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The Investigation of the Effects of Chlorpyrifos and 2,4-Dichlorophenoxyacetic Acid Application on Bovine Liver Catalase Activity

Hasan KARADAĞ

University of Adiyaman, Faculty of Science and Letters, Department of Chemistry, Adiyaman, TURKEY

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Abstract. In this study, it was investigated whether different concentrations of organophosphate insecticide chlorpyrifos (CPF) and systemic herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) on bovine liver catalase (CAT) activity cause any inhibitions or activations. For this purpose, 25, 50, 100, 250 and 500 ppm concentrations of CPF and 2,4-D were used. Following the applications of all tested concentrations of the both pesticides, the CAT activity elevated. Under the exposure of 25, 50, 100, 250 and 500 ppm concentrations, % CAT activity increases were calculated as 10.0; 6.2; 4.6; 6.9 and 6.0 in CPF apllications, while these increases were calculated as 13.1; 10.3; 17.0; 24.4 and 18.8 in 2,4-D applications, respectively. The present research indicated the elevations in CAT activity with 2,4-D were higher compared to CPF. This means that 2,4-D may have increased hydrogen peroxide production more than CPF.

***Keywords*:** Catalase, chlorpyrifos, 2,4-dichlorophenoxyacetic acid, pesticide.

Klorpirifos ve 2,4-Diklorofenoksiasetik Asit Uygulamasının Sığır Karaciğer Katalaz Aktivitesi Üzerine Etkilerinin İncelenmesi

Özet. Bu çalışmada, organofosfat insektisit klorpirifos (CPF) ve sistemik herbisit 2,4-diklorofenoksiasetik asit (2,4-D)'nin farklı konsantrasyonlarının, sığır karaciğer katalazı (CAT) üzerine herhangi bir inhibisyon ya da aktivasyona neden olup olmadığı araştırılmıştır. Bu amaçla CPF ve 2,4-D'nin 25, 50, 100, 250 ve 500 ppm konsantrasyonları kullanılmıştır. Her iki pestisitin test edilen tüm konsantrasyonlarının uygulanmasını takiben CAT aktivitesi artmıştır. 25, 50, 100, 250 ve 500 ppm konsantrasyonların etkisinde % CAT aktivite artışları CPF uygulamalarında sırasıyla 10.0; 6.2; 4.6; 6.9 ve 6.0 olarak hesaplanmışken bu artışlar 2,4-D uygulamalarında sırasıyla 13.1; 10.3; 17.0; 24.4 ve 18.8 olarak hesaplanmıştır. Sunulan araştırma CAT aktivitesi artışlarının CPF'ye oranla 2,4-D uygulamalarında daha yüksek olduğunu göstermektedir. Bu 2,4-D'nin hidrojen peroksit üretimini CPF'ye göre daha fazla arttırmış olabileceği anlamına gelmektedir.

***Anahtar Kelimeler*:** Katalaz, Klorpirifos, 2,4-diklorofenoksiasetik asit, pestisit.

1. INTRODUCTION

Chlorpyrifos (O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphonothioate) (CPF) is organophosphate insecticide that inhibits acetylcholinesterase activity. CPF has been used both in a domestic and agricultural (outdoor) environment. Due to the fact that it has been blamed of causing retardation of the mental development of children, it was banned in most houses in the U.S in 2001 [1]. 2,4-Dichlorophenoxyacetic acid (2,4-D) is systemic herbicide. It is used to control many types of broadleaf weeds. 2,4-D is possible carcinogen for humans and experimental animals [2] and leads to fertility problem at men [3].

Pesticides may cause formation of reactive oxygen species (ROS), which may lead to oxidative stress [4]. Antioxidant defense systems such as catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) are involved to prevent

* Corresponding author. *Email address:* *hkaradag@adiyaman.edu.tr*

ROS toxicity [5]. Hydrogen peroxide is non-radical species of ROS [6]. Catalase from bovine liver (CAT) (E.C.1.11.1.6.) catalysis decomposition of hydrogen peroxide to water and oxygen [7]. Hydrogen peroxide damages cells. To prevent this, catalase is often used by cells [8].

Pesticides increase agricultural production, however, bioaccumulation through the food chain can finally become a risk to mammals because of their negative effects [9–12]. CPF and 2,4-D are used in agricultural areas. Both pesticides can be found together in the environment. CPF and 2,4-D are toxic to humans and animals [1, 2, 3]. Both compounds can alter enzyme activities. Because of that, it was designed a study on the effects of both pesticides on bovine liver catalase activity.

2. MATERIAL AND METHOD

2.1. Chemicals

Catalase from bovine liver (C-1345), chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$) (45395) (Fig.1.a) and 2,4-dichlorophenoxyacetic acid ($C_8H_6Cl_2O_3$) (D70724) (Fig.1.b) were bought from Sigma-Aldrich. All other chemicals used were analytical grade.

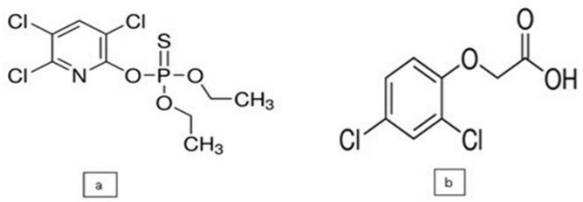

Figure 1. Structures of chlorpyrifos (a) and 2,4-dichlorophenoxyacetic acid (b).

2.2. Protein Determination

The protein concentration was measured spectrophotometrically at 750 nm by the method of Lowry et al., 1951 [13]. Bovine serum albumin was used as a standard for the determination of protein concentration. For this purpose, four solutions were prepared: 1. Solution (A): 0.5 g $CuSO_4.5H_2O$ and 1 g sodium citrate dihydrate were dissolved at distilled water and completed to 100 ml; 2. Solution (B): 20 g Na_2CO_3 and 4 g NaOH were dissolved at distilled water and completed to 1000 ml; 3. Solution (C): 1ml solution A was added to 50 ml solution B; 4. Solution (D): 10 ml Folin-Ciocalteu was added to 10 ml distilled water. After that, 2.5 ml solution C was added to 0.5 ml of prepared sample solution, vortexed, waited for 10 minutes at room temperature, mixed with 0.25 ml solution D, vortexed, waited for 30 minutes and read at 750 nm for protein concentration determination.

2.3. CAT Activity

CAT activity was measured according to the method of Lartillot et al., 1988 [14] which is studied by Bergmeyer, 1974 [15] previously. CAT activity was determined spectrophotometrically at 240 nm using a specific absorption coefficient at 0.040 cm^2 μmol^{-1} H_2O_2. 2.5 mL of substrate solution of 10 mM of H_2O_2 in 50 mM, pH=7.5 phosphate buffer (Tukel and Alptekin, 2004 [16]) and 20 µL of CAT solution of about 0.5 mg protein mL^{-1} were mixed at 20 °C for 2 min and the reaction was stopped by adding 0.5 mL of 1 M HCl. CAT activity was calculated as µmol H_2O_2 decomposition. mg $protein^{-1}$ min^{-1} or (U mg^{-1}).

2.4. Effect of Pesticides on Enzyme Activity

Firstly, 5000 ppm CPF was prepared in 2 ml absolute ethyl alcohol and 5000 ppm 2,4-D dissolved in 2 ml 95 % ethyl alcohol. Because of low solubility of CPF and 2,4-D in water, CPF was prepared in absolute ethyl alcohol and 2,4-D was prepared in 95 % ethyl alcohol, because 2,4-D is polar according to CPF. Then, these 5000 ppm pesticide concentrations were arranged to 25, 50, 100, 250 and 500 ppm [17]. 25, 50, 100, 250 and 500 ppm CPF with ethyl alcohol plus 700 µL sample solution of about 0.5 mg protein mL^{-1} were prepared and 25, 50, 100, 250 and 500 ppm 2,4-D with 95 % ethyl alcohol plus 700 µL sample solution of about 0.5 mg protein mL^{-1} were prepared. At 0 ppm or control, 300 µL absolute ethyl alcohol plus 700 µL sample solution for CPF were used and 300 µL 95 % ethyl alcohol plus 700 µL sample solution for 2,4-D were used. My solution volumes of enzyme and ethyl alcohol and pesticide were 1 ml [17]. The mixture was incubated at room temperature for half hour. Then, activities of CAT were determined.

2.5. Data Analysis

The data are presented as mean±standard deviation. For the statistical analyses, one-way analysis of variance (ANOVA) was used, followed by the Student Newman-Keul's test using the SPSS version 21 statistical software (SPSS Inc., Chicago, IL, USA). Differences were considered significant if $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of Chlorpyrifos on CAT Activity

Different CPF concentrations with CAT enzyme solutions were prepared, then, activities of CAT were measured as reported in Table 1. Compared to the control activity, there are statistical differences between all CAT activities which interacted with the CPF ($p<0.05$, n=3). Under the exposure of 25, 50, 100, 250 and 500 ppm CPF concentrations, % CAT activity increases were calculated as 10.0; 6.2; 4.6; 6.9 and 6.0 %, respectively.

3.2. Effect of 2,4-Dichlorophenoxyacetic acid on CAT Activity

Different 2,4-D concentrations with CAT enzyme solutions were prepared, then, activities of CAT were measured as reported in Table 1. When compared to the control activity, there are statistical differences between all CAT activities which interacted with the 2,4-D ($p<0.05$, n=3). Under the exposure of 25, 50, 100, 250 and 500 ppm 2,4-D concentrations, % CAT activity increases were calculated as 13.1; 10.3; 17.0; 24.4 and 18.8 %, respectively.

Table 1. Effect of chlorpyrifos and 2,4-dichlorophenoxyacetic acid concentrations on catalase activity.

Concentration (ppm)	Effect of CPF on CAT Activity ± SD (U mg^{-1})	Effect of 2,4-D on CAT Activity ± SD (U mg^{-1})
0	1426 ± 38^{a}	1297 ± 15^{a}
25	1569 ± 12^{b}	1467 ± 9^{b}
50	1514 ± 19^{c}	1430 ± 24^{c}
100	1491 ± 12^{c}	1518 ± 16^{d}
250	1524 ± 11^{c}	1614 ± 7^{e}
500	1512 ± 6^{c}	1541 ± 18^{d}

Data is expressed as mean ± standard deviation (n = 3). At the table “a, b, c, d and e” letters are used for differences of activity levels. There are statistical differences between data which are shown with different letters ($p< 0.05$).

3.3. Comparison of Chlorpyrifos and 2,4-Dichlorophenoxyacetic acid on CAT activity

The percentages of both CAT activities were increased under the exposure of 25, 50, 100, 250 and 500 ppm CPF and 2,4-D concentrations. But, increasing CAT activity with 2,4-D was higher compared to CPF. 2,4-D increased the percentages of CAT activity more than 3.13 times compared to that of CPF at 500 ppm (Fig.2).

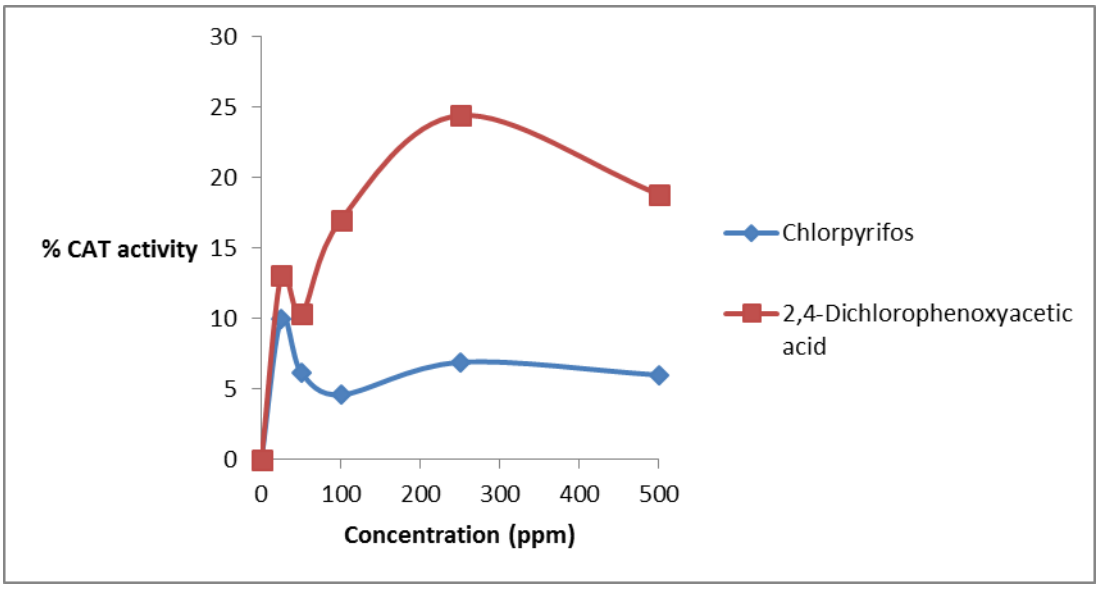

Figure 2. Comparison of chlorpyrifos and 2,4-dichlorophenoxyacetic acid on catalase activity.

Reactive oxygen species (ROS) generation increases in pesticide toxicity [18]. Hydrogen peroxide is a form of ROS. When hydrogen peroxide occurs, CAT converts hydrogen peroxide to water and oxygen. Increases at CAT activity might be response to production of hydrogen peroxide or ROS [19].

Karadag et al. [20-22] applied various pesticides (cyprodinil and fludioxonil; deltamethrin and alpha cypermethrin; lambda cyhalothrin and fenthion) on bovine liver catalase. When they applied pesticide concentrations from 0 to 500 ppm, they reported enzyme inhibitions. In contrast to these, in the present study, the enzyme activation was observed.

CPF increased CAT activity. For instance, Cacciatore et al., 2015 [23] found increased CAT activity (22 % of increase) in the soft tissues of freshwater gastropod (*Planorbarius corneus)* after 7.5 µg L^{-1} of CPF exposure. Similarly, Wu et al., 2011 [24] showed that CPF at the concentration of 0.12 µg $µL^{-1}$ increased significantly CAT activity by 85 % of the control in thunberg (*Oxya chinensis*). In the same way, Kaur and Sandhu, 2008 [25] exposed buffalo (*Bubalus bubalis)* calves to CPF at a dose of 0.05 mg kg^{-1} per day for 20 consecutive weeks. They found that the enzymatic activity of catalase (CAT) (63.8 %) elevated. Jin et al., 2015 [26] showed considerable increases of CAT activity at the 30 and 100 µg L^{-1} CPF groups in the larval zebrafish (*Danio rerio*). When Aly et al. 2010 [27] administered orally 13.495 mg/kg (1/10 LD_{50}) CPF to male mice, they reported 1.41 fold CAT activation. This activation is higher compared to the findings of this study. After application of 25, 50, 100, 250 and 500 ppm CPF concentrations, 1.100, 1.062, 1.046, 1.069, 1.060 fold CAT activations were found respectively.

24-D increased CAT activity as well. For example, Oruc et al., 2004 [28] treated 2,4-D to *Cyprinus carpio* for 96 h. They indicated 1.66 fold rise in catalase activity in kidney of *Cyprinus carpio*. At another work, Atamaniuk et al., 2013 [29] treated 100 mg L^{-1} of 2,4-D to goldfish (*Carassius auratus)*. 2,4-D increased catalase activity (by 41 % or 1.41 fold). This activations are higher compared to the findings of present study. After application of 25, 50, 100, 250 and 500 ppm 2,4-D concentrations, 1.131; 1.103; 1.170; 1.244 and 1.188 fold CAT activations were found respectively.

4. CONCLUSION

The experiments were carried out to determine the effect of CPF and 2,4-D pesticides on bovine liver catalase activity. Both pesticides activated CAT activity at 25, 50, 100, 250 and 500 ppm pesticide concentrations. These results indicate that after the exposure of CPF and 2,4-D pesticides in living organism, hydrogen peroxide which is harmful for cells might be produced. In conclusion, when hydrogen peroxide increases due to pesticide exposure, catalase enzyme activity increases too. These results at the same time important for defense system of cells.

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