Vol.66: e23210311, 2023
https://doi.org/10.1590/1678-4324-2023210311
ISSN 1678-4324 Online Edition

Article - Biological and Applied Sciences

Protective Effects of *Aloe Vera* Extract Administiration against Trifluralin in *Drosophila melanogaster* with Various Parameters

Özge Çeliktaş-Köstekçi[1]
https://orcid.org/0000-0001-9762-9144

Fahriye Zemheri-Navruz[2*]
https://orcid.org/0000-0003-1744-1091

[1]Bartın University, Institute of Science, Bartın, Turkey; [2]Bartın University, Faculty of Science, Department of Molecular Biology and Genetics, Bartın, Turkey.

Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Jane Manfron Budel

Received: 13-May-2021; Accepted: 02-Jul-2022.

*Correspondence: fahriyezmhr@hotmail.com; Tel.: +90-378-5011000 (F.Z.N.).

HIGHLIGHTS

- The longevity of the female and male *Drosophila melanogaster* is improved at doses given *Aloe vera*.
- Trifluralin has been observed to cause DNA damage, while *Aloe vera* has been shown to reduce the DNA damage.
- Trifluralin reduced larval survival rate, *Aloe vera* increased larval survival rate at high concentration.

Abstract: Pesticides are toxic chemicals used to control pests, plant diseases, or undesirable vegetation. Trifluralin (TRF), causing toxicity and mutagenicity, is a dinitroaniline class herbicide used to control undesirable vegetation in agriculture. *Aloe vera* is a widely used plant having rich gel content and a wide area of utilization. The present study aims to investigate the protective effect of *Aloe vera* on *Drosophila melanogaster* toxicity caused by TRF. For this purpose, life span, larval toxicity, and genotoxicity tests were performed on *D. melanogaster* administered with concentration of TRF (0.1 mM) and *Aloe vera* (2.5%, 5% and 10%). The results showed that life span was improved in the female and male populations when TRF and *Aloe vera* was administered (2.5%, 5% and 10%). Furthermore, the larval toxicity experiment showed that TRF decreased the survival rate of larvae; however, TRF and *Aloe vera* (10%) increased it at the highest concentration. According to the Comet Assay, TRF was found to cause an increase in the DNA damage; on the other hand, when *D. melanogaster* was administered with TRF and *Aloe vera*, the DNA damage decreased gradually in a dose-dependent manner. In conclusion, *Aloe vera* could be a good candidate to reduce the harmful effects of TRF, one of the most used herbicides.

Keywords: Trifluralin; *Aloe vera*; life span; DNA damage; larval toxicity.

INTRODUCTION

The use of modern agricultural techniques has been on the increase not only to enhance the productivity of agricultural products but also to obtain better products. Therefore, also the use of pesticides has increased day by day. Pesticides adversely affect human health and the environment as they persist in agricultural

products and the environment for a long time [1,2]. Herbicides are one of the pesticides used to kill or suppress the development of undesirable vegetation [3]. Trifluralin (TRF; 2,6-dinitro-N, N-dipropyl-4-trifluoromethylaniline) is a Class 3 carcinogenic herbicide that falls into the class of dinitroaniline. According to the data from EPA (United States Environmental Protection Agency), it is reported that TRF has toxic effects above 0.5 ppm [4] and is genotoxic in various experiments [5,6,7]. TRF has been used in agriculture since 1963. This herbicide is registered separately or in mixtures, and used in the following crops: *Glycine max, Gossypium hirsutum, Arachis hypogaea, Phaseolus vulgaris, Allium sativum, Ricinus communis, Manihot esculenta, Solanum melongena, Daucus carota, Abelmoschus esculentus, Brassica oleracea, Brassica oleracea capitata, Brassica oleracea botrytis, Capsicum annuum, and ornamental plants* [8]. TRF is greatly persistent in the environment and it can serve as a biotoxin and leads to genotoxicity in terrestrial organisms, including humans [9]. Furthermore it is an agent that endorses a cellular damage because of its direct action on the microtubules [10]**.** The genotoxicity of TRF herbicide on *Colossoma macropomum* was verified by the micronucleus test and comet assay [11]. TRF has caused the genotoxicity through the activation of oxidative stress pathway and chromosomal damage in Chinese hamster lung fibroblast (V79) cells [12].

Aloe vera (L.) Burm.f. is a clear and gel-like substance obtained from *A. vera* leaf [13]. *A. vera* plant is known to contain active components such as vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids [14]. It is reported that *A. vera*, which has anticarcinogenic, antibacterial, antiviral, anti-inflammatory and antioxidant activities, reduces the genotoxic effect of *D. melanogaster* reduced genotoxicity after *D. melanogaster* had mutated with ethyl methanesulfonate (EMS) [15]. In a study conducted it is revealed that the gel supplementation increased its reproductive performance [16] and enhances the productivity of *Oreochromis niloticus* [17]. This indicates that gel extract of *A. vera* includes phytochemical(s) that can be beneficial in the development of anticancer drug [18].

Single-cell gel electrophoresis (SCGE) or Comet Assay method was used in our study to determine the effects of genotoxic agents on cells and to examine the damages that might occur in the DNA of cells [19]. The simplicity of the Comet Assay and its use in all kinds of tissues/cells increases the applicability of the test [20]. Lifespan of the *D. melanogaster* is affected by genotoxic agents which is a quantitative feature of phenotypic variation in natural populations based on both genetic and environmental components [21]. *D. melanogaster* is a model organism preferred to examine the effects of chemicals with genotoxic effects [22].

In this study, it was aimed to determine the protective effect of *A. vera* against genotoxicity in *D. melanogaster* given TRF for the first time. For this purpose; larval toxicity, life span analyses and comet assay of fly tissues were performed.

MATERIAL AND METHODS

Organism Used

In this study, we used Oregon-R wild strain of *D. melanogasters* in laboratory stock, which are genetically homozygous and non-mutant and have been used for many years at the Genetics Research Laboratory within Bartın University, Faculty of Science, Department of Biology.

Chemical Substances Used

The following chemicals were used: Trifluralin (2,6-Dinitro-N, N-dipropyl-4-trifluoromethylaniline, Cas No: 1582-09-8, Santa Cruz, USA), agar (Sigma Aldric, USA), diethyl ether (IsoLab, Germany), propionic acid (Merck, Germany), orthophosphoric acid (Merck, Germany), xylene (Sigma Aldric, USA), sodium chloride (IsoLab, Germany), phosphate buffered saline (Multicell), ethylenediaminetetraacetic acid (Sigma Aldric, Germany), sodium hydroxide (IsoLab, Germany), trizma base (Sigma Aldric, USA), low melting agarose (BioShop Canada Inc.), normal melting agarose (Peqlab), triton-X (Sigma Aldric, USA), dimethyl sulfoxide (Merck, Germany), ethidium bromide (Molecula, USA). *A. vera* extract was obtained in laboratory according to the methods of YanMei (2011) and Chandrashekara and Shakarad (2011).

Drosophila melanogaster third-stage larvae and adults were exposed to concentrations of 0.1 mM TRF and *A. vera* (2.5, 5 and 10%). TRF was dissolved in xylene. *A. vera* gel extract was obtained according to the method of Saritha et al [23].

Life Span Experiment

For each experimental group, 15 individuals of the same age (1-3 days old) consisting of non-mated ♀♀ and ♂♂ flies were collected. During the experiment, *D. melanogaster* was fed in grouped culture flasks

(Group 1: Control, group 2: Xylene, group 3: 0.1 mM TRF, group 4: 2.5% Aloe vera +0.1 mM TRF, group 5: 5% Aloe vera +0.1 mM TRF, group 6: 10% Aloe vera + 0.1 mM TRF) containing different concentrations of TRF and *A. vera*. During the experiment, food was refreshed once a week. The number of individuals was tracked on a daily basis and this continued until the last individual died. The experiments were repeated twice [24].

Larval Toxicity

Larval toxicity experiments were performed using the 3rd stage (72±4 hours) larvae of *D. melanogaster*. Larvae were obtained by crossing 10 ♂♂ individuals with 10 ♀♀ individuals in culture medium containing standard growth medium. After mating of flies, the 3rd stage larvae were collected and 50 larvae were added to each group (Group 1: Control, group 2: Xylene, group 3: 0.1 mM TRF, group 4: 2.5% *Aloe vera* +0.1 mM TRF, group 5: 5% Aloe vera +0.1 mM TRF, group 6: 10% *Aloe vera* + 0.1 mM TRF) culture flask. Larvae development was monitored on a daily basis and the number of flies were noted by sex for 15 days. The experiment was repeated twice [25].

Comet Assay

Comet assay was performed on groups of homogenized fly tissues (~10,000 cells per slide). (Group 1: Control, group 2: Xylene, group 3: 0.1 mM TRF, group 4: 2.5% *Aloe vera* +0.1 mM TRF, group 5: 5% *Aloe vera* +0.1 mM TRF, group 6: 10% *Aloe vera* + 0.1 mM TRF) that were administered with TRF and Aloe vera extract for 15 days in order to determine the damage that occurred in DNA. According to the method of Dhawan et al. (2009), the fly samples were decomposed in HBSS (containing 20 mM EDTA and 10% DMSO) solution and the cells were suspended in 0.5% low melting agarose (LMA) [26]. It was added on slides coated with 1% normal melting agarose (NMA) and allowed to freeze. The preparations were kept in the lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris base, pH adjusted to 10, and 1% Triton X-100, 10% fresh DMSO to be added) at 4°C for 1 hour. The preparations were kept in electrophoresis buffer (10N NaOH, 200mM EDTA, pH> 13.0) at 4°C for 15 minutes. Alkaline electrophoresis was applied at 24 V and 300 mA for 40 minutes. The preparations were neutralized in 0.4 M Tris buffer (pH 7.5) for 5 minutes. The preparations were then stained with 100 µL Red Safe (10 µL/mL) and fluorescence microscopy (Zeiss, Germany) was used to examine them [27]. While examining the obtained images, damage records from 0 to 4 were noted in 100 randomly selected cells (0 = undamaged, 4 = highly damaged) [28].

Statistical Analysis

In the study, the Duncan's post-hoc test was applied to evaluate the data obtained from female and male life span and larval toxicity experiments and to evaluate the significance of the difference between the averages by one-way analysis of variance (One-Way ANOVA) (SPSS 20) in intergroup evaluations. The statistical significance of the average was considered as $p<0.05$.

RESULTS

When the data on life span were examined, it was observed that the average life span of male and female individuals increased compared to the control groups (Table 2). It was observed that the life span was reduced in the groups administered with TRF whereas the life span was improved in the TRF + *A. vera* (2.5-5-10%) groups. When female and male individuals were compared to each other, it was seen that male individuals lived longer than female individuals in control and all experimental groups.

When the effect of TRF and *A. vera* on the survival rate of *D. melanogaster* was examined, it was observed that the survival rate of the larvae was reduced at the specified concentration of TRF. In the control group, the 3rd stage larvae were obtained at 86±1.00% concentration. In the xylene group, the 3rd stage larvae were obtained at 18±1.50% concentration, in direct proportion, due to the high proportion of xylene. At TRF+5% *Aloe vera* concentration, the proportion of larvae reaching the 3rd stage decreased. At the TRF+10% *Aloe vera* concentration, the proportion of larvae reaching the 3rd stage increased. At TRF concentration and TRF+2.5% *A. vera* concentration, the proportion of larvae reaching the 3rd stage decreased (Table 1). The rate of *D. melonagaster*'s transition from pupa to adult was analyzed among all experimental groups. The rate of those that matured is 86% in control group, 82% in the TRF experimental group, 63% in the TRF+2.5% *A. vera* experimental group, 84% in the TRF+5% *Aloe vera* experimental group, and 97% in the TRF+10% *A. vera* experimental group (Table 1).

Table 1: Average life span (in days) of female (♀) and male (♂) *D.melanogaster* populations and significance controls between groups. (Group 1: Control, group 2: Xylene, group 3: 0.1 mM TRF, group 4: 2.5% *Aloe vera* +0.1 mM TRF, group 5: 5% *Aloe vera* +0.1 mM TRF, group 6: 10% *Aloe vera* + 0.1 mM TRF)

	♀				♂			
Experiment groups	**N**	**Max. Life (Days)**	**Mean Life span (Day)±S.E**	**Probability Levels Between Groups**	**N**	**Max. Life (Days)**	**Mean Life span (Day)±S.E**	**Probability Levels Between Groups**
Group 1	45	78	72.6±2.50`	1-2**	30	82	76±3	6-1**
Group 2	45	64	60.3±2.45	1-3**	30	77	72±2.25	4-2**
Group 3	45	60	58±0.57	5-2**	30	77	70±3.5	5-2**
Group 4	45	73	68.6±2.09	6-2**	30	94	89.5±2.25	4-1*
Group 5	45	75	71±1.87	4-3**	30	82	80±1.00	4-3**
Group 6	45	82	75.6±2.46	5-3**	30	92	91.5±0.25	6-3**
				6-3**				4-2*
				4-2*				4-5*
								6-5*

Max.: Maximum, S. E.: Standard error, **: The difference between groups is significant at the p<0.001 level. *: The difference between groups is significant at the *p*<0.05 level.

Table 2: The effect of TRF and TRF+ *Aloe vera* on the survival rate of *D.melonagaster*'s larvae. (Group 1: Control, group 2: Xylene, group 3: 0.1 mM TRF, group 4: 2.5% *Aloe vera* +0.1 mM TRF, group 5: 5% *Aloe vera* +0.1 mM TRF, group 6: 10% *Aloe vera* + 0.1 mM TRF)

Experiment groups	**Amount of larvae added**				**Rate of larvae reaching 3. instar (%)±S.E**	**Survival pupal Mean**	**Survival to pupal Mean±S.E**	**Probability Levels Between Groups**	**Survival to adult Mean**	**Survival to adult Mean±S.E**	**Probability Levels Between Groups**
	I. Run		**II. Run**								
	Rate	**Mean**	**Rate**	**Mean**							
Group 1	45/50	%90	41/50	%82	86±1	93.5	93.5±0.25	1-2*	43	43±1	1-2* 1-2**
Group 2[a]	24/50	%48	22/50	%44	18±1.50	9	9±1.50	3-2*	3	3±0.50	3-2* 3-2**
Group 3[b]	30/50	%60	39/50	%78	78±0.5	78.5	78.5±8.75	4-2*	41	41±2.00	4-2* 4-2**
Group 4[b]	50/50	%100	43/50	%86	69±2.25	87.5	87.5±5.25	5-2*	31.5	31.5±6.25	5-2* 5-2**
Group 5[b]	44/50	%88	44/50	%88	88±0	92	92±9.00	6-2*	42	42±2.50	6-2* 6-2**
Group 6[b]	40/50	%80	38/50	%76	93±1.75	99	99±2.00	6-2**	48.5	48.5±2.75	

[a]: During the medium preparation stage, 2% xylene, [b]: TRF was prepared with 1% xylene.
S. E.: Standard error, **: The difference between groups is significant at the *p*<0.001 level.
*: The difference between groups is significant at the *p*<0.05 level.

When examining the Comet images, the damage in tail length of 100 randomly selected cells was rated from 0 to 4. As a result, it was observed that TRF caused an increase in DNA damage in female and male flies whereas the DNA damage decreased, depending on the dose, at the concentrations of TRF+2.5% *A. vera*, TRF+5% *Aloe vera* and TRF+10% *A. vera* (Figure 1).

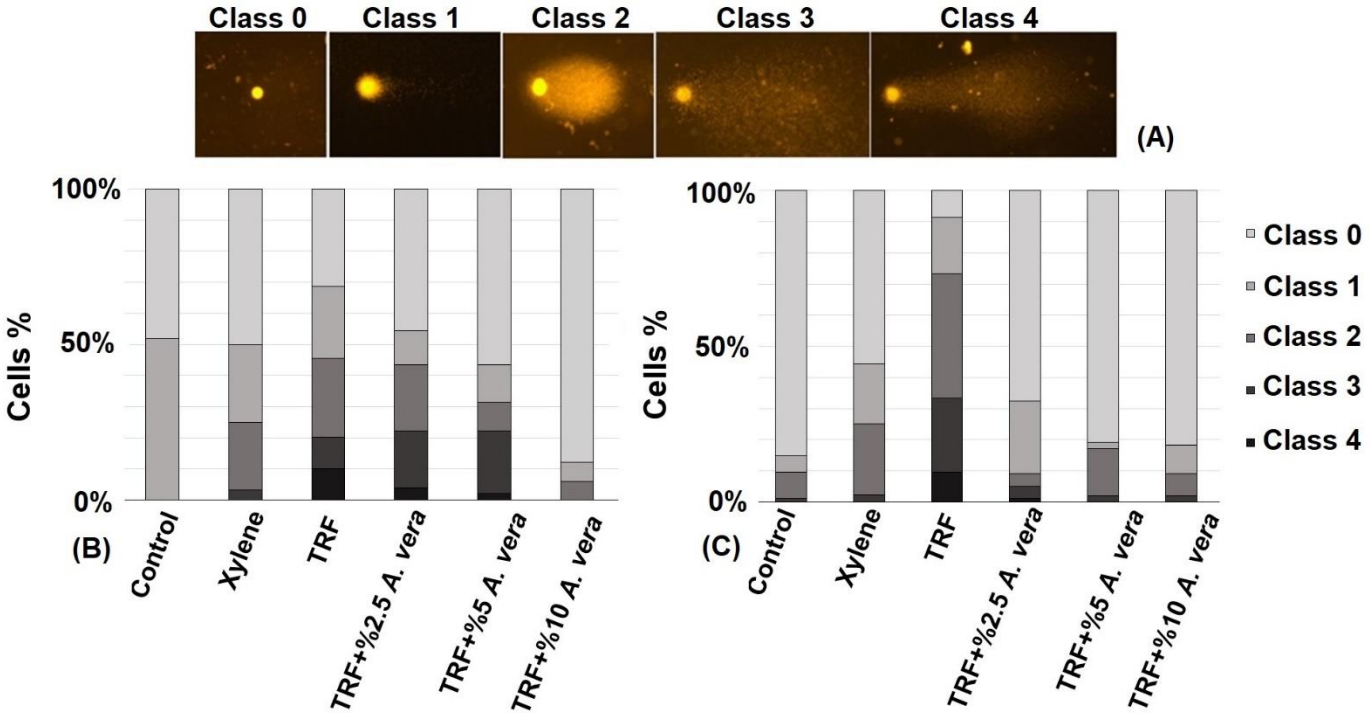

Figure 1. (A) Classified comet image showing DNA damage. (B-C) Graphical representation of the damage caused by exposure of adult female (B) and male (C) *D.melanogaster* populations to different concentrations of TRF (0.1 mM) and *Aloe vera* (2.5% - 5% and 10%) for 15 days (Group 1: Control, group 2: Xylene, group 3: 0.1 mM TRF, group 4: 2.5% *Aloe vera* +0.1 mM TRF, group 5: 5% *Aloe vera* +0.1 mM TRF, group 6: 10% *Aloe vera* + 0.1 mM TRF). (p<0.05)

DISCUSSION

In our study, the protective effect of *A. vera* extract against the toxic and genotoxic effects of TRF (0.1 mM) was investigated. In a study conducted on the genotoxic effect of TRF was investigated in lymphocyte cells of humans and bone marrow cells of mice by using chromosome aberration technique and it was concluded that the use of TRF in an amount that is genetically hazardous should be regulated [29]. It was seen that Olitref herbicide containing 26% TRF had mutagenic effects on the reproductive cells of mice [30]. TRF's genotoxic potential has been shown on maize (*Zea mays*) by using the random amplified polymorphic DNA (RAPD) technique [31]. It was also observed that TRF affected mitochondrial respiration at high concentrations [32]. Similarly, in a study regarding genotoxicity, immunotoxicity and reproductive toxicity of TRF, it was reported that TRF was genotoxic in line with the SMART test on *D. melanogaster* as is the case in our study [33]. It was observed that the survival rate of larvae decreased at the specified concentration of TRF in the larval toxicity study within the scope of our study.

The genotoxic effects of treflan, a commercial form of TRF, on *Oreochromis niloticus*, an economically important fish species, were studied by using the micronucleus test and morphological nucleus irregularity analysis and it was shown that ascorbic acid had a decreasing effect on the genotoxicity caused by TRF and Treflan [34]. It was found out in this study conducted coherently with these studies that *A. vera* extract, a natural antioxidant source, [35] It was seen that *A. vera* leaves had a curative effect against malathion toxicity in rats [36]. It was found that resveratrol and *A. vera* administration to *D. melanogaster* increased the life span [37]. In this study, it was found that the life span of the experimental groups administered with *A. vera* and TRF (0.1 mM) increased.

Protective effect of *A. vera* can be a therapeutic agent potentially for the clinical treatment of sepsis on polymicrobial sepsis in mice [38]. According to another study, *A. vera* gel extract can counteract the damaging effects of BPA on the reproductive system of rats and protects rats' testes against bisphenol-a (BPA)-induced toxicity [39]. It is seen in out study that *A. vera* which is known to have a protective effect counteracts harmful effects of TRF. The rate of larvae reaching the 3rd stage and the rate of maturity increased at TRF+*A. vera* (5-10%) concentration whereas they decreased in the TRF+*A.vera* (2.5%) group. According to the Comet

Assay, it was observed that TRF increased the DNA damage on *D. melanogaster*, and that, when used with TRF+*A. vera* (2.5, 5 and 10%), the DNA damage decreased.

As a result, it is seen that *A. vera* can be used as a preservative alongside TRF and can reduce the harmful effects of TRF, and it is considered that *A. vera* gel has a significant role for human health and vitality and our study contributes to the literature.

Funding: This study was supported by Bartın University, Scientific Research Projects Coordination Unit. Project Number: 2019-FEN-CY-005.
Conflicts of Interest: The authors declare no conflict of interest.
This study was a MSc thesis of O. Celiktas-Kostekci. We thank to Dr. Kemal Büyükgüzel (Bülent Ecevit Universtity, Zonguldak-Turkey).

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