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Role of Brain Lipid and Cholesterol Fluctuations in Malathion-Induced Mortality of *Blatta orientalis* (Linnaeus): Beyond Cholinesterase Inhibition

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Abstract

In Oriental cockroaches (*Blatta orientalis*), when Malathion is applied to the forefemur of both males and females separately, there are notable changes observed. These changes include significant variations in the levels of lipids and cholesterol in the brain, as well as the inhibition of cholinesterase activity. Importantly, the death of these cockroaches resulting from Malathion treatment is not solely attributable to the inhibition of cholinesterase activity but is also linked to the fluctuations in the brain's lipid and cholesterol content. Additionally, the study found that there is a minor, statistically non-significant increase in these effects at the 60-minute mark for both male and female cockroaches. However, a substantial and statistically significant increase ($P < 0.001$) in these effects is observed at the 90-minute mark in both sexes. Notably, in males, there is a significant increase ($P < 0.05$) at the 90-minute mark, while in females, there is a non-significant increase ($P < 0.05$) at both time intervals.

Keywords: *Blatta orientalis*, Brain, Malathion, Lipid , Cholesterol, Mortality

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Introduction

Cockroaches are an ancient group of insects, with their ancestors, called "roachoids," originating approximately 320 million years ago during the Carboniferous period. These early ancestors, however, did not possess the internal ovipositors found in modern roaches. Cockroaches are relatively unspecialized insects; they lack specific adaptations like the sucking mouthparts seen in aphids and other true bugs. Instead, they have chewing mouthparts and are considered among the most primitive of living Neopteran insects. Cockroaches are highly adaptable insects capable of thriving in a wide range of climates, from frigid Arctic conditions to scorching tropical environments. In tropical regions, cockroaches often grow much larger than their temperate counterparts. This insect order is one of the oldest, with a fossil history spanning over 300 million years. There are approximately 4,000 known cockroach species worldwide, categorized into six families: Blattidae, Cryptoceridae, Polyphagidae, Nocticolidae, with more than 445 genera.

In India, two common species of cockroaches are the American cockroach (*Periplaneta americana*) and the Oriental cockroach (*Blatta orientalis*). Nevertheless, the vast majority of cockroach species, more than 99%, inhabit natural ecosystems and likely play essential roles in forest food webs (as reported by Bhoopathy in 1997 and Jayakumar *et al.* in 1994).

While most cockroaches live in the wild, there are a few species that have adapted to human habitation. These species are considered pests because they can damage and contaminate food with their foul-smelling excretions.

The order Blattodea encompasses around thirty cockroach species that have adapted to human environments, distinguishing them from the thousands of species within this order. These particular cockroaches have a tendency to feed on human and pet food, often emitting an unpleasant odor. Notably, they can unwittingly carry disease-causing microorganisms on their bodies, which is especially concerning in settings like hospitals. Cockroaches have been associated with allergic reactions in humans, with certain proteins, such as tropomyosin, being a common trigger for allergies. This can result in cross-reactive allergies to substances like dust mites and shrimp. These allergens are also known to exacerbate asthma symptoms. Interestingly, some cockroach species possess remarkable survival abilities, with the capability to live for up to a month without a source of food. Consequently, the absence of visible cockroaches in a home does not necessarily indicate their absence altogether. Surprisingly, research has found that a significant percentage, approximately 20-48%, of homes devoid of observable cockroach presence still contain detectable cockroach allergens in the dust.

Organophosphates are substances that poison a wide range of creatures, including insects, birds, amphibians, and mammals. They primarily achieve this by targeting the acetylcholinesterase enzyme (AChE) found at nerve endings. The consequence of this interaction is the phosphorylation or chemical alteration of AChE, rendering it less effective. As a result, the effector organs, which respond to nerve signals, become excessively stimulated due to the buildup of acetylcholine (ACh), the chemical that transmits nerve impulses, at the nerve endings (Roberts DM, Aaron CK, 2007). AChE plays a vital role in regulating the transmission of nerve impulses from nerve fibers to various types of muscle cells, secretory cells, autonomic ganglia, and within the central nervous system (CNS). When a significant portion of AChE is rendered inactive through phosphorylation, symptoms and signs of cholinergic poisoning start to appear. At higher doses, the loss of AChE function leads to the accumulation of ACh in various parts of the nervous system, including cholinergic neuroeffector junctions (resulting in muscarinic effects), skeletal nerve-muscle junctions, and autonomic ganglia (resulting in nicotinic effects), as well as within the central nervous system.

In areas where ACh accumulates, it can cause a range of effects, including muscle contractions, secretions, muscle twitching, and even paralysis of muscles, especially in the diaphragm and thoracic skeletal muscles, which can lead to respiratory paralysis. In the central nervous system, high concentrations of ACh can result in sensory and behavioral disturbances, lack of coordination, reduced motor function, and respiratory depression. The increased production of pulmonary secretions, along with respiratory failure, is typically the cause of death in cases of organophosphate poisoning. Recovery hinges on the eventual restoration of enzyme function in critical tissues. The absorption of organophosphates can occur efficiently through inhalation and ingestion, while their penetration through the skin and subsequent absorption into the bloodstream can vary depending on the specific agents involved (Gallo MA & Lawryk NJ, 1991).

Organophosphorus insecticides are known for their potent toxicity and are widely used for insect pest control. The impact of these chemicals on esterase and cholinesterase enzymes in the head and fat bodies of *Schistocerca gregaria* (desert locust), *Musca domestica* (housefly), and *Apis mellifera* (honeybee) has already been well-documented in scientific research.

However, the present study aims to further our understanding by conducting a detailed biochemical analysis of the lipid and cholesterol levels in the brains of cockroaches *Blatta orientalis* (Linnaeus) treated with malathion. This investigation seeks to provide additional insights into the mechanisms through which malathion exerts its effects.

Materials and Methods

Newly emerged adult Oriental cockroaches (*Blatta orientalis*) from a genetically homogeneous colony were subjected to treatment with technical-grade malathion, which was dissolved in two different solvents: benzene (at a concentration of 1.5%) and acetone (also at a concentration of 1.5%). In each case, ten microliters of these solutions were carefully applied to the profemur, which is known to be the most vulnerable area for both male and female cockroaches. For each treatment, a group of twenty insects was utilized. As a control group, some insects were treated solely with the solvent, devoid of any active ingredient.

To evaluate the effects of these treatments, post-treatment examinations were conducted at two distinct time points: 60 minutes and 90 minutes following the application of the substances. During these examinations, the brains were meticulously removed from the insects and placed in a solution of physiological saline, maintaining a pH level of 7.2. Any residual tissues adhering to the brains were carefully removed, after which the brains were dried using filter paper. Subsequently, conventional biochemical methods and protocols were employed to analyze and quantify various components within the brain tissue. These analyses included the assessment of protein levels, the determination of total lipid content, the measurement of cholesterol levels, and the evaluation of cholinesterase enzyme activity.

The quantification of total lipids in the brain followed the methodology outlined by *Folch et al., (1951)* involving the homogenization of brain tissue with a mixture of chloroform and methanol in a ratio of 2:1 (volume/volume). The total lipid content was determined through gravimetric analysis.

Cholesterol levels were assessed following the method developed by *Zlatkis et al. (1953)* In this procedure, ferric chloride was utilized as a coloring reagent. The intensity of the resulting pinkish-colored complex was proportional to the amount of cholesterol present in the sample. The cholesterol activity was expressed in milligrams per gram of wet tissue.

The activity of cholinesterase was determined through an enzymatic reaction involving acetylcholine buffer salt. This buffer salt comprised a mixture of veronal buffer, acetylcholine solution, and salt that had been equilibrated at 37 degrees Celsius. To this mixture, hydroxylamine solution was added, resulting in the formation of a brown complex. The quantification of cholinesterase activity was achieved by plotting a standard curve, with densities as the ordinate and micromoles of acetylcholine hydrolyzed per gram of protein per half an hour as the abscissa. This curve allowed for the determination of cholinesterase activity in micromoles per gram of tissue.

Results

The study documented significant changes in the levels of total lipids, cholesterol, and cholinesterase enzyme activity in the brains of male and female Oriental cockroaches (*Blatta orientalis*) following exposure to malathion.

On treatment with 1.5 % in benzene

Cholesterol Levels: A notable and statistically significant rise ($p < .01$) in cholesterol content was observed at both the 60 and 90-minute time intervals for both male and female cockroaches. (Table and Figure 2).

Cholinesterase Enzyme Activity: There was a substantial and statistically highly significant decrease ($p < .001$) in the quantity of acetylcholine hydrolyzed per gram of protein per half an hour, observed in both male and female cockroaches. (Table and Figure 3).

Lipid Levels: In male cockroaches, there was an initial non-significant rise in lipid content observed at the 60-minute mark, which was followed by a remarkably significant increase ($p < .001$) at the 90-minute point. Conversely, in females, there was a significant increase ($p < .05$) in lipid content recorded at both the 30 and 60-minute intervals. (Table and Figure 1).

On treatment with 1.5 % malathion in acetone

Cholesterol Levels: In males, there was a significant increase ($p < .05$) in cholesterol content at the 60-minute mark, which was followed by a non-significant increase at the 90-minute point. In females, the increase in cholesterol content was not statistically significant ($p > .05$) at both the 60 and 90-minute intervals. (Table and Figure 2).

Cholinesterase Enzyme Activity: A notable decrease in cholinesterase activity was observed at both time intervals for both male and female cockroaches, and this decrease was statistically significant ($p < .01$) (Table and Figure 3).

Lipid Levels: Initially, there was a non-significant increase in lipid content observed at the 60-minute mark in both male and female cockroaches. However, at the 90-minute interval, there was a significant increase ($P < .001$) in lipid content noted in both sexes. (Table and Figure 1). The change in biochemical constituents including inhibition in cholinesterase activity confirm the penetration, movement to and action of malathion on the brain, the possible site of action.

Both males and females exhibited nearly identical responses to malathion in terms of the impact on the biochemical parameters of the brain.

Table: 1 Biochemical analysis of the lipid content in the brains of *Blatta orientalis* (Linnaeus) treated with malathion.

	Lipid Content (mg/g)							
	Male				Female			
	60 minutes		90 minutes		60 minutes		90 minutes	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
1.5 % malathion in benzene	35.01±0.01	54.00 ±0.22	27±0.61	52±0.51	35.61±0.11	45.1±0.19	24.0±0.16	47.2±0.09
1.5% malathion in acetone	51±0.18	68.1±0.16	51±0.06	72.50±0.14	46.5±0.02	74.3±0.12	47.5±0.10	88.20±0.33

*A non significant increase at 60 minutes in male and female is followed by a significant increase (P <.001) at 90 minutes in either of the sexes.

Figure: 1 Biochemical analysis of the lipid content in the brains of *Blatta orientalis* (Linnaeus) treated with malathion.

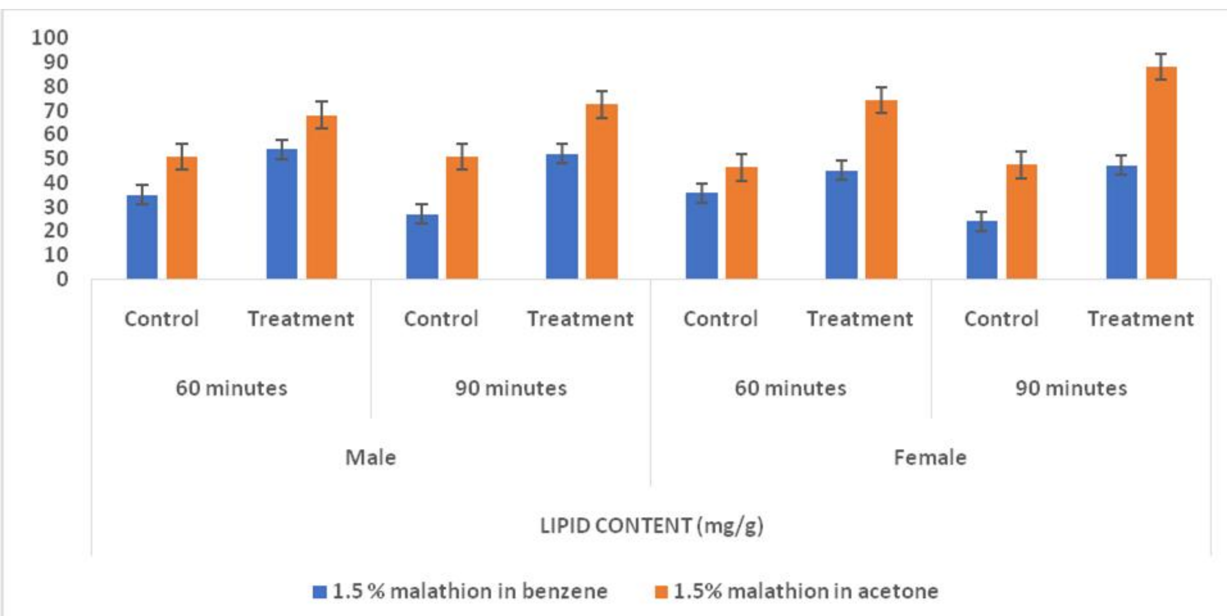


Table: 2 Biochemical analysis of the cholesterol content in the brains of *Blatta orientalis* (Linnaeus) treated with malathion.

	Cholesterol Content (mg/g)							
	Male				Female			
	60 minutes		90 minutes		60 minutes		90 minutes	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
1.5 % malathion in benzene	5±0.16	6.81±0.21	5.35±0.17	9.69±0.55	4.52±0.54	5.75±0.23	5.25±0.70	6.63±0.30
1.5% malathion in acetone	6.81±0.032	7.44±0.31	6.41±0.44	7.01±0.05	7.5±0.60	8.25±0.07	7.53±0.11	7.62±0.10

*A significant increase (P< .05) at 60 minutes is followed by a non- significant increase at 60minutes in male. In female it is non- significant increase (P<.05) at both the time intervals

Figure: 2 Biochemical analysis of the cholesterol content in the brains of *Blatta orientalis* (Linnaeus) treated with malathion.

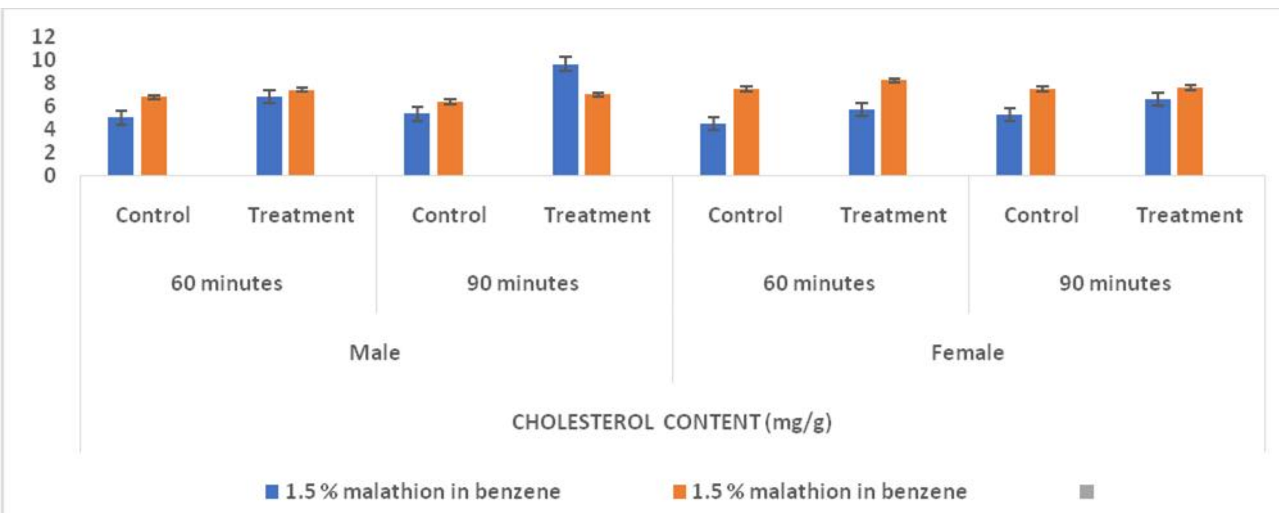
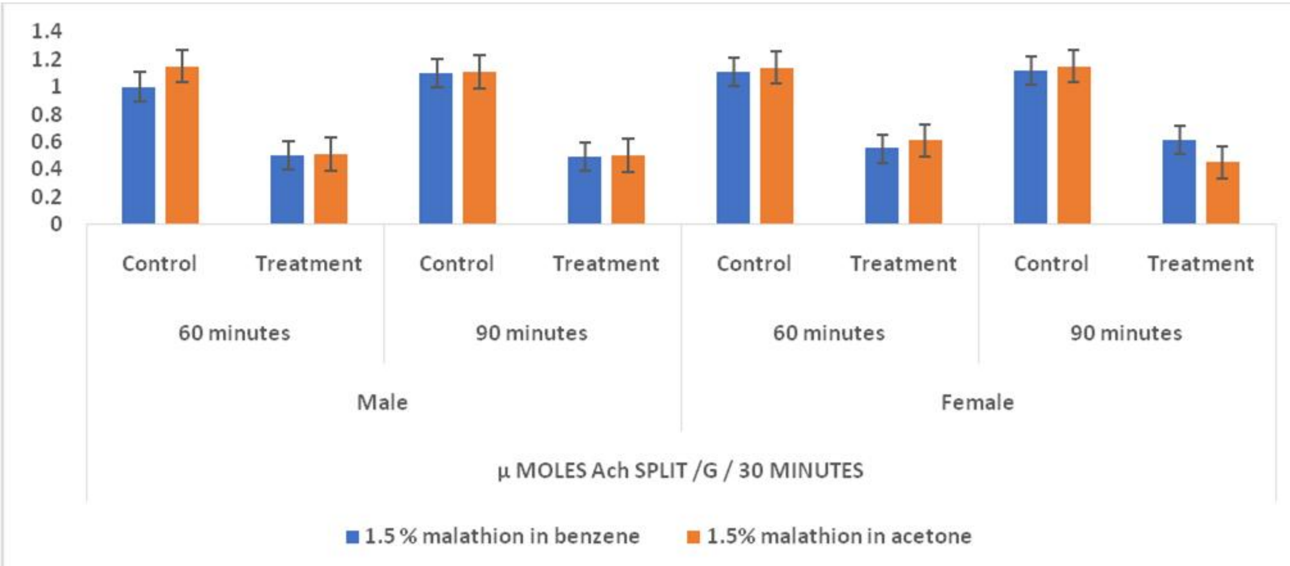


Table: 3 Biochemical analysis of the cholinesterase activity in the brain of *Blattaorientalis* (Linnaeus) treated with malathion.

	µ Moles Ach Split /G / 30 Minutes							
	Male				Female			
	60 minutes		90 minutes		60 minutes		90 minutes	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
1.5 % malathion in benzene	1±0.79	.5±0.77	1.1±06	.49±0.15	1.11±0.29	.55±0.02	1.12±0.67	.61±0.12
1.5% malathion in acetone	1.15±0.21	.51±0.31	1.11±0.61	.5±0.19	1.14±0.33	.61±0.66	1.15±0.07	.45±0.11

*A significant fall (p < .01) in cholinesterase activity is recorded at both the time intervals and in both the sexes

Figure: 3 Biochemical analysis of the cholinesterase activity in the brains of *Blatta orientalis* (Linnaeus) treated with malathion.



Discussion

The toxic effects of malathion on cockroaches depend largely on the biochemical processes within the insect and the physiochemical properties of the phosphorous compound. The observable toxic symptoms, including various levels of convulsions, are primarily a result of the inhibition of the enzyme cholinesterase. The continuous decrease in enzyme activity observed in both males and females at the 60 and 90 minute marks following treatment indicates that the primary mechanism of action of malathion is the inhibition of cholinesterase activity. This inhibition of enzyme activity due to organophosphorus poisoning is a well-established phenomenon. It occurs as a result of the isomerization of malathion, transforming it from a thiono group to a thiole group. Notably, the differences in cholinesterase content in the brains of the two sexes observed in this study align with the findings of Metcalf and March,(1949) who demonstrated a direct relationship between organophosphate poisoning and the levels of acetylcholinesterase (AChE) in the brain. The observed changes in Oriental cockroaches *(Blatta orientalis*) following the application of Malathion to their forefemurs raise intriguing questions about the mechanisms underlying pesticide toxicity. This discussion will delve into these findings and explore their significance in the context of insecticide action, with reference to relevant literature.

Firstly, the study identified significant variations in the levels of lipids and cholesterol in the brain of treated cockroaches. This finding is consistent with previous research on the impact of organophosphate pesticides like Malathion on insects. Organophosphates are known to disrupt lipid metabolism in insects, affecting lipid storage, mobilization, and utilization (Bass *et al.*, 2016). The altered lipid and cholesterol profiles observed in this study may be indicative of disruptions in energy metabolism and membrane integrity, which could contribute to the overall toxicity of Malathion.

Secondly, the inhibition of cholinesterase activity was noted in both male and female cockroaches. This outcome is in line with the well-established mode of action of organophosphate pesticides, which primarily target the cholinergic system by inhibiting acetylcholinesterase (AChE) activity (Casida and Quistad, 2005). Cholinesterase inhibition leads to the accumulation of acetylcholine at synapses, causing hyperstimulation of the nervous system and ultimately paralysis and death.

However, what makes this study particularly noteworthy is the observation that the mortality of the cockroaches could not be solely attributed to cholinesterase inhibition. Instead, it is closely associated with the changes in brain lipid and cholesterol content. This suggests that the effects of Malathion on these insects extend beyond its canonical target, indicating possible secondary or indirect mechanisms of toxicity. It is important to note that insects, including cockroaches, rely on lipid metabolism for energy production and membrane structure (Klowden, 2007). Disruptions in lipid homeostasis can have profound physiological consequences, which may synergize with cholinergic disruption, ultimately leading to insect mortality. Organophosphates function by irreversibly binding to cholinesterase enzymes, thereby disrupting the regulation of acetylcholine, a neurotransmitter essential for normal nerve signal transmission (Sogorb *et al.,* 2008). This disruption leads to the accumulation of acetylcholine, resulting in overstimulation of nerve cells, paralysis, and ultimately, the death of the insect (Eddleston *et al.,* 2008).

However, the present study unveils an additional dimension to Malathion's mode of action in Oriental cockroaches—the significant variations in brain lipid and cholesterol levels. The involvement of lipids and cholesterol in the response to pesticide exposure may be related to the fundamental roles these molecules play in cellular membrane structure, signal transduction, and overall cell function (Fahy *et al.,* 2011). The observed changes in lipid and cholesterol content may disrupt the integrity of neuronal membranes, influencing ion transport and receptor functions, which could contribute to the observed physiological effects.

The temporal analysis of the effects is particularly intriguing. The minor, statistically non-significant increase in these effects at the 60-minute mark suggests an initial response that may be related to the rapid absorption and distribution of Malathion within the cockroach's body (Burr *et al.,* 1970). However, the substantial and statistically significant increase observed at the 90-minute mark highlights a delayed and more pronounced impact. This time-dependent pattern of response may be due to various factors, including the metabolism of Malathion within the cockroach's body, the accumulation of toxic metabolites, and the cumulative disruption of physiological processes over time (Casida and Quistad, 2005).

The observed sex-specific differences in response to Malathion are noteworthy. While both males and females displayed significant effects at the 90-minute mark, males exhibited a statistically significant increase ($P < 0.05$) in response, whereas females showed a non-significant increase ($P < 0.05$) at both time intervals. These sex-specific differences may be attributed to variations in metabolic rates, enzyme activities, or even differences in the composition of neural tissues between male and female cockroaches (Feyereisen, 2006). Further investigation is warranted to elucidate the underlying mechanisms responsible for these sex-specific responses.

Furthermore, the temporal analysis of the data reveals intriguing dynamics. The minor, statistically non-significant increase in effects at the 60-minute mark implies that the initial response to Malathion exposure may not be immediately lethal but becomes progressively more pronounced. This observation aligns with the time-dependent nature of organophosphate toxicity, as the accumulation of neurotoxic effects takes time to manifest fully (Casida and Quistad, 2005). The substantial and statistically significant increase in effects at the 90-minute mark underscores the importance of considering exposure duration in pesticide risk assessments.

In summary, the findings of this study shed light on the complex mechanisms underlying the toxicity of Malathion in Oriental cockroaches. While cholinesterase inhibition plays a pivotal role, it is not the sole driver of mortality. The disruption of lipid and cholesterol metabolism emerges as another critical facet of Malathion's mode of action. These results highlight the multifaceted nature of pesticide toxicity in insects and underscore the importance of comprehensive assessments that consider both direct and indirect effects.

Additionally, the study also detected significant biochemical changes in protein, lipid, and cholesterol levels in the brains of malathion-treated insects. The increase in protein content when treated with 1.5 % malathion (in benzene) could be attributed to either an induction phenomenon or the blocking of messenger synthesis responsible for repressing protein production, ultimately stimulating protein synthesis. However, the simultaneous decrease in protein content when treated with 1.5 % malathion (in acetone) is not readily explainable and warrants further investigation.

The increase in lipid content may result from the release of certain lipoprotein macro-molecular structures from unspecialized cell walls in the hemolymph, which contribute to higher lipid levels in treated insects.

Based on a study by O'Brien in 1967, it is suggested that fat bodies store lipids and can dissolve and retain toxins like schradan, an organophosphate compound. This implies that malathion, another organophosphorus compound, may accumulate in fat bodies and then travel to its target site, the brain, via connective tissue. To counteract malathion's toxic effects on the brain, additional lipids may be supplied by the fat body, resulting in increased lipid content in the brain. A similar increase in lipid content due to paraoxon treatment has been reported previously.

An increase in lipid levels could be attributed to the insect's vigorous fanning behavior after being exposed to poison. Research by Weisforgh in 1964 suggests that fanning may release lipids in the form of diglycerides. The absence of inhibition of lysosomal acid hydrolases, enzymes capable of breaking down accumulated compounds, might lead to lipid accumulation. Interference with mitochondrial fatty acid oxidation could induce lipid accumulation.

The increase in cholesterol content observed in this study indicates a greater association of phospholipids with tissues. Phospholipids play a crucial role in cholesterol mobilization and metabolism, aligning with Sarma's findings in 1950.In summary, these findings suggest that in addition to the peripheral region of the thoracic ganglion, the brain may also be a target of malathion's action. Malathion, beyond its cholinesterase inhibiting properties, appears to affect various biochemical components. This view is supported by the observed ultrastructural changes in the brain following malathion treatment.

Further, these biochemical changes in , lipid and cholesterol within 60 minutes of application of malathion after entering the insect body arrives and acts on brain within such a short period.

Conclusion

In conclusion, the application of Malathion to the forefemur of Oriental cockroaches (*Blatta orientalis*) has revealed significant alterations in their physiological responses. These alterations encompass substantial variations in brain lipid and cholesterol levels, alongside the inhibition of cholinesterase activity. It is important to emphasize that the demise of these cockroaches following Malathion treatment cannot be attributed solely to cholinesterase inhibition; instead, it is intricately linked to the observed fluctuations in brain lipids and cholesterol content. Furthermore, the study's temporal analysis revealed intriguing dynamics in these effects. While a minor, statistically non-significant increase was observed at the 60-minute mark for both male and female cockroaches, a remarkable and statistically significant escalation ($P < 0.001$) in these effects became apparent at the 90-minute mark in both sexes. Notably, in males, there was a significant increase ($P < 0.05$) at the 90-minute mark, while in females, an increment was observed at both time intervals, although it did not reach statistical significance ($P < 0.05$).

These findings underscore the complexity of the impact of Malathion on Oriental cockroaches, suggesting that its mode of action involves more than just cholinesterase inhibition. The time-dependent nature of these effects further highlights the need for a comprehensive understanding of the biochemical and physiological mechanisms involved in pesticide toxicity. This study provides valuable insights into the intricate and time-dependent effects of Malathion on Oriental cockroaches. The dual impacts on cholinesterase activity and brain lipid/cholesterol content suggest a multifaceted mode of action for this organophosphate pesticide. Understanding these complex interactions is crucial not only for pest management strategies but also for a broader understanding of the biochemical responses of insects to pesticide exposure. This research contributes valuable insights into the nuanced responses of these pests to chemical control, shedding light on potential avenues for more effective pest management strategies.

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