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Araştırma Makalesi / Research Article

Potential Mutagenicity of Udimo 75 WG Herbicide in *Salmonella typhimurium* with Ames Test

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Abstract

In addition to the benefits of pesticides frequently used in the agricultural sector, there are also many negative effects on the environment and living organisms. We aimed this study was to investigate mutagenic activity of Udimo 75 WG herbicide that commonly used in the agricultural sector by Ames/*Salmonella* microsome short-time test system. Experiments were applied in the absence (-S9) and presence (+S9) of enzymes in the metabolic activation by using TA98 and TA100 strains of *Salmonella typhimurium.* At first, the cytotoxic doses of the test substances were detected and then, 5 non-cytotoxic dose for each test substances were selected. For the mutagenicity of Udimo 75 WG 500, 250, 125, 62.5 and 31.25 µg/plate doses were studied. The obtained data were statistically analysed by Dunnett's t-test and the results were evaluated by compare with spontaneous control plates. According to the results of this study, Udimo 75 WG herbicide showed mutagenic activity both in the absence and presence of S9 fraction at the dose of 500 µg/plate concentration in both strains. Therefore, this herbicide may be in the genotoxic risk group for humans and other living things.

Udimo 75 WG Herbisitinin Ames Testi ile *Salmonella typhimurium'da* Potansiyel Mutajenitesi

Anahtar kelimeler
Ames testi; Mutajenite; Sülfonilüre herbisitleri; Udimo 75 WG

Öz

Tarım sektöründe sıklıkla kullanılan pestisitlerin faydalarının yanı sıra çevreye ve yaşayan canlılara olumsuz birçok etkisi de vardır. Bu çalışmada, tarım sektöründe yaygın olarak kullanılan bir herbisit olan Udimo 75 WG'nin Ames/*Salmonella* mikrozom kısa süreli test sistemi ile mutajenik aktivitesinin araştırılması amaçlanmıştır. Deneyler *Salmonella typhimurium* TA98 ve TA100 suşları kullanılarak metabolik aktivasyon yokluğunda (-S9) ve varlığında (+S9) gerçekleştirilmiştir. İlk olarak test maddelerinin sitotoksik dozları tespit edilmiş ve ardından her test maddesi için 5 sitotoksik olmayan doz seçilmiştir. Çalışmada Udimo 75 WG 500, 250, 125, 62.5 ve 31.25 µg/plak dozları çalışılmıştır. Elde edilen veriler, Dunnett t-testi ile istatistiksel olarak analiz edilmiş ve sonuçlar, spontan kontrol plakları ile karşılaştırılarak değerlendirilmiştir. Bu çalışmanın sonuçlarına göre, Udimo 75 WG herbisiti, her iki suşta da 500 µg/plak konsantrasyonunda S9 fraksiyonunun hem yokluğunda hem de varlığında mutajenik aktivite göstermiştir. Dolayısıyla bu herbisit, insanlar için genotoksik bir risk oluşturabilir.

1.Introduction

Identification of chemical substances that cause mutation has become an important parameter in the risk assessment of that substance in recent years, and such chemicals cause fertility problems and cause problems in future generations. These substances also have the potential to cause cancer, and mutagenicity tests have also gained importance at this stage (Moulas *et al.* 2013). Earlier studies have showed that some pesticides are clastogenic and mutagenic in different biological test systems (Siroki *et al.* 2001, Celik 2003, Stivaktakis *et al.* 2010, Moulas *et al.* 2013, Akyıl and Konuk 2014, Özkara 2017). The Ames test is an important test method for determining the mutagenic and anti-mutagenic effects of various chemical substances and also has an effect of over 90% in detecting genotoxicity (Kauffmann *et al.* 2020). In this test system, different *Salmonella typhimurium* strains with mutations in his operon are used to define the mutagenic activity of substances (Maron and Ames 1983).

Sulfonylurea herbicides are very strong, highly selective chemicals that are widely used in agriculture and can act at low application rates (Mora *et al.* 2019) and it is estimated that there will be even more sales in the market in further. These herbicides have weak toxicity for animals and humans, and are effective on a broad range of weeds (Myhre *et al.* 2004). Sulfonylurea herbicides inhibit the activity of acetolactate synthase (ALS) enzyme, and the biosynthesis of isoleucine, leucine and valine causing the death of the plant (Jin *et al.* 2012, Delye *et al.* 2018). It has been shown that sulfonylurea herbicides could bind to active site of the ALS and blocked the substrate binding (Zhang 2021).

Udimo 75 WG is a systemic sulfonylurea herbicides that controls varies plants from pests. The increasing use of herbicides around the world is very important to demonstrate the potential risk effects on non-target living things through Ames test. We aimed of this study is to determine the mutagenic activity of Udimo 75 WG herbicide with the Ames test, depending on its widespread use.

2.Materials and methods

2.1. Chemicals and test strains

The test substance Udimo 75 WG was purchased from a local market in Afyonkarahisar/Turkey and dissolved in sterile distilled water. S9 from the liver of rats (Sprague Dawley) were obtained from Sigma Aldrich. The *S. typhimurium* test strains TA98 and TA100 were kindly supplied by Prof Nuran Diril, of Hacettepe University, Turkey. These strains were incubated for 16h in liquid nutrient broth and kept at -80°C. Their genetic markers and other properties including the numbers of spontaneously induced revertants and responses to positive controls, were controlled as designed by Maron and Ames (Maron and Ames 1983).

2.2.Determination of cytotoxic doses

For the test of cytotoxic doses, 0.1 ml test suspension added for each concentration and 0.1 ml bacterial suspension of TA100 from overnight culture to 2 ml top agar which maintained in water bath at 45°C. After shaking for 3 s, the mixture was added to the nutrient agar. All plates were maintained in incubator at 37°C for 24 h, and then the revertant colonies were counted for each plate and toxic and non-toxic doses were determined. Samples were evaluated on triplicate plates in two independent parallel experiments and all results of the experiment were analyzed by the statistical analysis.

2.3. Ames Salmonella/Microsome Assay

In order to determine the mutagenic activity of Udimo 75 WG, the plate incorporation test was applied using the TA98 and TA100 strains of *S. typhimurium* in the absence and presence of S9 fraction (Maron and Ames 1983). Genetic controls of test strains described according to Maron and Ames (1983). The test concentrations of the Udimo 75 WG (500, 250, 125, 62.5, 31.25 µg/plate) were determined according to Dean *et al.* (1985). Five different concentrations of Udimo 75 WG to be used during the experiment were freshly prepared with sterile distilled water, and stock solutions were kept

at 4 °C. The strains were maintained at 37 °C in nutrient broth for 16 h with orbital shaking. Positive controls were also used in parallel with the application.

The test petri dishes for the assays without the S9 fraction were carried out with different concentrations of 100 µL of test material, 100 µL of overnight bacterial culture and 500 µl of phosphate buffer into 2 mL top agar in a hot water bath at 45 °C.

This prepared mixture was shaken with vortex for 3 seconds and dumped into minimal glucose agar. The petri dishes with the S9 fraction were carried out with 500 µL of S9 mix instead of the phosphate buffer. All petri dishes with minimal glucose agar were maintained at 37 °C for 72 hours and revertant colonies were recorded at the end of the incubation. The assay was applied with three replicate for each dose and two individual applications were designed and the results were evaluated statistically.

2.4. Statistical analysis

The significant differences among the treatment groups were determined by using the SPSS ver. 15.0. In the analyses, the Dunnett-t test (2 sided) was performed on Ames tests.

3. Results

Ames tes method contains plating His– *Salmonella typhimurium* onto media including trace amounts of histidine and adding different concentrations of substances to be tested for mutagenicity, resulting colonies that if the compound is capable of reversing the mutation in *S. typhimurium.* His- mutation is consequently converted to His+ (Ames *et al.* 1973, Flückiger-Isler *et al.* 2004).

Results of the Ames test are presented in Table 1. At the end of the experiment, all concentrations showed a mutagenic effect with both strains. In addition to the mutagenic response, the increase in revertant colony number was dose-dependent in both TA98 and TA100 strains. Besides, amount of spontaneous revertant colonies of the negative control was observed to be less than that of all the applied doses of the test material. Additionally, positive control plates caused a significant increase in the rate of spontaneous mutation in two strains. The test concentrations below 500 µg/plate showed no significant increases in the amount of spontaneous revertant colonies with compared to the negative control. Udimo 75 WG herbicide has mutagenic activity both in the absence and presence of S9 fraction of 500 µg/plate dose in both strains.

Table 1. The mutagenic activity of Udimo 75 WG for *S. tyhimurium* TA98 and TA100 strains

Substance	Dose (µg/plate)	Revertant Colony Mean ± Standard Deviation			
		TA98		TA100	
		- S9	+ S9	- S9	+ S9
Udimo	500	102.13±7.6*	132.51±3.37*	209.24±6.16*	326.27±12.49*
	250	76.25±5.4	108.21±6.21	172.42±5.24	300.16±9.28
	125	59.47±3.21	94.27±3.31	159.30±4.25	279.21±10.45
	62.5	52.29±2.72	80.09±4.41	121.31±2.47	241.54±6.41
	31.25	44.25±4.58	52.27±3.30	101.12±3.35	210.48±7.12
NC (dH_2O)	100	41.12±4.14	57.24±6.23	90.17±5.21	152.21±7.13
SA	10			2641.15±31.18*	
2AA	5				2824.13±37.18*
2AF	200		1165.13±21.08*		
NPD	200	1320.25±32.85*			

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

4. Discussion

In the Ames test, bacterial mutants (mutated to the histidine gene that produces amino acids necessary for bacteria to survive) have been developed to be used to determine the mutagenic effects of chemical substances. For this, a number of strains of *S. typhimurium* bacteria are used (such as TA98, TA100, TA1535 and TA1537). Without the synthesis of histidine in bacteria, no growth and colony form can occur. After the strains are treated with the suspect chemical, they become able to produce histidine and grow to form a colony. In the study, Ames test with the different doses of Udimo 75 WG displayed a mutagenic activity at the highest concentration of 500 µg/plate both TA98 and TA100 strains (Maron and Ames 1983, Malev 2012, Kumar *et al.* 2013).

S. typhimurium TA98 strain has spontaneous mutations at the frameshift hisD3052 allele which consists of a -1 deletion. This allele can be reverted by frameshift mutagens. The base-pair substitution hisG46 allele in TA100 strain consists of a CCC codon (leucine) instead of the wild-type CTC codon (proline). This mutation can be reversed by mutagens that cause base change at G-C pairs (Di Sotto et al. 2008). In this context these bacterial properties, our results can be said that Udimo 75 WG mutagenicity in TA98 strain is caused by frameshift mutations and that of TA100 strain is due to base change (Di sotto *et al.* 2008).

Many studies have been conducted on sulfonylurea herbicides from past to presents with different microorganisms. Boldt and Jacobsen (1998) reported that metsulfuron methylin, a sulphonyl urea herbicide, is toxic at low concentrations in *Pseudomonas* strains, while chlorsulfuron has toxic effects at only higher concentrations. Thifensulfuron methylin was found to be toxic in some strains of *Pseudomonas* in the same study (Boldt and Jacobsen 1998). This study showed that the herbicides caused different results among strains (Boldt and Jacobsen, Burnet and Hodgson 1991). In the studies of many other researchers, sulphonylurea herbicides generally showed toxic effects. Chlorsulfuron is a sulphonylurea herbicide, and it has been shown to be toxic in some microorganisms with concentration ranging from 3 µM (Forlani *et al.* 1995) to 2.8 mM (Blair and Martin 1988). Burnet and Hodgson (1991) reported that sulfometuron methyl and chlorsulfuron, which are suphonylurea herbicides, have different toxic effects. In this study, sulfometuron methyl reduced the growth in many strains compared to methyl culorsulfuron. All these studies mentioned above have results that support our study. Unlike our study, the sulphonylurea herbicide monosulfuron does not have a mutagenic effect with the Ames test (Man-yi *et al.*2008).

The achievement of such different results in different studies varies depending on the different physiological and genetic properties of the microorganisms. Microorganisms may also include different isoenzymes of ALS (Burnet and Hodgson 1991). In addition, these differences play an important role in the physical interaction between herbicide and enzyme. It has been also shown that the membranes function as a barrier for sulphonylurea herbicides (Burnet and Hodgson 1991). Additionally, the tested chemicals can be genotoxic, mutagenic or not genotoxic due to their biological activity and chemical structure such as positions of the binding location and having rings in the structure (Kutlu *et al.* 2011, Ema *et al.* 2012, Kaur *et al.* 2014).

5. Conclusion

In conclusion, Udimo 75 WG was found to mutagenic at some doses in the Ames test. This and many similar herbicides may pose genotoxic risks to humans and other microorganisms. In order to increase the reliability of a study, it is considered appropriate to support it with other different test methods. It has been recommended that various chemical substances may enhance the number of revertants in *Salmonella* strains due to mechanisms that would correlate to eukaryotes (Gocke and Albertini 1996). In addition to these methods, comet test can be applied to detect DNA damage (Sasaki *et*

al. 2000). Studies like this will support the Ames test results and give us more insight into the chemical substances.

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