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Araştırma Makalesi / Research Article

Assessment of Mutagenic Activity of Karate Zeon Pesticide by Ames Test

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Abstract

Pesticides widely used in the agricultural market led to an increase in the quality and yield of crops. However, pesticides are a major concern in the environment as they also harm non-target creatures, because of pesticide resistance, and mutagenic and carcinogenic effects. In this study, we aimed to research the potential mutagenicity of Karate Zeon which is a synthetic pyrethroid insecticide. *Salmonella typhimurium* mutagenicity assay (Ames test) was used to evaluate the mutagenicity of Karate Zeon insecticide in the absence and presence of the S9 fraction on two strains (TA98 and TA100). In this study, doses of 250, 25, 2.5, 0.25, 0.025 µg/plate of Karate Zeon were used. Karate Zeon pesticide showed mutagenic activity at 250 µg/plate concentration on both TA98 and TA100 with and without metabolic activation.

Karate Zeon Pestisitinin Mutajenik Aktivitesinin Ames Testi ile Değerlendirilmesi

Anahtar kelimeler
Ames Testi; Karate zeon; Mutajenite; Pestisit

Öz

Pestisitler, ürünlerin kalitesi ve veriminin artmasında tarımda yaygın olarak kullanılırlar. Ancak pestisitler, hedef olmayan organizmalar üzerinde pestisit direnci, mutajenik ve karsinojenik etkilerinden dolayı çevre için büyük bir endişe kaynağı haline gelmiştir. Bu çalışmada, sentetik bir piretroid insektisit olan Karate Zeon'un potansiyel mutajenitesinin araştırılması amaçlanmıştır. *Salmonella typhimurium* mutajenite testi (Ames testi), S9 fraksiyonunun varlığı ve yokluğunda Karate Zeon insektisitinin iki suş (TA98 ve TA100) üzerindeki mutajenitesini değerlendirmek için kullanılmıştır. Bu çalışmada, Karate zeonun 250, 25, 2.5, 0.25, 0.025 µg/plak dozları kullanılmıştır. Karate Zeon pestisiti, hem TA98 hem de TA100 üzerinde metabolik aktivasyon varlığı ya da yokluğunda 250 µg/plak konsantrasyonunda mutajenik aktivite göstermiştir.

1.Introduction

Insecticides are produced to eliminate pests and enhance the quality and quantity of crops in agricultural soils. Although the use of insecticides increases day by day, their use is of concern due to their cytotoxic/mutagenic and carcinogenic effects and they can damage non-target organisms or cause pest resistance (Asita and Mokhobo 2013). The mutagenic effect of pesticides on non-target organisms and their unfavorable effect on the world's environment is a serious problem (Pimental *et al.* 1998). In addition, the residues from pesticides are understood to accumulate in the water, soil, (Medina *et al.* 1999) and further fruits and vegetables (Osman *et al.* 2010, Ahouangninou *et al.* 2012) which create a threat to the environment and living health.

Pyrethroids are one of the commonly used pesticides in cultivation. They have been also used in pet shampoo, insect repellent, and louse cure (Saillenfait *et al.* 2015). These chemicals are synthetic derivatives of pyrethrins which are botanical insecticides and have been utilized for many years. However, The World Health Organization (WHO, 2009) has identified most of the pyrethroids as moderately dangerous (Class II) (Jensen *et al.* 2011, Özkara 2019). Pyrethroids affect the nervous system of insects by inhibiting the activity of acetylcholinesterase enzyme found at postsynaptic neuromuscular junctions. This action results in continued nerve signaling in overstimulation of the nerve cells. The insects exposed to pyrethroids show tremors and convulsions and eventually die (Atsdr 2003, Valles and Koehler 2003). Additionally, this pesticide is also important in terms of human health as it is only slightly effective on mammals. (Oulhote and Bouchard 2013, Viel *et al.* 2015).

Lambda-cyhalothrin (50 g/L) is a synthetic pyrethroid insecticide, sold by Syngenta Limited with trade names Karate Zeon in the local market. Except for the present paper, there is no other mutagenicity study of Karate Zeon found in the literature. *In vitro* studies on mutagenic tests allow us to investigate the toxicity of various substances. For this reason, this research aimed to examine the mutagenic effects of different doses of Karate Zeon by using the Ames test with or without a metabolic activation system in *S. typhimurium* TA98 and TA100 strains.

2. Material and Method

2.1. Chemicals

Karate Zeon pesticide was purchased from a local market in Afyonkarahisar, Turkey and dissolved in sterile distilled water. The test chemicals were obtained from Riedel and Merck.

2.2. Test strains

The histidine-dependent strains (TA100 and TA98) of *S. typhimurium* were obtained from Prof. B. N. Ames (California University, Berkeley). These strains were incubated for 16 h in liquid nutrient broth and kept at -80°C. Their genetic markers and other properties, such as the number of spontaneous revertants and responses to positive controls, were controlled as defined by Maron and Ames (1983).

2.3. Ames Salmonella/Microsome Assay

The mutagenic effects of the Karate Zeon on *S. typhimurium* strains were determined by the standard plate incorporation method (Ames *et al.* 1975, Maron and Ames 1983).

The cytotoxic concentrations of the Karate Zeon (250, 25, 2.5, 0.25, 0.025 µg/plate) for Salmonella strains used in this study were evaluated according to Dean *et al.* (1985). Karate Zeon stock solutions were prepared by dissolving it in sterile distilled water and keeping the solutions at 4°C. The test strains were maintained in nutrient broth at 37°C for 16 h with shaking. In the presence of S9, the positive controls 2-aminoanthracene (2-AA) and 2-aminofluorene (2-AF) were used for TA100 and for TA98, respectively. Additionally, sodium azide (SA) and 4-nitro-o-phenylenediamine (NPD) were used in the absence of S9.

The plates without the S9 mix consisted of 0.1 mL bacterial suspension from overnight culture, 0.1 mL of each concentration of Karate Zeon, and 0.5 mL phosphate buffer to 2 ml top agar. The mixture was shaken with the help of a vortex and then discharged into the minimal agar. Instead of phosphate buffer, the petri dishes with the S9 mix were consisted of 0.5 mL of S9 mix. All petri dishes used in the experiment were incubated for 72 h at 37°C, and the revertant colonies on each petri dish were numbered at the end of the assay. All the experiments were carried out in the absence and presence of S9 fractions, and each dose was examined with three parallel plaques, and these were replicated at different times.

2.4. Statistical analysis

The significant differences between the experimental groups were determined statistically by the SPSS ver. 15.0. In the analyses, the Dunnett's-t test (2-sided) was performed for Ames tests.

3. Results

Table 1 summarizes the results of the Ames method. The numbers of spontaneous revertant colonies were within the normal amounts in both two strains examined. All of the doses with and without S9 mix slightly increased the revertant colonies in both two strains as compared to the negative control. However, the positive control substances significantly increased the numbers of revertant colonies in both strains. In the present experiment, only the 250 µg/plate doses of the Karate Zeon pesticide resulted in an increase in the mutation rate in both the TA98 and TA100 strains with or without S9 mix (P<0.05).

Table 1. Plaque incorporation test results of *S. typhimurium* TA98 and TA100 strains were examined with Karate Zeon pesticide in the presence and absence of S9.

Test Material	**Dose (µg/plate)**	**Revertant colony number Mean ± SD**			
		TA98		**TA100**	
		- S9	**+ S9**	**- S9**	**+ S9**
Karate Zeon	250	72.51±5.7*	118.56±6.58*	176.65±7.32*	263.21±10.41*
	25	56.27±3.16	89.28±5.45	157.20±6.28	238.13±9.71
	2.5	48.25±3.51	80.41±3.65	155.21±5.58	214.09±7.27
	0.25	44.52±2.71	67.21±3.54	126.81±3.42	201.14±6.11
	0.025	33.41±2.67	52.24±2.53	106.26±2.53	181.12±5.41
Negative Control (dH_2O)	100	29.21±4.11	46.18±6.31	79.09±7.24	117.20±6.31
SA	10			2654.13±40.41*	
2-AA	5				2237.24±25.40*
2-AF	200		1285.11±14.17*		
NPD	200	1240.25±32.72*			

* Mean statistically significant at p<0.05 (Dunnett t-test), NC: Negative control, SA: Sodium azide, 2-AA: 2-aminoanthracene, 2-AF: 2-aminofluorene, NPD: 4-nitro-o-phenylendiamine.

4. Discussion

Pyrethroid insecticides are highly toxic for insects and cause a low degree of toxicity in mammals. These insecticides are often used in both agriculture and veterinary medicine, and to control pests in confined spaces (Casida and Quistad 1998). In addition, these substances have been replaced by organophosphorus pesticides because of their effective role in most areas of applications (Ministry of the Environment in Japanese 2011). Due to their many advantages, it is very important to research the biological effects of pyrethroid insecticides, which are included the Karate Zeon. Previous studies have reported their toxicity on living organisms (Grossman 2007, Surralles *et al.* 1995).

The Ames test is a test system used to understand both the mutagenic and anti-mutagenic effects of various chemicals and provides over 90% correlation in predicting genotoxicity (Weisburger 2001). In the Ames test, *S. typhimurium* strains that have a mutation in the *his*-operon are used to detect the mutagenicity of substances (Maron and Ames 1983). The prokaryotic bioassays give information about primary DNA damage and the gene mutations induced by any agent (Leme and Marin-Morales 2009). In this study, the mutagenic activity of Karate Zeon was determined by the Ames test and results showed that Karate Zeon pesticide induces mutations in the TA98 and TA100 strains at 250 µg/plate doses with or without S9 mix. This result is consistent with the findings of Özkara (2017) who reported a significant increase in the mutagenic activity at only 250 µg/plate dose of Siperkor (a pyrethroid group pesticide) in both strains in the presence and absence of S9. In contrast, Deltamethrin has no genotoxic effects on *S. typhimurium* and V79 Chinese hamster ovary cells (Pluijmen et al. 1984). Depending on the genetic system or the assay, data on the Deltamethrin carcinogenicity and genotoxicity are quite controversial (Shukla and Taneja 2000). Basri *et al.* (2008) investigated the mutagenicity of Cyfluthrin, a pyrethroid insecticide with a wide spectrum on harmful insects used in agriculture and homes, on TA98 and TA100 strains, and they did not find any mutagenic effects in the presence and absence of S9. Moreover, Miadokova *et al.* (1992) investigated the mutagenicity of Superspermetrin, an insecticide included in the pyrethroid group, and concluded that this substance was not mutagenic in the presence and absence of S9 on TA1535, TA1538, TA97, TA98 and TA100 strains. In addition, pyrethroid group pesticides have been searched by many investigators with different test systems (Taju *et al.* 2014, Song *et al.* 2015, Vardavas *et al.* 2016, Yun *et al.* 2017).

5. Conclusion

In conclusion, studies on pesticides reveal very different results. The safety of a pesticide on other non-target organisms is as important as its effectiveness. For this reason, it is of great importance to have information about the reliability of short-time test systems and pesticides and to make risk assessments. Therefore, pesticides should be used in accordance with their prospects, it should not be forgotten that unconscious use and excessive consumption will harm the environment and all living things. In addition, pesticide accumulations should also be eliminated from the environment by biological methods. For this reason, improvement studies (bioremediation) are also important today.

6. References

Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for pyrethrins and pyrethroids. Atlanta, GA: Department of Health and Human Services, Public Health Service, Toxicology (2003).

Ahouangninou, C., Martin, T., Edorh, P., Bio-Bangana, S., Samuel, O., St-Laurent, L. and Fayomi, B., 2012. Characterization of health and environmental risks of pesticide use in market-gardening in the rural city of Tori-Bossito in Benin, West Africa. *Journal of Environmental Protection*, **3**, 241-248.

Ames, B.N., McCann, J., Yamasaki, E., 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutation Research*, **31**, 347-364.

Asita, A.O. and Mokhobo, M.M., 2013. Clastogenic and cytotoxic effects of four pesticides used to control insect pests of stored products on root meristems of *Allium cepa*. *Environment and Natural Resources Journal*, **3(2)**, 133-145.

Basri, H., Topaktaş, M., Rencüzoğulları, E., Kayraldız, A., Dönbak, L., Dağlıoğlu, Y.K., 2008. Genotoxic potential of cyfluthrin. *Mutation Research*, **1(2)**, 49-50.

Casida, J., Quistad, G., 1998. Golden age of insecticide research: past, present, or future? *Annual Review of Entomology*, **43**, 1-16.

Grossman, N., 2007. Influence of pyrethroids and piperonylbutoxide on histamine release from isolated rat mast cells. *Journal of Inflammation Research*, **56,** 473-478.

Jensen, H.K., Konradsen, F., Dalsgaard, A., 2011. Pesticide use and self-reported symptoms of acute pesticide poisoning among aquatic farmers in Phnom Penh, Cambodia. *International Journal of Toxicology*, 639814.

Leme, D.M. and Marin-Morales, M.A., 2009. *Allium cepa* test in environmental monitoring: A review on its application. *Mutation Research*, **682**, 71-81.

Maron, D.M., Ames, B.N., 1983. Revised methods for the Salmonella mutagenicity test. *Mutation Research*, **113**, 173-215.

Medina, D., Prieto, A., Ettiene, G., Buscema, I. and Abreu de, V.A., 1999. Persistence of Organophosphorus Pesticide Residues in Limón River Waters. *Bulletin of Environmental Contamination and Toxicology*, **63(1)**, 39-44.

Miadokova, E., Vickova, V., Duhova, V., Trebaticka, M., Garajova, K., Grolmus, J., Podstavkova, S., Vicek, D., 1992. Effects of supercypermethrin, a synthetic developmental pyrethroid, on four biological test systems. *Mutation Research*, **3**, 161-168.

Ministry of the Environment Compilation of pollutant release and transfer register data Ministry of the Environment; in Japanese, 2011.

Osman, K.A., Al-Humaid, A.M., Al-Rehiayani, S.M. and Al-Redhaiman, K.N., 2010. Monitoring of pesticide residues in vegetables marketed in Al-Qassim region, Saudi Arabia. *Ecotoxicology and Environmental Safety*, **73(6)**, 1433-1439.

Oulhote, Y., Bouchard, M.F., 2013. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. *Environmental Health Perspectives*, **121**, 1378-1384.

Özkara, A., 2017. Evaluation of Siperkor Pesticide Mutagenicity with Ames Test. *Afyon Kocatepe University Journal of Sciences and Engineering*, **17**, 393-398.

Özkara, A., 2019. Assessment of cytotoxicity and mutagenicity of insecticide Demond EC25 in Allium cepa and Ames Test. *Caryologia*, **72(2)**, 21-27.

Pimentel, D., Greiner, A. and Bashore, T., 1998. Economic and environmental costs of pesticides use, Environmental Toxicology, Current Developments, London UK, Gordon and Breach Science Publisher, 121-187.

Pluijmen, M., Drevon, C., Montesano, R., Malaveille, C., Hautefeuille, A., Bartsch, H., 1984. Lack of mutagenicity of synthetic pyrethroids in *Salmonella typhimurium* strains and in V79, Chinese hamster ovary cells. *Mutation Research*, **137**, 7-15.

Saillenfait, A.M., Ndiaye, D., Sabaté, J.P., 2015. Pyrethroids: exposure and health effects an update. *International Journal of Hygiene and Environmental Health*, **218(3)**, 281-92.

Shukla, Y., Taneja, P., 2000. Mutagenic evaluation of deltamethrin using rodent dominant lethal assay. *Mutation Research*, **467**, 119-127.

Song, Y., Kai, J., Song, X., Zhang, W., Li, L., 2015. Long-term toxic effects of deltamethrin and fenvalerante in soil. *Journal of Hazardous Materials*, **289**, 158-164.

Surralles, J., Xamena, N., Creus, A., Catalan, J., Norppa, H., Marcos, R., 1995. Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures. *Mutation Research*, **341**, 169-184.

Taju, G., Abdul Majeed, S., Nambi, K.S.N., Farook, M.A., Vimal, S., Sahul Hameed, A.S., 2014. In vitro cytotoxic, genotoxic and oxidative stress of cypermethrin on five fish cell lines. *Pesticide Biochemistry and Physiology*, **113**, 15-24.

Valles, S.M. Koehler, P.G., 2003. Insecticides used in the urban environment: mode of action (ENY282) Gainesville, FL: Department of Entomology and Nematology University of Florida.

Vardavas, A.I., Stivaktakis, P.D., Tzatzarakis, M.N., Fragkiadaki, P., Vasilaki, F., Tzardi, M., Datseri, G., Tsiaoussis, J., Alegakis, A.K., Tsitsimpikou, C., Rakitskii, V.N., Carvalho, V., Tsatsakis, A.M., 2016. Long-term exposure to cypermethrin and piperonyl butoxide cause liver and kidney inflammation and induce genotoxicity in New Zealand white male rabbits. *Food and Chemical Toxicology*, **94**, 250-259.

Viel, J.F., Warembourg, C., Le Maner-Idrissi, G., Lacroix, A., Limon, G., Rouget, F., Monfort, C., Durand, G., Cordier, S., Chevrier, C., 2015. Pyrethroid insecticide exposure and cognitive developmental disabilities in children: the PELAGIE mother child cohort. *Environment International*, **82**, 69-75.

Weisburger, J.H., 2001. Antimutagenesis and anticarcinogenesis, from the past to the future. *Mutation Research*, **480**, 23-35.

Yun, X., Huang, Q., Rao, W., Xiao, C., Zhang, T., Mao, Z., Wan, Z.A., 2017. Comparative assessment of cytotoxicity of commonly used agricultural insecticides to human and insect cells. *Ecotoxicology and Environmental Safety*, **137**, 179-185.