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Research Article

EFFECT OF PYRACLOSTROBIN-BASED HERBICIDE ON DNA DAMAGE AND REPRODUCTIVE PERFORMANCE IN *DROSOPHILA MELANOGASTERS*

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ABSTRACT. The increasingly uncontrolled proliferation of pesticide use today affects the life of all creatures negatively. Pyraclostrobin (Pyra) is a strobilurin class fungicide, which has been widely used in recent years and is known to be toxic to aquatic species. In this study, the effects of Pyra at increased concentrations between 25-1000 µg/L on reproductive performance and genotoxicity of a model organism, *Drosophila melanogaster*, were investigated. It was found that Pyra decreased reproductive performance and pupa formation from larvae in *D. melonagasters* in parallel with the dose increase. In addition, it was found that Pyra concentrations (between 200-1000 µg/L) administered to male and female adults caused DNA damage in *D. melonagasters*, in parallel with their increased concentrations. As a result, it was concluded that Pyra at high concentrations may have a negative effect on *D. melanogasters* and therefore on the ecosystem of the living creatures.

Keywords: *Drosophila melanogaster, DNA damage, larval toxicity, pyraclostrobin, reproductive performance.*

INTRODUCTION

Pesticides are omnipresent environmental pollutants that have adverse effects on water quality, biodiversity and human health [1]. The effects of chronic exposure to low-dose pesticides are not clearly known, but high-dose pesticide exposure increases risk of neurological diseases such as parkinson's disease and cancer [2].

Strobilurins are a new broad-spectrum fungicide that are the most widely used in the world, and attract attention due to their toxicity on non-target organisms [3, 4]. Pyra is in the chemical form of methyl N-{2-[1-(4-chlorophenyl)-1H-pyrazol-3-yloxymethyl]phenyl}(N-methoxy) carbamate [5]. The most significant feature is that it has the ability to effectively eliminate various plant fungi due to the inhibition of mitochondrial respiration [6]. As a result of some studies investigating its concentrations in waters, it was reported that the concentration of Pyra was 0.239 µg/L in Maine, Idaho

and Wisconsin in the USA [7], 1.61 µg/L in Nebraska [8], and 17.24 µg/L in paddy water of China [9] In addition, as a result of some studies, it was reported that Pyra caused oxidative stress together with DNA damage and conditions such as cardiotoxicity and neurotoxicity [10, 11].

In this study conducted on *D. melanogasters*, it was aimed, for the first time, to determine the risk status that Pyra might pose on living organisms with its effect on larval toxicity, reproductive performance and DNA damage, depending on the increased dose.

MATERIALS AND METHODS

In the study was used Prya (Seltima®, BASF, Turkey) which commercially available herbicide containing 100 g/L. It was used Oregon-R wild strain of *D. melanogasters* in laboratory stock, which are genetically homozygous and non-mutant. *D. melanogaster* cultures were grown in a ventilated incubator at a constant temperature of 24 °C in and humidity of 60-70% [14], culture medium cornflour (104), sugar (94), beer yeast (9 g), agar (6 g), distilled water (1020) and acid mixture (to be 6 ml; 8.36 ml propionic acid + 7.83 ml orthophosphoric acid + 1081 ml distilled water) and in the appropriate medium to which the determined Pyra doses are added [12, 13]. Groups of *D. melanogaster* carried out with Pyra at concentrations of 0-1000 µg/L by dissolving in water [15] are shown in Table 1.

Table 1. *Experimental groups*

Group 1	Control
Group 2	Pyra (25 µg/L)
Group 3	Pyra (50 µg/L)
Group 4	Pyra (75 µg/L)
Group 5	Pyra (100 µg/L)
Group 6	Pyra (150 µg/L)
Group 7	Pyra (175 µg/L)
Group 8	Pyra (200 µg/L)
Group 9	Pyra (250 µg/L)
Group 10	Pyra (400 µg/L)
Group 11	Pyra (500 µg/L)
Group 12	Pyra (750 µg/L)
Group 13	Pyra (1000 µg/L)

Larval Toxicity Analysis

For this analysis were performed using (72±4 hours) larvae of *D. melanogaster* which were acquired by crossing male and female individuals in culture medium comprising standard growth medium. The 3rd stage larvae were selected to be used in all experimental groups created. The 100 larvae were inserted in each experimental group from the larvae selected from the stage same. Adult flies were collected from the larval stage to the pupal stage in fifteen-day periods and followed for 60 days. The experiment was repeated three times [16].

Reproductive Performance Analysis

In the experimental stage, a total of 20 flies, including 10 female and 10 male individuals age 1-3 days old which were exposed to Prya at 0-1000 µg/L concentrations for 60 days [17]. The number of live and dead individuals over fifteen-day periods was followed up a daily basis and this continued until the latest individual died and survival rate of live flies expressed as a percentage [18, 19].

Comet Assay

Comet test was performed to determine the effect of Pyra on DNA damage in *D. melanogasters*. For this test male and female individuals were exposed to the medium containing each experimental group treated with Pyra at concentrations of 0-1000 µg/L for 15 days and comet analysis was performed by preparing separate slides from adult *D. melanogasters.* Primarilly 0.5% agarose (LMA) with a low melting temperature was liquided in a 40 °C. Then the slides were covered with 1% normal melting agarose (NMA). Five samples each, which were decomposed in 100 µl of HBSS solution pre-prepared, were mixed with 100 µl of 0.5% LMA, added to each slide coated with NMA, and incubated at 4 °C for 5 minutes. The preparations were kept in the lysis solution (to be added 100 mM EDTA, 2.5 M NaCl, 10 mM Tris base, (pH. 10), and 1% Triton X-100, 10% fresh DMSO) at 4 °C for 1 hour. The slides 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. And then stained with 100 µL Red Safe (10 µL/mL) and fluorescence microscopy (Zeiss, Germany) was used to examine them [20]. When examining the acquired images, damage records from 0 to 4 were noted in 100 haphazardly choosed cells (value of 0—undamaged, 1, 2, 3, or 4—the highest damaged) [21].

Statistical Analysis

In the evaluation of the data obtained as a result of the analyzes; pupal development and reproductive performance data expressed as numbers and percentages. Analysis of DNA damage results was completes using the GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) package program and was used determine the differences between groups One Way ANOVA, for comparisons between groups Dunnett's multiple comparisons test. Significance was attributed at $p<0.05$.

RESULTS AND DISCUSSION

Effect on Larval Toxicity

It was found that, as a result of administration of Pyra, the number of pupae decreased by 32-39% with the amounts administered between 100-400 µg/L and by more than 40% with the amounts administered between 500-1000 µg/L at the end of the 15-day period. It was found that the number of pupae decreased by approximately 30% with the amounts administered between 25-250 µg/L and 31-40% with the amounts administered between 400-1000 µg/L at the end of the 30-day period. It was observed that the number of pupae decreased by 40% with the amounts administered between 25-150 µg/L and 43-52% with the amounts administered between 175-1000 µg/L at the end of the 45-day period. It was

observed that the number of pupae decreased by 40% with the amounts administered between 25-200 µg/L and 44-53% with the amounts administered between 250-1000 µg/L at the end of the 60-day (Table 2).

Effect on reproductive performance

As a result of Pyra administrations, it was found that the number of adult males and females decreased by 50% and more with the amounts of 250 and 400 µg/L and more, respectively, at the end of 15-day period (Table 3), by 50% or more with the amounts of 75 and 50 µg/L and more, respectively, at the end of 30-day period (Table 4), by 50% or more with the amounts of 200 µg/L and more, respectively, at the end of 45-day period (Table 5), and by 50% and more with the amounts of 175 and 100 µg/L and more, respectively, at the end of 60-day period (Table 6).

Table 2. *Number and percentage of pupae formed at the end of 15, 30, 45 and 60 days as a result of Pyra administration*

(µg/L)	15		30		45		60	
	pupa	%	pupa	%	pupa	%	pupa	%
Control	75	100	99	100	148	100	148	100
25	64	85.33	78	78.79	108	72.97	110	74.32
50	63	84.00	78	78.79	99	66.89	103	69.59
75	55	73.33	75	75.76	96	64.86	97	65.54
100	51	68.00	74	74.75	95	64.19	96	64.86
150	50	66.67	73	73.74	90	60.81	92	62.16
175	50	66.67	72	72.73	85	57.43	91	61.49
200	48	64.00	71	71.72	83	56.08	90	60.81
250	46	61.33	70	70.71	83	56.08	84	56.76
400	46	61.33	68	68.69	77	52.03	81	54.73
500	43	57.33	63	63.64	74	50.00	77	52.03
750	40	53.33	61	61.62	72	48.65	77	52.03
1000	39	52.00	60	60.61	72	48.65	70	47.30

Table 3. *Number and percentage of adults formed from pupa after 15 days*

(µg/L)	male	%	female	%	total	%
Control	34	100	31	100	65	100
25	31	91.18	30	96.77	61	93.85
50	31	91.18	28	90.32	59	90.77
75	28	82.35	27	87.1	55	84.62
100	22	64.71	26	83.87	48	73.85
150	20	58.82	24	77.42	44	67.69
175	20	58.82	19	61.29	39	60.00
200	18	52.94	18	58.06	36	55.38
250	17	50.00	17	54.84	34	52.31

Table 3. *(Continues)*

(µg/L)	male	%	female	%	total	%
400	17	50.00	15	48.39	32	49.23
500	16	47.06	15	48.39	31	47.69
750	15	44.11	15	48.39	30	46.15
1000	14	41.18	9	29.03	23	35.38

Table 4. *Number and percentage of adults formed from pupa after 30 days*

(µg/L)	male	%	female	%	total	%
Control	26	100	23	100	49	100
25	14	53.85	16	69.57	30	61.22
50	14	53.85	11	47.83	25	51.02
75	13	50.00	9	39.13	22	44.90
100	13	50.00	10	43.48	23	46.94
150	13	50.00	9	39.13	22	44.90
175	12	46.15	6	26.09	18	36.73
200	12	46.15	8	34.78	20	40.82
250	8	30.77	7	30.43	15	30.61
400	8	30.77	7	30.43	15	30.61
500	7	26.92	5	21.74	12	24.49
750	7	26.92	7	30.43	14	28.57
1000	5	19.23	4	17.39	9	18.37

Table 5. *Number and percentage of adults formed from pupa after 45 days*

(µg/L)	male	%	female	%	total	%
Control	18	100	22	100	40	100
25	14	77.78	18	81.82	32	80.00
50	13	72.22	16	72.73	29	72.50
75	12	66.67	15	68.18	27	67.50
100	12	66.67	14	63.64	26	65.00
150	11	61.11	7	31.82	18	45.00
175	10	55.56	13	59.09	23	57.50
200	9	50.00	10	45.45	19	47.50
250	9	50.00	13	59.09	22	55.00
400	5	27.78	8	36.36	13	32.50
500	5	27.78	10	45.45	15	37.50
750	5	27.78	9	40.91	14	35.00
1000	4	22.22	6	27.27	10	25.00

Table 6. *Number and percentage of adults formed from pupa after 60 days*

(µg/L)	male	%	female	%	total	%
Control	11	100	11	100	22	100
25	10	90.91	10	90.91	20	90.91
50	10	90.91	10	90.91	20	90.91
75	8	72.73	6	54.55	14	63.64
100	7	63.64	5	45.45	12	54.55
150	7	63.64	5	45.45	12	54.55
175	5	45.45	5	45.45	10	45.45
200	5	45.45	5	45.45	10	45.45
250	5	45.45	3	27.27	8	36.36
400	4	36.36	5	45.45	9	40.91
500	4	36.36	1	09.09	5	22.73
750	2	18.18	3	27.27	5	22.73
1000	2	18.18	3	27.27	5	22.73

Effect on DNA damage

DNA damage findings in the groups administered with Pyra in parallel with the increased concentrations were examined, it was found that the administrations between 25-150 µg/L did not cause DNA damage when compared to the control group ($p>0.05$). On the other hand, DNA damage was found to be higher in the groups administered with Pyra between 200 ($p<0.05$), 250 ($p<0.01$) and 400-1000 µg/L ($p<0.001$) compared to the control group (Figure 1).

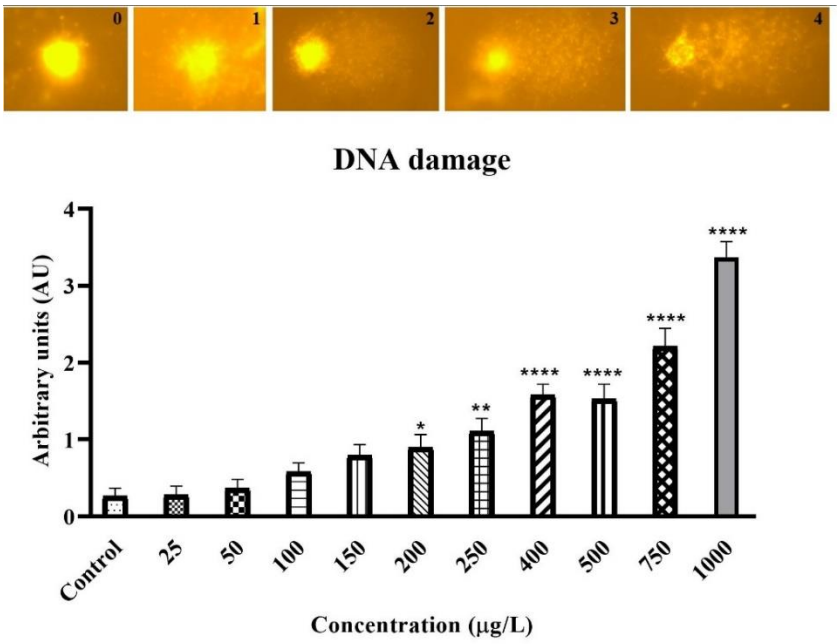

Fig. 1. *The effect of Pyra on DNA damage in D. melanogaster. Values with different letters are statistically significant (p<0.05*, p<0.01**, p<0.001****).*

It has been stated in many studies that pesticides reduce pupal development and reproductive performance on *D. melanogasters*. In this respect, Kissoum et al. (2020) investigated the effect of spiromesifene (Oberon® 240 SC), a pesticide widely used to control pests such as mites and whiteflies, using *D. melanogaster* Meigen, 1830 (Diptera, Drosophilidae) [22]. Morphometric measurements of progeny of surviving adults were evaluated when spiromesifene was topically administered to newly molted pupae at two concentrations (LC10:21.45 and LC25:39.53µg active ingredient/pupa). As a result, it was stated that spiromesifene significantly reduced the number of progeny of parents surviving until fertility, fecundity and pupal treatment. In a study evaluating the effects of nitenpyram, one of the neonicotinoid insecticides, on metabolic parameters in *D. melanogaster*, it was found that nitenpyram, used in one-third and one-tenth of the acute LC_{50} values, significantly reduced the lifespan, pupation rate, occlusion rate, and egg production of *D. melanogasters* [23]. It was stated that the acute dose administration of 6.75 µg/mL (1/3 LC_{50}) of thiamethoxam to *D. melanogaster* extended the time needed for the growth and development of the flies and reduced fertility, pupal and eclipse rates, and life span [24]. It was reported that topical administration of azadirachtin, which is a leading natural pesticide and offers an alternative to traditional insecticides, to *D. melanogasters* in an amount of LD50 (0.63 µg) increased mortality and decreased fertility with mating [25], and that azadirachtin altered reproductive behavior of both male and female in *D. melanogasters* through mating and post-mating periods [26]. In another study investigating the effect of pymetrozine, which is used to control the brown pest, on *D. melanogasters*, it was emphasized that the compound administered at 10 mg/ml and for 24 hours effectively reduced the mating behavior and female fertility of the fruit fly [27]. Similarly, it was found in this study that Pyra, a fungicide, especially at high concentrations (>200 µg/L) reduced the reproductive performance and pupa development of *D. melanogasters*, thus Pyra could have significant effects on the reproduction of insects.

In this study, it was found that the administration of Pyra in amounts >200 µg/L and more might cause DNA damage in *D. melanogasters* and cause genotoxicity. Similarly, it was reported that the administration of 6.75 µg/mL (1/3 LC_{50}) of Thiamethoxam to *D. melanogasters* in acute doses triggered DNA damage [28]. In addition, in a study investigating the genotoxicity of diflubenzuron and spinosad, known as insecticides, in somatic cells of Drosophila, it was stated that diflubenzuron did not cause genotoxic effects, whereas spinosad was effective on hemocytes and caused significant increases in DNA damage [29]. In a study in which *D. melanogasters* were exposed to endosulfan (0.02–2.0 µg/ml) for 12-48 hours through food, it was stated that endosulfan caused DNA damage and its adverse effects were revealed at both cellular and organism level [30]. In a study examination the *in vivo* genotoxicity of artificial pyrethroid cypermethrin in the basal ganglia and anterior midgut of *D. melanogaster*, it was reported that administration of unlike concentrations of cypermethrin (0.0004, 0.0008, 0.002, 0.2 and 0.5 mg/kg) mixed in Drosophila feed caused a significant dose-dependent increase in DNA damage in basal ganglia and anterior midgut cells of *D. melanogasters* [31]. Rajak et al. (2017) reported that acute non-lethal exposure of *D. melanogasters* to Acephate (5 µg/mL) caused DNA fragmentations determined by the comet assay, and acephate triggered genotoxicity [32].

CONCLUSION

As a result, it was found that Pyra administered between 25-1000 μg/ml decreased larval development and reproductive performance of *D. melonagasters* and caused DNA damage in parallel with the increased concentrations. This showed that Pyra, which is widely used as a fungicide, may have negative effects on insects and damage the ecosystem of the living creatures.

Conflict of Interest. The author declared that there is no conflict of interest.

Authorship Contributions. Concept: S.I., F.Z.N, Design: S.I., F.Z.N., Data Collection or Processing: F.Z.N, O.C.K., Analysis or Interpretation: S.I., F.Z.N, O.C.K., Literature Search: S.I., Writing: S.I., F.Z.N.

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