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Effect of pesticides after 24 hours of treatment on transaminase, Alkaline phosphatase and Cholinesterase in *Callosobruchus analis* by filter paper impregnation method

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Abstract
Enzyme estimation after 24 hours of treatment with three pesticides, the *Acorus calamus* (essential oil), Biosal (Neem pesticide) and Deltamethrin (Pyrethroid) was carried out (GOT, GPT, ALP and CHE) against *Callosobruchus analis* by Filter paper Impregnation Method. The LC_{50} dose was calculated of *Acorus calamus*, Biosal and Deltamethrin 0.9999 µl/cm^2, 6.945603 µl/cm^2 and 0.03472 µl/cm^2. Enzyme activity after 24 hours of treatment *A. calamus* on *C. analis* GOT, GPT, ALP and CHE was 76.12%, 69.96% 21.18% and 67.97% respectively. Enzyme activity of Biosal on *C. analis* GOT, GPT, ALP and CHE was 75.77%, 67.84%, 16.84% and 48.64% respectively. Enzyme activity after 24 hours treatment of Deltamethrin against *C. analis* for GOT, GPT, ALP and CHE was 63.11%, 72.84%, 57.7% and 63.81% respectively.

Keywords: Pesticides, treatment, alkaline phosphatase and cholinesterase, C*allosobruchus analis*

1. Introduction
Many researchers worked on enzymes activity [1] worked on provisional recommendation on IFCC methods for measurement of catalytic concentration of enzymes asparate. [2] worked on provisional recommendation on IFCC methods for the measurement of catalytic concentration of enzyme alanine aminotransferase. [3] reported biochemicalchanges in 6th instar larvae of *Tribolium castaneum* by apply pyrithroid pesticides. [4] reported the level of esterase in the various developmental stages of *Tribolium castaneum*. [5] reported the effect of Decis-D on the esterase of red flour beetles *Tribolium castanem*. [6] worked on macromolecular and enzymatic abnormalities induced by a synthetic pyrethroid Ripcord cypermethrin in adults beetles of stored grain pest Tribolium castaneum (Herbest) (Coleoptera: Tenebnonidae). [7] worked o ultraviolet spectrophotometery for determination of insecticides and aromatic hydrocarbon pollutants. [8] worked on permethrin and cypermethrin on the toxicity of gamma – HCH+ studies on some biochemical component of adult beetles of *Tribolium castaneum*. [9] reported the effect of starvation on protease in insecticide resistant and susceptible strain of *Tribolium castaneum*. [10] worked on screening of Korean herbel medicines used to improve conginitive function for anti-cholinesterase activity. [11] reported of *clerodendum ineme* extract and cyhalothrin against *Rhizopertha dominica* PARC strain and their effects on acid phosphatase and cholinesterase activity. [12] worked on the effect of α-cypermethrin on esterase of sixth instar larvae and 10 days old adults of three different strains of *Triboliun castaneum*. [13] reported acetylcholinesterase inhibition of oil from *Acorus calamus* rhizome. [14] worked on asarone in *Acorus calamus* and their acetylcholinesterase inhibition. [15] reported the effect of an organophosphate, pirmiphos-Methyl. On esterase of different developmental stages of stored grain pest *Tribolium castaneum*. The present study work on enzyme effect of phytopesticides *Acorus calamus*, Biosal as compared to pyrethroid deltamethrin.

2. Material and Methods

2.1 Biochemical Estimation

Determination of Enzymatic Activities under the Effect of Pesticides: Freshly emerged adults of known age were used fifty grams mung (*Vigna radiata*) were taken in each petri dish and mixed with pesticide at LC 50 doses than 122 insects 0.5 g (500 mg) adult insects were released separately. Untreated insects of the same number of adults were kept as control. After 24 hours, O.5 g of treated alive and untreated adults were taken separately and crushed and homogenized for biochemical analysis of enzymatic activity.

2.2 Preparation of the Homogenate

For the biochemical analysis, the homogenate was prepared by following method.

Callosobruchus analis weighing about 0.5 g (500 mg) for each batch in each replicated were crushed in 2.5 ml bi-distilled water with pestle and mortor and homogenized in "Labofuge 200" tissue grinder "Laboratory dispresser OSK 9258" for five minute at 1000 rpm. The homogenates were then centrifuged in "Lobofuge 200" at 3500 rpm for 20 minutes placed in cold chamber. Supernatants were taken in separate tubes and were used for biochemical experiments. During experiment the homogenate and reaction mixture were kept in ice at 5-8 °C approximately. The extracts were used for the estimation of Glutamate pyruvate transaminase (GPT), Glutamate oxaloacetate transaminase (GOT), Cholinesterase (CHE), and Alkaline phosphatase (ALP).

2.3 Method of Enzyme Estimation:

A) Estimation of Glutamate Oxaloacetate Transaminase (Got)

The activity of enzyme GOT was estimated by chromatest Kit no. 13427, the reagent composition consisiting of AST substrate TRIS buffer mmol/L. PH. 7.8, L-asparate 362 mmo/L, malate dehydrogenase > 460 U/L, lactate dehydrogenase> 600U/L and the AST coenzyme NADH 1,3 mmol/L, 2- oxoglutrate 75 mmol/L Biocides which was measured 340 nm UV min 1240 UV- VIS Spectophotometer Shimadzu.

Principle

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

Oxaloacetate + NADH + H^+ $\xrightarrow{\text{MDH}}$ L- malate + NAD

Aspartate aminotransferase (AST/GOT) catalyzes the transfer of amino group from aspartate to oxoglutarate with the formation glutamate and oxaloacetate. The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+, proportional to the activity of AST present in the sample.

B) Estimation of Glutamate Pyruvate Transaminase (Gpt)

The activity of enzyme GPT was estimated by chromatest Kit no. 13361 the reagent composition was consisting ALT substrate, TRIS buffer 150 mmol/L lactate dehyrogenase> 1350 U/L and the ALT coenzyme. NADH 1:3 mmol/L, 2-oxogularate 75 mmol/L Biosides which was measured 340 nm, UV min 1240 UV-VIS spectophotometer Shimamadzu:.

Principle

L- Alanine + 2 oxoglutrate $\xrightarrow{\text{ALT/GPT}}$ L- Glutamate + Pyruvate + NADH + Pyruvate H^+ $\xrightarrow{\text{LDH}}$ Lactate+NAD^+

Alanine aminotransferase (ALT/GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD +, Proportional to the activity of ALT present in the sample.

C) Estimation of Alkaline Phosphatase

The activity of enzyme alkaline phosphatase (ALP) was estimated by chromatest Kit no. 12915. The reagent composition was consisting ALP buffer, DEA buffer 1.25 mol/L PH 10.2, magnesium chloride 0.6 mmol/L Biosides and substrate 4-NPP 50 mmol/L Biosides which was measured 405 nm, UV min 1246 UV-VIS Spectrophotometer Shimadzu.

Principle

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

Alkaline phosphatase (ALP) Catalyze the Hydrolysis of 4-nitophenylphosphate (4-NPP) with the formation of free 4-nitrophenol and inorganic phosphate, acting the alkaline buffer as a phosphate- group acceptor. The reaction is monitored kinetically at 405 nm by the rate of formation of 4-nitrophenol, proportion to the activity of ALP present in the sample.

D) Estimation of Butryl Cholinesterase

The activity of enzyme cholinesterase (CHE) was estimates by RANDOX Kit no. 188259. The reagent composition Buffer/chromogen phosphate buffer 50 mmoi/l, PH 7.7. DTND 0.25 mmol/l and the substrate Butyrylthiocholine Iodide 6 mmol/l. Butyrylcholinesterase hydrolysis hydrolysis burylthiocholine to give thiocholine and butyrate. The reaction between thiocholine and DTND gives 2-nitro 5-mercaptobenzoate a yellow compound which can be measured at 405 nm UV min 1246 UV-VIS Spectophotometer Shimadzu.

Principle

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

Thiocholine + DTNB → 2-nitro-5 mercaptobenzoate.

Dtn = Dithiobis (nitrobenzoate)

3. Result and Discussion

Enzyme activity after 24 hours of treatment *A. calamus* on *C.analis* GOT,GPT,ALP and CHE was 76.12%, 69.96% 21.18% and 67.97% respectively. Enzyme activity of Biosal on *C. analis* GOT, GPT, ALP and CHE was 75.77%, 67.84%, 16.84% and 48.64% respectively. Enzyme activity after 24 hours treatment of Deltamehrin against *C. analis* for GOT, GPT, ALP and CHE was 63.11%, 72.84%, 57.7% and 63.81% respectively. [16] worked on pirimiphos-methyl treatment significantly decreased the carboxylesterase (CE)

activity in all development stage 4th and 6th instar larvae newly emerged and 15 days old beetles of pak strain (56, 29, 76, 66%) FSS II (81, 75, 64 and 27%) and CTC-12 strain (61,58,51 and 48%). Author observed the acetylcholinesterase (AchE) activity decreased significantly with the treatment in all stages of three strain. The cholinesterase (ChE) increased (65, 59 and 37%) only in the 4th and 6th instar larvae and 15 days old adult beetles of pak.strain, while it deceased (31%) in newly emerged adult. ChE activity of 15 days old beetles increase (21 and 80%) while the other stages decrease after treatment with pirmiphos-methyl. In the present study activity of enzyme cholinesterase after 24 hours treatment of deltamethrin against *Callosobruchus analis* by Filterpaper Impregnation Method observed as 63.81%. Present study was comparable showed that deltamethrin effective on enzyme inhibition. [17] reported the enzyme inhibition Danitol-treated *Alphitobus diaperinus* adult showed 44.66% and 45.91% in GPT and GOT activity respectively, similarly inhibition of 52.48% and 12.15% in GPT and GOT activity respectively. In the present study GOT and GPT activity after 24 hours treatment of detltamethrin was observed as 63.11% and 72.84%.Present study was not comparable may be due to different insects species and pesticides. [18] Author used the prospectus of utilizing Lantana camara as apotent fumigant insecticides. They observed inhibition of acetylcholinesterase (AchE) by coumaran. In the present study cholinesterase activity was observed after treatment of phytopesticides Biosal and *Acorus calamus* by Filter paper impregnation method cholinesterase activity was observed after treatment of *A. calamus* 67.97% and after the treatment of Biosal 48.68%. Present study was shows that phytopesticides effective on enzyme activity. [19] collected field strain were susceptible to spinosad. They observed high resistance to deltamethrin in cross resistance (380 foldes) against spinosad just upto 8 generation. They observed enzymatic assay acetylcholinestrase amylase and catalase. In the present study cholinesterase activity was observed after 24 hours treatment of deltamethrin by Filter paper Impregnation method. Present study showed the inhibition of enzyme activity.

Table 1: Enzyme inhibition of GOT, GPT, ALP and CHE against *Callosobruchus analis* after 24 hours treatment by *A. Calamus* at LC_{50} 0.09999 µl/cm^2.

S.NO.	Enzyme	Treatment	U/L	Inhibition	Activity
1.	GOT	Control	431.58	00	100.00
		Treated	328.48	23.88	76.12
2.	GPT	Control	80.93	00	100.00
		Treated	56.61	30.04	69.96
3.	ALP	Control	8.19	00	100.00
		Treated	14.65	78.81	21.18
4.	CHE	Control	97.72	00	100.00
		Treated	127.72	32.05	67.97

Table 2: Enzyme inhibition of GOT, GPT, ALP and CHE against *Callosobruchus analis* after 24 hours treatment by Biosal at LC_{50} 6.945603 µl/cm^2.

S. No	Enzyme	Treatment	U/L	Inhibition	Activity
1.	GOT	Control	38.47	00	100.00
		Treated	29.14	24.23	75.77
2.	GPT	Control	41.49	00	100.00
		Treated	28.14	32.16	67.84
3.	ALP	Control	23.57	00	100.00
		Treated	43.30	83.71	16.28
4.	CHE	Control	74.07	00	100.00
		Treated	112.08	51.31	48.68

Table 3: Enzyme inhibition of GOT, GPT, ALP and CHE against *Callosobruchus analis* after 24 hours treatment by Deltamethrin at LC_{50} 0.03472 µl/cm^2.

S. No	Enzyme	Treatment	U/L	Inhibition		Activity
1.	GOT	Control	8.802	00		100.00
		Treated	5.55	36.89		63.11
2.	GPT	Control	106.88	00		100.00
		Treated	77.84	27.16		72.84
3.	ALP	Control	38.63	00		100.00
		Treated	54.97	42.30		57.7
4.	CHE	Control	363.66	00		100.00
		Treated	495.26	36.18		63.81

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