on macro and microorganisms are increasing rapidly [36,37]. Though the acute toxic effects of glyphosate and AMPA on mammals are low, there are animal studies of the accumulation of these compounds in the environment that raise the possibility of health effects due to chronic low-dose exposure in organisms. Intensive use of glyphosate has led to the selection of weeds and microorganisms resistant to glyphosate. Changes in microbial composition due to glyphosate may have led to the proliferation of plant and animal pathogens. It is thought that selective pressure for glyphosate resistance developing in bacteria leads to changes in microbiome composition, triggering the development of antibiotic resistance against clinically crucial antimicrobial agents [36,38,39]. Interdisciplinary research examining associations between prolonged exposure to low doses of glyphosate, disruptions in microbial communities, increased antibiotic resistance, and the occurrence of animal, human, and plant diseases may be useful. Independent research is needed to reconsider tolerance thresholds for glyphosate residues in water, food, and animal feed to consider all possible health risks.

In our study, the affinity levels of the plant protection products frequently used in the Çanakkale region according to the results of the docking analysis to the DNA molecule were determined as Alpha Cypermethrin>Malathion>Quinclorac>Roundup/Glyphosate, respectively. In accordance with the literature, alpha-cypermethrin is the molecule with the highest toxicity and gene damage potential. Considering the variety of products, it is necessary to pay attention to the use of both single and multiple plant protection products. The importance of complying with the healthy and safe usage instructions in terms of farmer health and safety and public health should not be forgotten. During the use of this molecule, plant protection products with lower DNA affinity and toxicity can be preferred as an alternative. In addition, periodic training can be conducted to ensure that farmers use pesticides in accordance with the instructions of the Ministry of Agriculture and Forestry and at the appropriate time, duration, and doses for the product grown.

According to studies examining gene damage from pesticides, although pesticide exposure has not been shown in the blood, it indicates that gene damage increases depending on the dose and duration of pesticide use, healthy and safe use habits, and personal protective use. In addition, in a study examining the relationship between different plant protection products and genetic damage in Greece, it was found that there were significant differences in micronucleus (MN) frequencies in exposed farmers and that pesticides had possible clastogenic and aneugenic effects on genetic materials [7]. In a study examining the role of malathion and glyphosate use in the development of Hodgkin lymphoma cancer in farmers, whose DNA damage level was examined by docking analysis, it was found that malathion use was three times higher in cases under the age of 40 compared to the control group [8]. Malathion is associated with thyroid cancer and non-Hodgkin lymphoma [40].

As in our study, it may be useful to select alternative products by docking analysis of different plant protection products that are in use and whose DNA affinity level and genetic damage relationship have not been examined before. In addition, we think that the results of docking analysis will guide the planning of local and national situation determination studies examining the gene damage of plant protection products, further analyses, and follow-up studies.

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