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Inhibition and Restoration of Esterase Activity in Freshwater Snails after Exposure to the Pesticides; Profenofos, Carbofuran, and Thiamethoxam

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Abstract

Pesticides are important in farming operations as they ensure crop yields of high quality and quantity. Aerial drifts of these chemicals during spraying direct them to non-target aquatic reservoirs where they adversely affect aquatic fauna and flora. The recovery of aquatic snails Helisoma duryi from toxicological effects of exposure to pesticides was investigated. The snails were exposed to 0.5 ppb of profenofos, carbofuran and thiamethoxam for up to 28 days. Fifteen snails were removed from each exposure tanks on days 1, 14 and 28 and stored. After 28 days of exposure the remaining snails were transferred to pesticide-free water for an additional 28 days. On days 14 and 28, snails were sampled from each exposure tank and stored. Esterase activity was analysed in all samples. Inhibitions of esterase activities were observed in all pesticide-exposed snails. Carbofuran caused the highest inhibitions. Esterase activity returned to ≥ 90% of normal levels after 28 days of recovery in snails exposed to profenofos and thiamethoxam. The results of this study indicate that aquatic snails can recover from effects of pesticides exposure. Proper management and spacing of pesticide applications are suggested to protect the health of non-target aquatic organisms.

Keywords

Pesticides, Freshwater Snails, Enzymes, Water Pollution

1. Introduction

The use of pesticides continues to be the most cost-effective and efficient approach to controlling pests in farming systems. The growing global population has placed

significant pressure on agricultural systems, driving increased demand for pesticides to maintain both the quality and quantity of food crops. Pesticides, which are chemical agents specifically designed to eliminate plant and animal pests, play a crucial role in enhancing crop yields and ensuring food security in many nations [1]-[3].

Pesticides are intentionally used in agriculture and public health to safeguard crops and livestock from diseases caused by pests [4]-[6]. In Zimbabwe, pesticides like organophosphates, carbamates, and neonicotinoids are commonly used to control various pests. Farmers typically apply pesticides to farmland through spraying and aerial drifts disperse and transports these agrochemicals long distances. Consequently they end up in aquatic and terrestrial environments and exert toxic effects on living organisms in these ecosystems. Most farmers in Zimbabwe, particularly those in the small-scale category, practice monoculture, which promotes the extensive use of pesticides to avoid productivity losses due to diseases.

Extensive use of agrochemicals negatively impacts living organisms in their environments. Al-Ghanim *et al.* [5] demonstrated that fenvalerate induces oxidative stress in zebrafish, significantly reducing the activity of antioxidant enzymes such as superoxide dismutase and catalase. Similarly, organophosphorus insecticides have been found to be toxic to fish, causing internal organ damage, immunotoxicity, and endocrine dysfunction [7]. Wee and Aris, [8] reported developmental defects and oxidative stress effects in zebrafish exposed to chlorpyrifos. Despite the significant increase in pesticide use in Zimbabwe's agro-land, there is insufficient research on the effects of these agricultural chemicals on both aquatic and terrestrial ecosystems.

Research on the effects of agrochemicals, including thiamethoxam a pesticide widely used in Zimbabwe for its effectiveness during drought conditions is highly limited. Regular monitoring of pesticide levels and their impacts on terrestrial and freshwater ecosystems is crucial to safeguard the health of both aquatic and terrestrial organisms. This study aimed to evaluate the effects of three commonly used pesticides in Zimbabwe on freshwater ecosystems, using esterase activity in the water snail *Helisoma duryi* as a biomarker of exposure.

2. Materials and Methods

2.1. Chemicals

All enzymes, and chemicals were of analytical grade and were supplied by Sigma Aldrich Chemical Company, Germany.

2.2. Snail Breeding and Exposure

Snails were bred in outdoor cement aquaria filled with municipal water and were fed pesticide-free lettuce twice a week. In this study, groups of 80 freshwater snails were exposed to sublethal concentrations (0.5 ppb) of chlorpyrifos, profenofos, and carbofuran over a 28-day period. The control snails were exposed to pesticide-free water. Due to the lack of existing data on the specific concentrations for the pesticides under investigation, preliminary 96-hour screening tests were first con-

ducted to determine the LD50 values. Based on these findings, a sublethal concentration of 0.5 ppb equivalent to 10% of the LD50 was selected for use in the exposures. On days 1, 14, and 28, 15 snails were sampled from each exposure group to prepare post-mitochondrial fractions. After the 28-day exposure period, the remaining snails were transferred to pesticide-free water for a recovery phase lasting 28 days. During the recovery phase, additional samples of 15 snails were collected from each group on days 14 and 28 to prepare post-mitochondrial fractions.

2.3. Homogenate Preparation and Protein Determination

Snails from each group were combined and homogenized in an ice-cold 0.1 M potassium phosphate buffer (pH 7.4). The homogenates were then centrifuged at 10,000 × g for 10 minutes, and the resulting supernatant (S-10 fraction) was collected and stored at −80˚C until analysis. Protein content in the post-mitochondrial fractions was determined using the Lowry *et al.* [9] method, with bovine serum albumin (BSA) as the calibration standard. Absorbance was measured at 750 nm.

2.4. Cholinesterase Activity

Cholinesterase activity was measured using the method of Ellman *et al.* [10] adapted for a microplate reader as described by Kallander *et al.* [11]. In each well of a microplate, the following reagents were added: 50 µL of 0.1 mg/mL post-mitochondrial fraction (PMF), 110 µL of 0.01 M Tris-HCl buffer (pH 8.0), and 50 µL of 0.4 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). The mixture was incubated for 3 minutes, after which 30 µL of 0.5 mM acetylthiocholine iodide was added. The formation rate of the thiocholine-DTNB complex was monitored for 3 minutes at 412 nm using a SpectraMax 340pc plate reader.

2.5. Carboxylesterase Activity

Non-cholinesterase activity was measured using the method of Mackness *et al.* [12]. In a 5 mL test tube, the following reagents were combined: 4 mL of reagent A (prepared by mixing 1 mL of 25 mg/mL α-naphthyl acetate with 100 mL of 50 mg Fast Blue RR salt dissolved in 0.1 M Tris-HCl buffer, pH 7.4) and 20 µL of 0.1 mg/mL post-mitochondrial fraction (PMF). The mixture was incubated in the dark for 10 minutes. The reaction was terminated by adding 1 mL of 20% (v/v) acetic acid, and absorbance was measured at 605 nm against an appropriate blank.

2.6. Statistical Analysis

Data were presented as mean ± SD. Statistical differences among the various exposure groups and between exposure groups and the control were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, performed with GraphPad Prism 8 software.

3. Results

3.1. Carboxylesterase Activity

All the different pesticides caused inhibitions of carboxylesterase activity in pesti-

cide-exposed snails and the inhibitions increased with an increase in exposure durations (Figure 1). The degree of inhibitions generally followed the trends, carbamates > organophosphates > neonicotinoids (Figure 1).

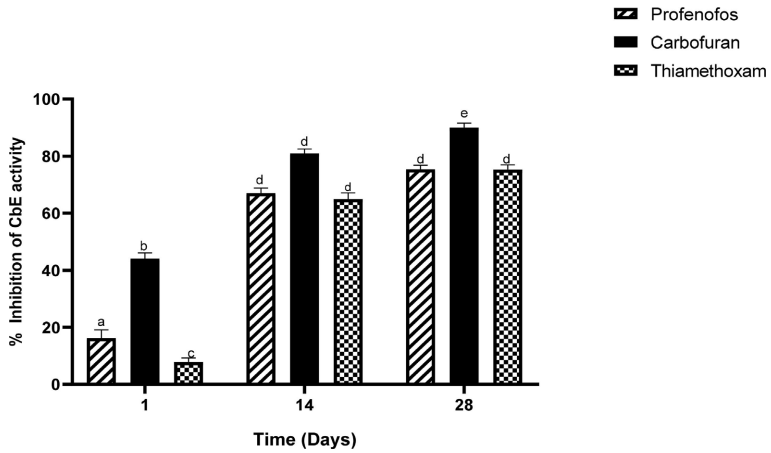

Figure 1. Effect of different exposure duration of different pesticides on carboxylesterase activity of the aquatic snail Helisoma duryi. Values represent the average of triplicate samples which are expressed as mean + SD. The different letters on the bars indicate significant differences and bars with the same letters indicate there was no significant difference.

3.2. Cholinesterase Activity

Time-dependent increases in inhibition of cholinesterase activities were observed in snails exposed to all the different classes of pesticides (Figure 2). For all exposure durations, the highest enzyme activity was caused by carbofuran (Figure 2).

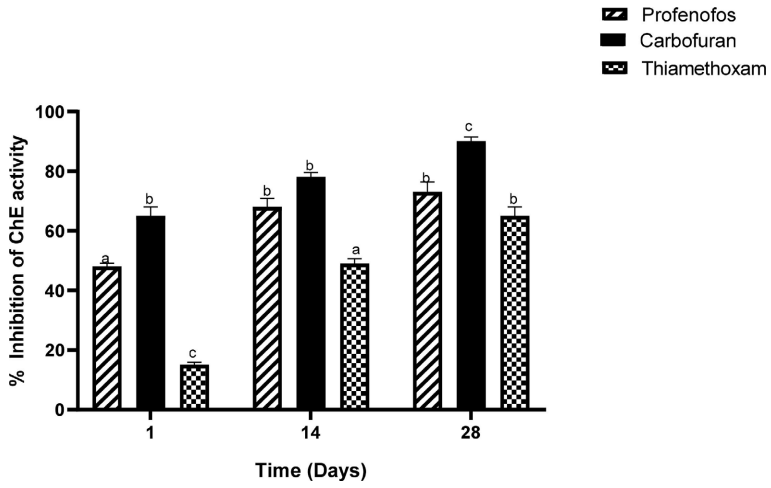

Figure 2. Effect of different exposure duration of different pesticides on cholinesterase activity of the aquatic snail Helisoma duryi. Values represent the average of triplicate samples which are expressed as mean + SD. The different letters on the bars indicate significant differences and bars with the same letters indicate there was no significant difference.

3.3. Recovery of Carboxylesterase Activity

Carboxylesterase activity was inhibited in all snails exposed to pesticides (Figure

3). Among the pesticides tested, the carbamate carbofuran caused the highest inhibition of carboxylesterase at each exposure duration. Inhibition of carboxylesterase significantly decreased ($p < 0.05$) in snails allowed to recover compared to those exposed for 14 and 28 days (Figure 3).

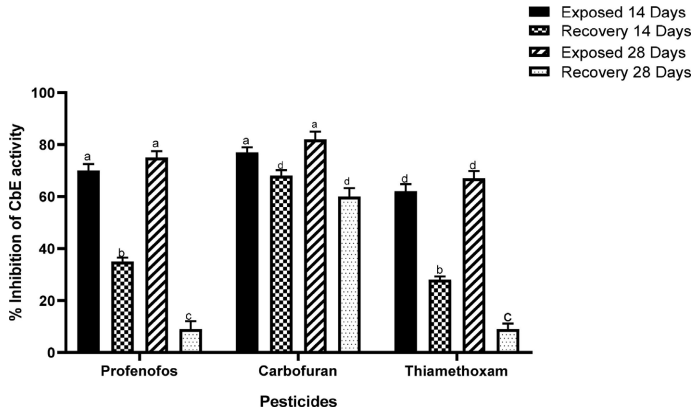

Figure 3. Inhibition of carboxylesterase activity in snails that were exposed for 14 days; snails that were allowed to recover for 14 days after being exposed to pesticides for 14 days; snails that were exposed for 28 days and snails that were allowed to recover for 28 days after being exposed to pesticides for 28 days. Values represent the average of triplicate samples which are expressed as mean + SD. The different letters on the bars indicate significant differences and the same letters indicate there was no significant differences.

3.4. Recovery of Cholinesterase Activity

Time-dependent inhibition of cholinesterase activity was observed in all snails exposed to pesticides (Figure 4). The highest cholinesterase inhibition occurred in snails exposed to carbofuran for 28 days (Figure 4). Cholinesterase inhibition significantly decreased ($p < 0.05$) in snails exposed to profenofos and thiamethoxam after a recovery period of 14 or 28 days (Figure 4).

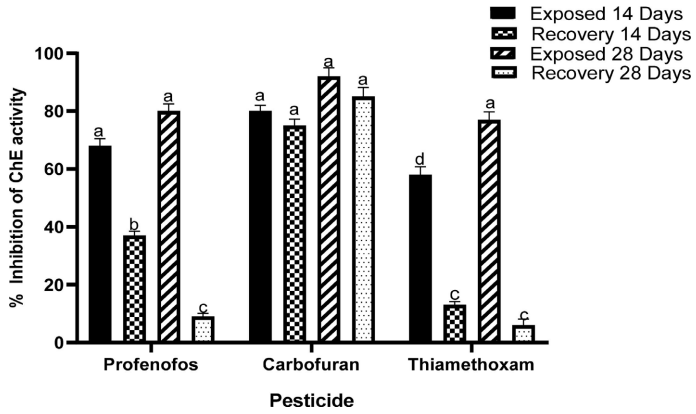

Figure 4. Inhibitions of cholinesterase activity in snails that were exposed for 14 days; snails that were allowed to recover for 14 days after being exposed to pesticides for 14 days; snails that were exposed for 28 days and snails that were allowed to recover for 28 days after being exposed to pesticides for 28 days. Values represent the average of triplicate samples which are expressed as mean + SD. The different letters on the bars indicate significant differences and the same letters indicate there was no significant differences.

4. Discussion

All the pesticides tested caused significant time-dependent reductions in the activities of non-cholinesterase enzymes (**Figure 1**) and cholinesterase enzymes (**Figure 2**) in the freshwater snails *Helisoma duryi* exposed to the organophosphate, carbamate, and neonicotinoid. For all pesticides, cholinesterase inhibition observed in snails exposed for 28 days was greater than the inhibition recorded in snails exposed for 14 days. Organophosphates are well-known to reduce acetylcholinesterase activity in both aquatic and terrestrial organisms [13]-[15]. In fact, cholinesterase inhibition is widely used as a biomarker of exposure to organophosphorus pesticides. In this study, profenofos inhibited cholinesterase activity by up to 70% and 80% after 14 and 28 days of exposure, respectively (**Figure 4**). Similar findings were reported by Gagnaire *et al.* [16] who observed reduced cholinesterase activity in the freshwater snails *Potamopyrgus antipodarum* and *Valvata piscinalis* exposed to chlopyrifos. Kumar *et al.* [17] found a 30% - 50% reduction in cholinesterase activity in *Paratya australiensis* exposed to profenofos, while Nillos *et al.* [18] reported decreased acetylcholinesterase activity in *Daphnia magna* exposed to the pesticides profenofos, fonofos and crotoxyphos. Khalil [19] observed a 78% reduction in acetylcholinesterase activity in snails exposed to 0.29 ppb of the organophosphate chlorpyrifos for 28 days. Additional confirmation of the anticholinesterase effects of organophosphates is provided by Duarte-Restrepo *et al.* [20] who noted reduced acetylcholinesterase activity in shrimps exposed to chlorpyrifos.

Neonicotinoids, which are neurotoxic chemicals targeting the central nervous systems of pest insects, also inhibit cholinesterase [21] [22]. Shaker *et al.* [23] reported significant cholinesterase inhibition in snails exposed to thiamethoxam and imidacloprid. Similarly, Vehovszky *et al.* [24] documented reduced cholinergic neurotransmission in *Lymnaea stagnalis* exposed to neonicotinoids. In this study, thiamethoxam caused up to a 74% reduction in cholinesterase activity in *Helisoma duryi*. Ewere *et al.* [25] also reported significant cholinesterase inhibition in snails exposed to neonicotinoids.

Carbamates have been identified as anticholinesterase agents [13] [26]. Khalil [19] observed reduced cholinesterase activity in the land snail *Eobania vermiculata* exposed to the carbamate methomyl lannate. Gohary *et al.* [27] reported similar reductions in cholinesterase activity in *Monacha cantiana* and *Eobania* vermiculata exposed to Mesurol 75-W.

In this study, the highest cholinesterase and carboxylesterase inhibition was observed in snails exposed to the carbamate carbofuran. The degree of enzyme inhibition decreased over time (**Figures 1 and 2**), likely due to the depletion of unbound enzymes. A comparison of the three pesticides revealed the order of potency in inhibiting enzyme activities to be carbofuran > profenofos > thiamethoxam. In contrast, Yadav *et al.* [28] found chlorpyrifos to be more potent than carbofuran in reducing acetylcholinesterase activity in *Eisenia fetida* earthworms, suggesting differing trends among pesticides.

All snails transferred to pesticide-free water after exposure showed signs of recovery, as evidenced by reduced inhibition of enzyme activities (**Figure 3** and **Figure 4**). Snails exposed to carbofuran displayed 75% inhibition after 14 days, increasing to 90% after 28 days of exposure, and only recovered less than 8% in the 28 day-duration in pesticide-free water (**Figure 3** and **Figure 4**). The slower recovery of enzymatic activity observed in snails exposed to carbofuran, even after a 28-day period in pesticide-free water, compared to the recovery of enzymatic activity in those exposed to profenofos or thiamethoxam, may be attributed to the specific mode of action and persistence of carbofuran as a carbamate pesticide. In *Helisoma duryi*, the carbamylation of enzymes likely results in the formation of more stable bonds that take longer to dissociate, thereby delaying enzymatic recovery. Also, the *Helisoma* snails probably have a higher sensitivity and affinity for carbofuran such that even at low concentrations of 0.5 ppb, extended of 28 days led to extensive binding, which may have overwhelmed the snails' detoxification and repair mechanisms resulting in the markedly low recovery of acetylcholinesterase and carboxylesterase activities observed.

Recovery in non-carbamate pesticide-exposed snails was nearly complete, with less than 10% inhibition observed after 28 days in clean water. The recovery patterns observed in organophosphate-exposed snails align with studies on aquatic mollusks [29] and fish [30] [31], where acetylcholinesterase activity inhibited by organophosphates required 2 - 4 weeks to return to near-control levels. This recovery likely results from de novo synthesis of the enzyme, as de-phosphorylation at the enzyme's active site is slow and considered essentially irreversible. In contrast, Dhainaut and Scaps [32] attributed recovery from carbamate or organophosphate exposure to the gradual release of free enzymes through aging of the enzyme-pesticide complex.

Despite recovery, snails exposed to carbamates exhibited 60% and 85% inhibition of carboxylesterase and cholinesterase activities, respectively, after 28 days. The slow recovery suggests a highly stable enzyme-pesticide complex that decarbamylates slowly, necessitating de novo enzyme synthesis. Recovery may also involve adaptations such as increased production of detoxifying enzymes like glutathione S-transferase, as suggested by Hernandez *et al.* [33]. Declining pesticide concentrations in clean water may further facilitate recovery by diluting residual pesticide levels within the snails.

5. Conclusions

Pesticides commonly used in agricultural pest control often enter aquatic ecosystems through aerial drift and leaching, where they can significantly impact the health of aquatic organisms. In this study, the pesticides profenofos, carbofuran, and thiamethoxam commonly used in Zimbabwean agriculture were found to significantly inhibit carboxylesterase and cholinesterase activities in the freshwater snail *Helisoma duryi*, with the degree of inhibition increasing over time. Among the tested pesticides, carbofuran (a carbamate) exhibited the strongest anti-ester-

ase effects, followed by the organophosphate profenofos and the neonicotinoid thiamethoxam. Notably, *Helisoma duryi* snails showed partial to substantial recovery of enzyme activity after being transferred to pesticide-free water, indicating some capacity for physiological recovery and detoxification over time.

These findings highlight the ecological risks associated with pesticide contamination in freshwater ecosystems, particularly in light of their widespread use and the potential for runoff from agricultural areas. The study also supports the use of esterase activity in *Helisoma duryi* as a valuable biomarker for detecting pesticide exposure and toxicity in aquatic environments. However, the study had several limitations: First, it was conducted under controlled laboratory conditions, which may not fully capture environmental complexities such as temperature variations and pollutant levels. Second, the study focused solely on one biomarker endpoint (sublethal enzymatic effects) without evaluating other important biological parameters such as survival, behaviour and reproduction, which are critical for assessing population-level impacts. Third, only one freshwater snail species was used, limiting the generalizability of the findings to other aquatic organisms.

Future research could address these limitations by:

1) Conducting field studies to assess real-world exposures and validate laboratory findings under natural conditions, including additional biomarkers such as antioxidant enzyme activities, genotoxicity, histopathological changes, and reproductive toxicity.

2) Investigating the effects of pesticide mixtures and examining chronic and multigenerational impacts of pesticide exposure on *Helisoma duryi* and other representative aquatic species.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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