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Comparative Evaluation of Enzyme Inhibition in Callosobruchus analis Following Exposure to Botanical and Synthetic Pesticides via the Glass Film Method

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Abstract

Enzymatic activity was assessed 24 hours after exposure to three pesticides: Deltamethrin (a synthetic pyrethroid), Biosal (a neem-based biopesticide), and Acorus calamus essential oil applied to adult Callosobruchus analis using the Glass Film Method. The enzymes analyzed included cholinesterase, glutamate pyruvate transaminase, alkaline phosphatase, and glutamate oxaloacetate transaminase. The calculated LC_{50} values for A. calamus, Biosal, and Deltamethrin were 0.15614 µL/cm², 12.174 µL/cm², and 0.04390 µL/cm², respectively. Following treatment with A. calamus, enzyme activity levels recorded were 77.54% for GOT, 73.84% for GPT, 72.61% for ALP, and 75.32% for CHE. In the case of Biosal, enzyme activity levels were 80.18% for GOT, 73.05% for GPT, 64.23% for ALP, and 60.19% for CHE. The activity of cholinesterase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and alkaline phosphatase was found to be 79.75%, 79.89%, 74.30%, and 62.17%, respectively, after exposure to Deltamethrin. These results indicate that inhibition of the enzyme in all three treatments was observed, particularly reductions in CHE and ALP activities, suggesting interference with key neurophysiological and metabolic processes in Callosobruchus analis.

Keywords: Callosobruchus analis, Deltamethrin, A. calamus, Biosal, Glass film method.

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Introduction:

Enzyme inhibition is significant because it regulates natural metabolic pathways, treats medical conditions, and aids in pest control. It stops overactive cellular reactions, blocks harmful bacteria, and allows scientists to design life-saving drugs. Enzymes are vital biological proteins that act as natural catalysts to speed up essential chemical reactions, build muscle, break down food, and copy DNA during cell division. Many insecticides act by inhibiting enzymes essential for insect survival. Organophosphates and carbamates inhibit acetylcholinesterase, causing paralysis and death of insect pests. Enzyme inhibition helps improve crop protection and agricultural productivity. Enzyme inhibition has been extensively studied due to its critical role in biochemical regulation and applications in pest control and toxicology. Studies on Sitophilus oryzae revealed significant changes in enzymatic activities following exposure to Acorus calamus extract and cypermethrin (Ahmed et al., 2000a). Subsequent research expanded these findings to Tribolium castaneum (PARC strain), demonstrating altered total protein content and cholinesterase activity after treatment with neem extract, cypermethrin (10EC), and methyl parathion (50EC) (Ahmed et al., 2000b). Later investigations highlighted how permethrin and cypermethrin exposure disrupted biochemical parameters in adult T. castaneum, particularly in relation to gamma-HCH-II toxicity (Saleem et al., 2001). Kok et al. (2002) observed the construction of an acetylcholinesterase—Choline oxidase biosensor for aldicarb determination. This work was extended by comparing enzyme activity profiles between malathion-resistant and susceptible strains of T. castaneum, with a focus on the in vivo effects of cyfluthrin on proteolytic enzymes (Saleem et al., 2004). Together, these studies underscore the complex interplay between insect physiology and chemical stressors, paving the way for more targeted pest control strategies. Recent research has provided valuable insights into the cytotoxic effects of various pesticidal compounds. In neural cell cultures, pyrethroid compounds were shown to significantly impact cellular function, as demonstrated through assessments of total ATP content, mitochondrial enzyme activity, and microscopic imaging techniques (Irmark et al., 2004). Schulze et al. (2004) reported the activation of phosphorothionate pesticides based on a cytochrome P450 BM-3 (CYP102 A1) mutant for expanded neurotoxin detection in food using an acetylcholinesterase biosensor. Ferat et al. (2005) worked on a novel matrix for the immobilization of acetylcholinesterase. Min et al. (2006) investigated the development of a dipstick enzyme-linked immunosorbent assay for the organophosphorus insecticide parathion-methyl. Zhang et al. (2008) observed the enzyme biosensor for monitoring carbamate pesticides in seawater. No et al. (2007) investigated the cholinesterase-based dipstick assay for the detection of organophosphate and carbamate pesticides. These findings highlight the neurotoxic potential of pyrethroids at the cellular level. The investigations focused specifically on Callosobruchus analis and demonstrated that botanical extracts — including dimilin, neem, and nimolocine — can significantly alter enzymatic activity in this pest species (Tabassum et al., 2008). Together, these studies provide a comprehensive picture of how different classes of pesticidal compounds affect organisms at multiple biological levels, from cellular to whole-organism responses.

Further studies by Sami and Shakoori 2008) first investigated the biochemical composition of Aulacophora foveicollis (the pumpkin beetle), with particular focus on endo-1,4-β-D-glucanase activity. This work was complemented by (Lopez and Pascual 2010) important discovery of acetylcholinesterase inhibition by monoterpenoids, revealing their promising role in integrated pest management strategies. Further expanding our knowledge of insect digestive enzymes, Sami et al. 2011) conducted detailed analyses of the salivary glands and gut in Mylabris pustulata (blister beetles), specifically characterizing their endo-β-1,4-D-glucanase systems. Khosravi and Sendi (2013). Observed the effect of neem pesticide (Achook) on midgut enzymatic activities and selected biochemical compounds in the hemolymph of lesser mulberry pyralid, Glyphodes pyloalis Walker (Lepidoptera: Pyralidae). Lopes and Furlaneto (2018). Worked on the standardization of homogenization protocols for insect biochemical analyses. Chaudhary and Sharma (2019). Worked on Plant-derived non-protein amino acids as insect growth regulators. Hamed and Ahmed (2019). Worked on the standardization of the glass film method for evaluating contact toxicity of insecticides to stored-product insects. Millán (2019) observed the alkaline phosphatases: Structure, substrate specificity, and functional relatedness to other members of a large superfamily of enzymes. Ibrahim and Kim (2020). Reported the biochemical responses of stored grain pests to sublethal insecticide exposure. Fournier and Mutero (2020). Worked on insecticide resistance and acetylcholinesterase. Pridgeon and Liu (2021). Reported the mechanisms of insecticide resistance and synergism in pest management. Pavela and Benelli (2022). Worked on Essential oils as eco-friendly biopesticides. Challenges and constraints: Guedes et al. (2023) observed pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Ferreira et al. (2024) reported the Aphis gossypii Nymphs and Spodoptera frugiperda Larvae treated with the combination of conidia of Entomopathogenic Fungi and Enzyme. Vommaro et al. (2024) reported the Combined effect of Bacillus thuringiensis and herbicide exposure on development delays in the Red Flour Beetle. Understanding the enzymatic changes in C. analis post-treatment provides insight into the mode of action and relative toxicity of botanical and synthetic pesticides. This knowledge is essential for developing environmentally friendly pest control strategies and evaluating the biochemical safety of natural pesticide alternatives in stored grain pest management.

Material and Methods: Biochemical Estimation:

Analysis of Enzymatic Responses to Pesticide Application:

Following pesticide exposure, 0.5 g of live adults from both treated and control groups were homogenized in 2.5 mL of bi-distilled water using a Labogauge 200 tissue grinder (OSK 9258) at 1000 rpm for five minutes, followed by centrifugation at 3500 rpm for 20 minutes.

Freshly emerged adults of known age were used. Fifty grams of mung (Vigna radiata) were taken in each petri dish and mixed with pesticide at LC50 doses. Homogenates were prepared using freshly emerged adults of known age, with 0.5 g (approximately 122 insects) per treatment replicate, following the methodology of Arif and colleagues (2012, 2015).

Procedure for Enzyme Quantification:

(a) Determination of Glutamic-Oxaloacetic Transaminase (GOT)

Activity:

Activity of Glutamic-Oxaloacetic Transaminase was determined by the colorimetric method of Chromatest Kit no. 13427, which is based upon the method of Bergmeyer et al. 1986. GOT activity can be measured spectrophotometrically at 340 nm.

Principle:

L-Aspartate + 2- oxoglutarate $\xrightarrow{AST/GOT}$ L -Glutamate + oxaloacetate.

Oxaloacetate + NADH + H^+ MDH L-malate + NA

(b) Determination of Glutamate Pyruvate Transaminase (GPT) Activity:

Activity of Glutamate Pyruvate Transaminase was determined by the colorimetric method of Chromatest Kit no. 13361, which is based upon the method of Bergmeyer et al. 1986. GPT activity can be measured spectrophotometrically at 340 nm.

Principle:

L- Alanine + 2-oxoglutarate $\xrightarrow{ALT/GPT}$ L- Glutamate + Pyruvate

+ NADH + Pyruvate H^+ $\xrightarrow{LDH}$ Lactate+ NAD^+

(c) Determination of Alkaline Phosphatase Activity:

Activity of Alkaline Phosphatase was determined by the colorimetric method of Chromatest Kit no. 12915. which is based upon the method of (IFCC 1983). ALP activity can be measured spectrophotometrically at 405 nm.

Principle:

4-Nitrophenlphosphate + H2O $\xrightarrow[pH>g]{ALP,Mg++}$ 4- Nitrophenol +P_1

(d) Determination of Butyrylcholinesterase Activity:

Activity of Butrylcholinesterase was determined by the colorimetric method of Randox Kit no. 188259, which is based upon the method of Ellman et al. 1961. BChE activity can be measured spectrophotometrically at 405 nm.

Principle:

Butyrylthiocholine + H2O $\xrightarrow{}$ cholinesterase thiocholine + butyrate

Thiocholine + DTNB → 2-nitro-5-mercaptobenzoate.

Dtn = Dithiobis (nitrobenzoate)

Result:

The enzyme inhibition data (Tables 1-3) revealed differential susceptibility among the four enzymes, with cholinesterase (CHE) and alkaline phosphatase (ALP) demonstrating the highest sensitivity to pesticide exposure. The enzyme activities for GOT, GPT, ALP, and CHE following exposure to A. calamus were found to be 77.54%, 73.84%, 72.61%, and 75.32%, respectively. In the case of Biosal treatment, the enzyme activities were 80.18% (GOT), 73.05% (GPT), 64.23% (ALP), and 60.19% (CHE). After Deltamethrin exposure, the enzymatic activities were recorded as 79.89% (GOT), 74.30% (GPT), 62.17% (ALP), and 79.75% (CHE). The observed inhibition patterns suggest that the three pesticides affect distinct but overlapping biochemical pathways in C. analis, with implications for their combined use in pest management.

Table 1: Inhibition of key enzymes (CHE, ALP, GPT, and GOT) in Callosobruchus analis after 24-hour exposure to A. Calamus extract at a lethal concentration (LC_{50}) of 0.15614 µl/cm².

S. No	Enzyme	Treatment	U/L	Inhibition	Activity
1.	GOT	Control	657.66	00	100.00
		Treated	509.94	22.46	77.54
2.	GPT	Control	35.11	00	100.00
		Treated	25.92	26.16	73.84
3.	ALP	Control	24.35	00	100.00
		Treated	31.01	27.38	72.61
4.	CHE	Control	175.62	00	100.00
		Treated	218.95	24.67	75.32

Table 2: Inhibition of key enzymes (CHE, ALP, GPT, and GOT) in Callosobruchus analis after 24-hour exposure to Biosal

extract at a lethal concentration (LC_{50}) of 12.174 µl/cm²

S.No	Enzyme	Treatment	U/L	Inhibition	Activity
1.	GOT	Control	65.58	00	100.00
		Treated	52.58	19.82	80.18
2.	GPT	Control	21.29	00	100.00
		Treated	15.55	26.95	73.05
3.	ALP	Control	11.31	00	100.00
		Treated	15.35	35.76	64.23
4.	CHE	Control	37.29	00	100.00
		Treated	52.13	39.80	60.19

Table 3: Inhibition of key enzymes (CHE, ALP, GPT, and GOT) in Callosobruchus analis after 24-hour exposure to Deltamethrin extract at a lethal concentration (LC_{50}) of 0.04390 µl/cm².

S. No	Enzyme	Treatment	U/L	Inhibition	Activity
1.	GOT	Control	21.00	00	100.00
		Treated	17.03	20.13	79.89
2.	GPT	Control	71.28	00	100.00
		Treated	52.95	25.70	74.3
3.	ALP	Control	16.29	00	100.00
		Treated	22.38	37.82	62.17
4.	CHE	Control	149.41	00	100.00
		Treated	179.85	20.44	79.55

Annexure (A)

Annexure (B)

Annexure (C)

Statistical Analysis

Statistical analysis of data was calculated by following the formula

$X_2 = \sum (O - E)^2 / E$

Where, O = Observed (Treated value), E = Expected (Control value)

Table 4. Critical χ^2 of the enzymes affected by A. Calamus is 9.488 (df=4, α=0.05). So, there is a statistically significant difference in enzyme activity between the Treated and Control groups ($p < 0.05$), indicating that A. Calamus affects enzyme inhibition because 48.11 > 9.488; we reject the null hypothesis. GOT shows the highest χ^2 contribution (33.19), suggesting it is most affected by A. Calamus. In contrast, CHE shows the second-highest chi-square value (χ^2 = 10.69). ALP and GPT show decreased activity (χ^2 = 1.82 and 2.41, respectively). Meanwhile, GOT has the chi-square value (χ^2 = 2.58).

Table 5 shows that the critical χ^2 of the enzymes affected by Biosal is 9.488 (df=4, α=0.05). So, there is a statistically significant difference in enzyme activity between the Treated and Control groups ($p < 0.05$), indicating that Biosal affects enzyme inhibition because 11.48 > 9.488; we reject the null hypothesis. CHE shows the highest χ^2 contribution (5.91), suggesting it is most affected by Biosal. While ALP and GPT show decreased activity (χ^2 = 1.44 and 1.55, respectively). Meanwhile, GOT has the chi-square value (χ^2 = 2.58).

Table 6 shows that the critical χ^2 of the enzymes affected by deltamethrin is 9.488 (df = 4, α=0.05). So, there is a statistically significant difference in enzyme activity between the treated and Control groups ($p < 0.05$), indicating that deltamethrin affects enzyme inhibition because 13.94 > 9.488; we reject the null hypothesis. CHE shows the highest χ^2 contribution (6.20), suggesting it is most affected by deltamethrin. While ALP and GPT show increased activity (χ^2 = 2.28 and 4.71, respectively). Meanwhile, GOT has the smallest effect of the pesticide (χ^2 = 0.75).

Discussion:

Comparative analysis indicates that despite their different modes of action, the three pesticides share the common effect of disrupting neurophysiological (CHE) and metabolic (ALP, GOT, GPT) enzyme systems. Tribolium castaneum larvae's enzyme profiles treated with sublethal Dimilin and Ambush concentrations. They observed increased activities of lactate dehydrogenase and phosphatase and a decrease in amylase activity. Furthermore, led to ALAT activity increases and

decreased in trehalase levels with Dimilin treatment. Their study concluded that, on multiple enzymes, there were synergistic or antagonistic effects of Dimilin and Ambush by combined application (Saleem and Shakoori 1989). The present study demonstrates that both plant-derived and synthetic pesticides effectively inhibit key metabolic enzymes, with Deltamethrin showing superior efficacy against CHE and ALP, while A. Calamus oil exhibited more consistent inhibition across all tested enzymes. The impact of mimosine on T. castaneum was reported to be a significant inhibition of trehalase, invertase, and amylase activities, while protease activity increased. This work primarily focused on carbohydrase and protease enzymes in larval stages (Ishaaya et al. 1991). The differential inhibition patterns observed between botanical and synthetic pesticides suggest that the latter may offer more targeted neurotoxic effects, whereas plant-derived compounds provide broader metabolic disruption. Mujeeb and Shakoori (2012) reported a significant reduction in cholinesterase activity in larval and adult stages of the CTC-12 strain, except for a notable increase in older adults of certain strains of T. castaneum. The enzyme inhibition data support the hypothesis that pesticide efficacy can be assessed through biochemical markers, providing a quantitative framework for comparing the potency of different insecticidal compounds. Sublethal doses of Deltamethrin significantly decreased ALP (33%), amylase (40%), and GPT (32%) activity in resistant populations of T. castaneum. In susceptible populations, there was a significant increase in GOT activity (513%), trehalase (348%), and acid phosphatase (135%) (Ali et al., 2014). These results partially correspond with the current study, where GOT and GPT activity in C. analis decreased moderately after Deltamethrin exposure, indicating that pesticide susceptibility and species variation are important factors influencing enzyme response. The present study confirms that both botanical (e.g., A. calamus and neem) and synthetic (Deltamethrin) pesticides can significantly alter key metabolic enzyme activities in C. analis. Among the tested pesticides, Deltamethrin exhibited the most potent inhibitory effects, especially on ALP and CHE. The findings contribute to the growing body of evidence that enzyme inhibition can serve as a biochemical marker for pesticide exposure and efficacy in stored grain pests. The current study demonstrates that both botanical and synthetic insecticides can significantly disrupt key metabolic enzymes in C. analis. The reduction in ALP and CHE activities suggests interference with essential physiological processes, potentially leading to impaired detoxification and neural function. Susceptible and resistant strains of Tribolium castaneum show a decrease in proteolytic enzyme activities after exposure to Deltamethrin (Saleem et al., 2004), supporting our observation about CHE and ALP activity reduction in Callosobruchus analis subsequent to Deltamethrin treatment. Combining inhibition of enzyme with Deltamethrin-like piperonyl butoxide toxicity significantly enhanced against Tribolium castaneum (Mansee and Montasser 2003). This proposes that the observed inhibition of the enzyme in our study might be further augmented through synergistic formulations, possibly improving the efficacy of pest control. The neem-derived saponins inhibit acetylcholinesterase activity in Tribolium castaneum (Ashfaq et al., 2016), which is consistent with our findings of CHE activity reduction in Callosobruchus analis by Biosal treatment.

These results support the probable integration of botanical-based pesticides into pest management strategies, offering environmentally friendly alternatives with comparable efficacy.

Conclusion:

In conclusion, Acorus calamus oil, Biosal, and Deltamethrin significantly inhibited key enzymatic activities, cholinesterase (CHE) and alkaline phosphatase (ALP), in Callosobruchus analis. Reductions suggest that metabolic disruptions and neurotoxicity, with Deltamethrin showing the strongest inhibitory effect. These findings highlight the potential of these pesticides in pest control by targeting vital physiological processes in the insect. Since many drugs are enzyme inhibitors, biochemists and pharmacologists are actively trying to discover and improve inhibitors. These inhibitors are judged based on two factors: potency and specificity. Enzyme inhibition by phytopesticides offers a highly targeted, ecologically sustainable, and biosafe approach to crop protection. By disrupting specific physiological pathways within pests, such as digestion, neural signaling, or hormonal development, these plant-derived metabolites mitigate the severe ecological and toxicological downsides associated with synthetic chemical alternatives.

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Annexure (A)

Table 4: Statistical analysis of key enzymes (CHE, ALP, GPT, and GOT) using Chi-Square in Callosobruchus analis after 24-hour exposure to A. Calamus extract.

S.No.	Enzyme	Treatment	U/L (O)	Expected (E)	(O - E)	(O - E) ²	(O - E) ²/E
1	GOT	Control	657.66	657.66	0	0	0
		Treated	509.94	657.66	147.72	21821.20	33.19
2	GPT	Control	35.11	35.11	0	0	0
		Treated	25.92	35.11	-9.19	84.46	2.41
3	ALP	Control	24.35	24.35	0	0	0
		Treated	31.01	24.35	+6.66	44.36	1.82
4	CHE	Control	175.62	175.62	0	0	0
		Treated	218.95	175.62	+43.33	1877.49	10.69

Annexure (B)

Table 5: Statistical analysis of key enzymes (CHE, ALP, GPT, and GOT) using Chi-Square in Callosobruchus analis after 24-hour exposure to Biosal extract.

S. No	Enzyme	Treatment	U/L (O)	Expected (E)	(O - E)	(O - E) ²	(O - E) ²/E
1	GOT	Control	65.58	65.58	0	0	0
		Treated	52.58	65.58	-13.00	169.00	2.58
2	GPT	Control	21.29	21.29	0	0	0
		Treated	15.55	21.29	-5.74	32.95	1.55
3	ALP	Control	11.31	11.31	0	0	0
		Treated	15.35	11.31	+4.04	16.32	1.44
4	CHE	Control	37.29	37.29	0	0	0
		Treated	52.13	37.29	+14.84	220.23	5.91

Annexure (C)

Table 6: Statistical analysis of key enzymes (CHE, ALP, GPT, and GOT) using Chi-Square in Callosobruchus analis after 24-hour exposure to Deltamethrin extract.

S. No	Enzyme	Treatment	U/L (O)	Expected (E)	(O - E)	(O - E) ²	(O - E) ²/E
1	GOT	Control	21.00	21.00	0	0	0
		Treated	17.03	21.00	-3.97	15.76	0.75
2	GPT	Control	71.28	71.28	0	0	0
		Treated	52.95	71.28	-18.33	335.99	4.71
3	ALP	Control	16.29	16.29	0	0	0
		Treated	22.38	16.29	+6.09	37.09	2.28
4	CHE	Control	149.41	149.41	0	0	0
		Treated	179.85	149.41	+30.44	926.59	6.20