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In Vitro effects of pesticide exposure on Bovine liver catalase activity

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In this work we investigated the inhibitory effect of Deltamethrin, Dichlorvos, Malathion and Lambda-cyhalothrin on bovine liver catalase (CAT) activity. We observed that the inhibition of enzyme increased with increasing concentrations of the pesticides from 0 to 250 ppm. The Kinetics conformed to the Michaelis-Menten model and a Lineweaver-Burk graph of CAT was drawn. To identify the inhibition type, Vmax and Km were calculated at different concentrations of Deltamethrin, Dichlorvos, Malathion and Lambda-cyhalothrin. It was documented that dichlorvos and malathion inhibited CAT competitively, whereas deltamethrin and Lambda-cyhalothrin inhibition over CAT were non-competitive.

Keywords: deltamethrin; dichlorvos; lambda-cyhalothrin; malathion; catalase; inhibition; pesticide

INTRODUCTION

Catalase (CAT, EC 1.11.1.6) is an important antioxidant enzyme in organisms which can catalyze H_2O_2 to H_2O and O_2 to maintain the redox balance. Catalase has one of the highest turnover rates of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen [1]. The intake of pollutants might change the activity of CAT due to the co-effects of oxidative stress or damages on enzyme structure and function caused by direct binding [2].

Dichlorvos is a highly volatile organophosphate that is extensively used as an insecticide to control household pests, in public health and to protect stored products from insects. Additionally, dichlorvos damages the DNA of insects [3].

Deltamethrin is a pyrethroid and a broad-spectrum insecticide. Deltamethrin is registered for use on various crops including cotton, corn, cereals, soybeans and vegetables for pests such as mites, ants, weevils and beetles [4].

Malathion is an organophosphate parasympathomimetic which binds irreversibly to cholinesterase. Malathion is an insecticide of relatively low human toxicity. Malathion is widely used in agriculture, residential landscaping, public recreation areas and in public health pest control programs such as mosquito eradication [5].

Lambda-cyhalothrin is an insecticide that belongs to the pyrethroid chemical class of pesticides. It is a mixture of highly active isomers of cyhalothrine and is used to control a wide range of pests in a variety of applications [6].

In this study the inhibition of CAT by deltamethrin, dichlorvos, malathion and Lambda-cyhalothrin was observed.

EXPERIMENTAL

Catalase from bovine liver (lyophilized powder, ≥10,000 units/mg protein), deltamethrin, dichlorvos, malathion, lambda-cyhalothrin and all reagents and chemicals were purchased from Sigma-Aldrich and were prepared analytically.

CAT activity measurement

CAT activity was measured in accordance with the method of Aebi [7]. The principle of the assay is based on the determination of the rate constant (s^{-1}, k) of hydrogen peroxide decomposition by the CAT enzyme at 240nm.

Protein measurement

The protein concentration was determined using the method of Lowry et al. [8].

Effects of pesticides on enzyme activity

All pesticides were dissolved in ethyl alcohol. Stock pesticide solutions were arranged to match 0, 50, 100 and 250 ppm and then each of these was mixed with a 750 µl enzyme solution. The final volume of the mixture was 1 ml. The mixture was incubated at room temperature for 30 min and then the activities of CAT were measured.

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RESULTS AND DISCUSSION

Inhibition type of deltamethrin to CAT

The activities of CAT were measured in the presence of different concentrations of *deltamethrin* (0, 50, 100 and 250 ppm) for different substrate concentrations of H_2O_2 (5, 10, 15, 20, 25 and 30 mM)(n=3). The activity of CAT decreased with increasing concentrations of *deltamethrin*. The Kinetics conformed to the Michaelis-Menten model and a Lineweaver-Burk graph of CAT was drawn by using the obtained results (Figure 1).

To identify the inhibition type, according to Figure 1, Vmax and Km were calculated at different concentrations of *deltamethrin* and are shown in Table 1. From the Lineweaver-Burk graph, we determined that the Km values were close to each other but we found that the Vmax values decreased. Thus, we suggest that *deltamethrin* inhibited CAT non - competitively.

Inhibition type of lambda-cyhalothrin to CAT

The activities of CAT were measured in the presence of different concentrations of Lambda-cyhalothrin (0, 50, 100 and 250 ppm) for different substrate concentrations of H_2O_2 (5, 10, 15, 20, 25 and 30 mM)(n=3). The activity of CAT decreased with the increasing concentrations of lambda-cyhalothrin.

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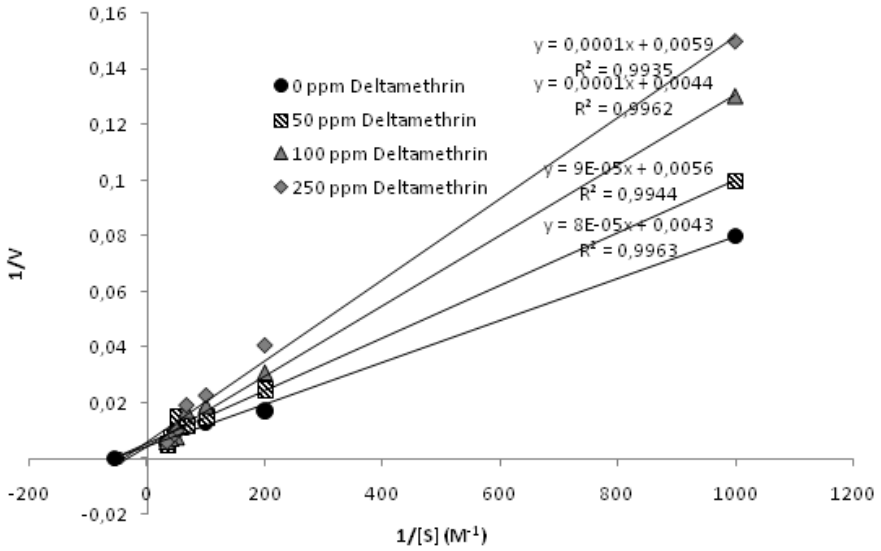

Fig 1. Lineweaver-Burk graph of CAT treated with deltamethrin.

Table 1. Effects of deltamethrin, lambda-cyhalothrin, dichlorvos and malathion on the kinetic parameters of CAT.

Concentrations of Pesticides	V_{max} (U/mg)	K_m (M H_2O_2)
0ppm Deltametrin	222.22	0.0181
50ppm Deltametrin	178.57	0.0161
100ppm Deltametrin	227.27	0.0227
250ppm Deltametrin	169.49	0.0169
0ppm Lambda-cyhalothrin	222.22	0.0181
50ppm Lambda-cyhalothrin	256.41	0.026
100ppm Lambda-cyhalothrin	238.09	0.0238
250ppm Lambda-cyhalothrin	181.81	0.036
0ppm Dichlorvos	222.22	0.0181
50ppm Dichlorvos	238.09	0.0238
100ppm Dichlorvos	250.00	0.05
250ppm Dichlorvos	285.71	0.0571
0ppm Malathion	222.22	0.0181
50ppm Malathion	250.00	0.025
100ppm Malathion	208.33	0.021
250ppm Malathion	227.27	0.045

The Kinetics conformed to the Michaelis-Menten model and a Lineweaver-Burk graph of CAT was drawn by using the obtained results (Figure 2).

To identify the inhibition type, according to Figure 2, Vmax and Km were calculated at different concentrations of lambda-cyhalothrin and are shown in Table 1. From the Lineweaver-Burk graph, we determined that the Km values are close to each other but we found decreased Vmax values. Thus, we suggest that lambda-cyhalothrin inhibited CAT non-competitively.

Inhibition type of dichlorvos to CAT

The activities of CAT were measured in the presence of different concentrations of dichlorvos (0, 50, 100 and 250 ppm) for different substrate concentrations of H_2O_2 (5, 10, 15, 20, 25 and 30mM)(n=3). The activity of CAT decreased with the increasing concentrations of dichlorvos. The Kinetics conformed to the Michaelis-Menten model and a Lineweaver-Burk graph of CAT was drawn by using the obtained results (Figure 3).

To identify the inhibition type, according to Figure 3, Vmax and Km were calculated at different concentrations of dichlorvos and are shown in Table 1. From the Lineweaver-Burk graph, we determined that the Vmax values are close to each other but we found increased Km values. Thus, we suggest that dichlorvos inhibits CAT competitively.

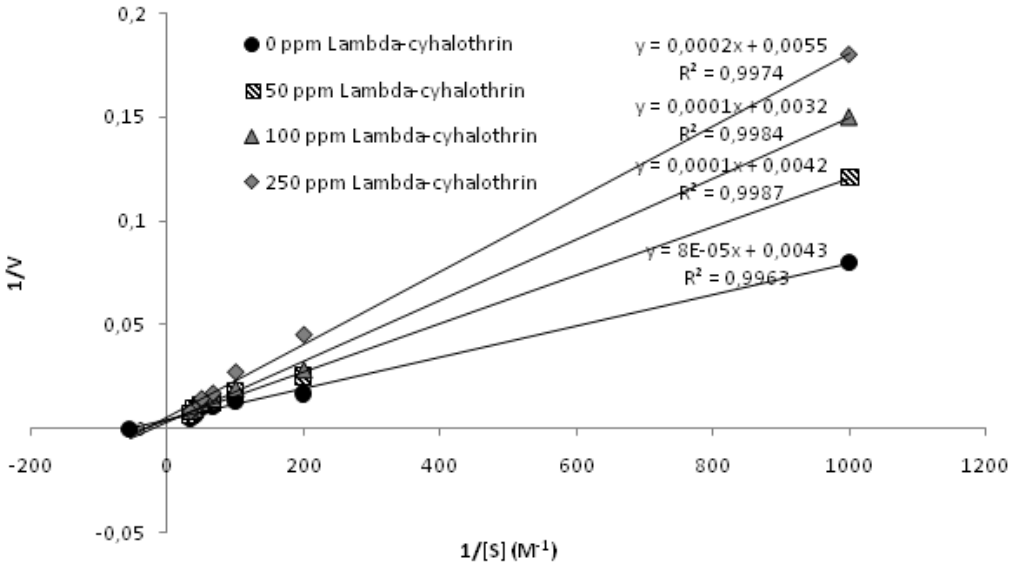

Fig 2. Lineweaver-Burk graph of CAT treated with lambda-cyhalothrin

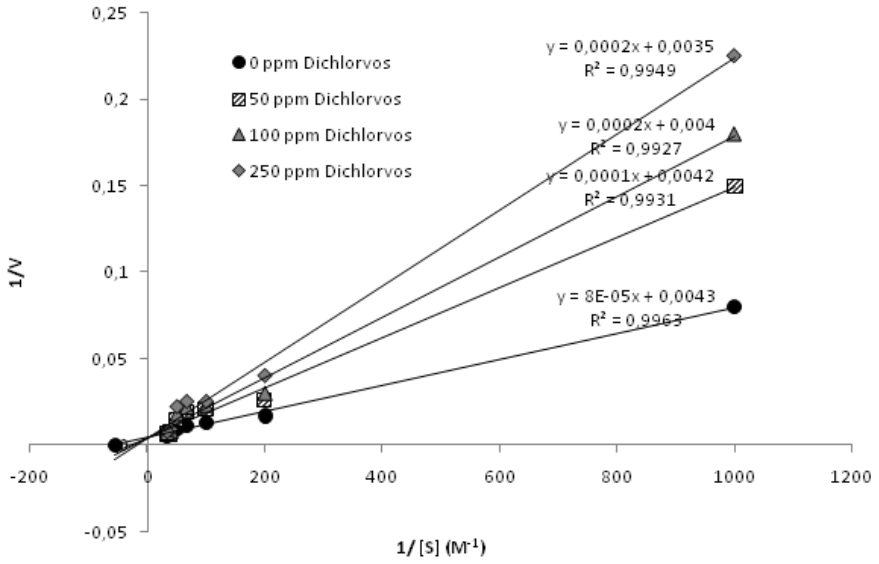

Fig 3. Lineweaver-Burk graph of CAT treated with dichlorvos

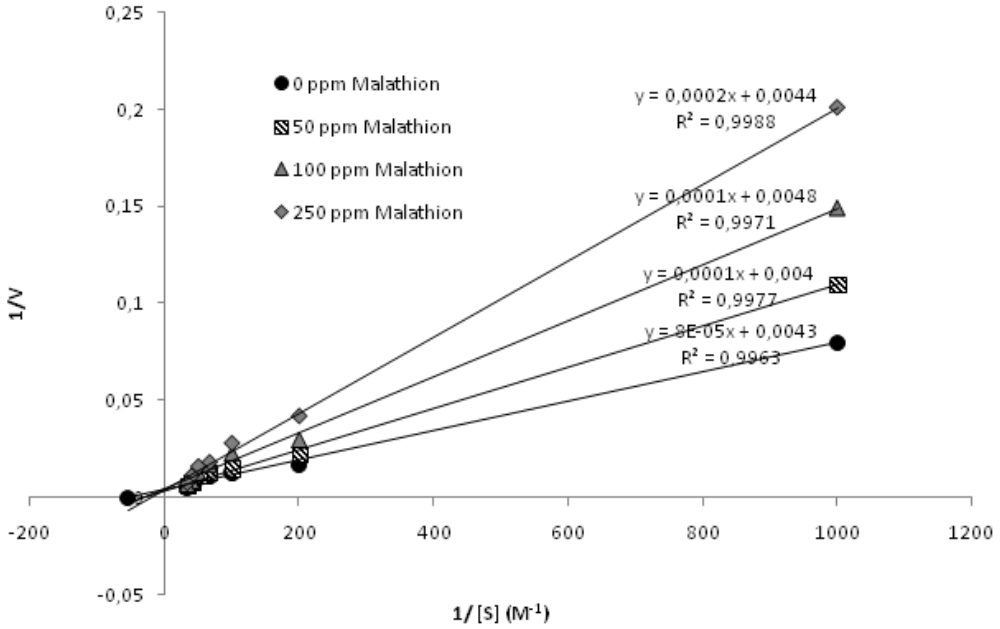

Fig 4. Lineweaver-Burk graph of CAT treated with malathion.

Inhibition type of malathion to CAT

The activities of CAT were measured in the presence of different concentrations of malathion (0, 50, 100 and 250 ppm) for different substrate concentrations of H_2O_2 (5, 10, 15, 20, 25 and 30 mM)(n=3). The activity of CAT decreased with increasing concentrations of malathion. The Kinetics conformed the Michaelis-Menten model and a Lineweaver-Burk graph of CAT was drawn by using the obtained results (Figure4).

To identify the inhibition type in accordance with Figure 4, Vmax and Km were calculated at different concentrations of malathion and are shown in Table 1. From the Lineweaver-Burk graph, we determined that the Vmax values are close to each other but we found increased Km values. Thus, we suggest that malathion inhibits CAT competitively.

The effects of different chemical substances, drugs, metal ions and anions on CAT have been investigated in many *in vitro* and *in vivo* studies, performed with various organisms.

T. Hamers et. al. observed that Dichlorvos at low concentrations inhibited acetylcholinesterase (AChE) competively [9]. M. A. Kamal observed malathion inhibited AChE non-competitively [10]. They reported similar results for AChE but there isn't any kinetic study for CAT inhibition by these pesticides. In addition to this, other studies also examined the inhibition of CAT by pesticides.

D. Julka et. al. [11] observed that treatment by dichlorvos resulted in significant decreases in the activities of the antioxidant enzymes SOD and CAT which were accompanied by a decrease in the values of lipid peroxidation. B. Mazmancı et. al. [12] and M. Berköz et. al. [13] have reported deltamethrin to cause a decrease in the activities of CAT, SOD and GPx.

E. Büyükgüzel [14] observed that treatment by malathion resulted in decreases of the activities of CAT and GPx. H. Fetoui et. al. [15] have reported the activities of CAT, SOD, GPx, glutathione reductase (GR) and glutathione-S-transferase (GST) were significantly decreased due to lambda-cyhalothrine exposure. We obtained similar results in the present study.

CONCLUSION

In this study we wanted to elucidate the kinetic parameters of H_2O_2 as a substrate and some pesticides as inhibitors, as well as the inhibition type. Kinetic studies were performed at very low concentrations of H_2O_2 and very short reaction times. The results showed that bovine liver catalases are inhibited by malathion, dichlorvos, lambda cyhalothrin and deltamethrin *in vitro*. It was shown that the catalytic degradation of H_2O_2 is inhibited competitively by malathion and dichlorvos but non-competitively by deltamethrin and lambda cyhalothrin.

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In vitro ЕФЕКТ НА ПЕСТИЦИДИ ВЪРХУ АКТИВНОСТТА НА КАТАЛАЗА ОТ ГОВЕЖДИ ЧЕРЕН ДРОБ

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(Резюме)

В тази работа е изследван инхибиращия ефект на пестицидите делтаметрин, малатион, дихлорвос и ламбда-цихалотрин върху активността на каталаза (CAT), изолирана от говежди чер дроб. Забелязахме, че инхибирането на ензима нараства с нарастването на концентрациите на пестицидите от 0 до 250 ppm. Кинетиката е в съгласие с уравнението на Михаелис-Ментен, което е потвърдено в координатите на Lineweaver-Burk. Характерът на инхибирането е различен, като при дихлорвосът и малатионът са конкурентни инхибитори, а делтаметринът и ламбда-цихалотринът - неконкурентни.