COMPARATIVE EFFECT OF BIOSAL® AND PYRETHROIDS (DELTAMETHRIN AND LAMBDA CYHALOTHRIN) ON ENZYMATIC ACTIVITY AND TOTAL PROTEIN CONTENTS IN *HERMOLAUS MODESTUS*

SYED IKHLAQ HUSSAIN[1] AND MUHAMMAD ZAHID[1]

[1]Department of Zoology, Federal Urdu University of Arts Science and Technology, Karachi
Corresponding author e-mail: ikhlaq.hussain@yahoo.com

Abstract

Pesticides Biosal, Deltamethrin and Lambda cyhalothrin were used for the determination of total protein contents and GOT, GPT activity and inhibition in *Hermolaus modestus.* Observations showed that LC_{50s} of biosal, deltamethrin and lambda cyhalothrin were 6.45μg/cm^2, 0.00004 μg/cm^2 and 0.349 μg/cm^2, respectively after 24 hours of treatment of adults of *Hermolaus modestus* by Filter Paper Impregnation Method. The activity of enzyme GOT was observed 98.67%, 33.95%, and 83.61% after the treatment of biosal, deltamethrin and lambda cyhalothrin, respectively. The activity of enzyme GPT after 24 hours of treatment with biosal, deltamethrin and lambda cyhalothrin were calculated 89.26%, 73.07% and 47.58%, respectively. The estimation of total protein contents after 24 hours of treatment of biosal, deltamethrin and lambda cyhalothrin in *H. modestus* was observed 31.05%, 4.60%, and 24.57%, respectively.

Introduction

The pesticides are used throughout the world to control the pests in most agricultural system. According to Ballantine (1992), One-third of the world crops destroyed by the pests every year. In different countries the use of pesticides has increased rapidly and one fifth of all pesticides are used in Pakistan (Tahir, 2000). Different types of pesticides save our crops from the pests in large scale and their implementation have long been recognized and regulated. The synthetic pesticides are vulnerable as compared to phytopesticides. Different groups of synthetic pesticides play an important role in pest control such as *chrysanthemum indicum* is a development inhibitor of *Dysdercus similes* (Kaur *et al.* 1989). The use of phytopesticide is very common to control the pests (Naqvi *et al.* 1996) and has minimum side effects (Johri *et al.* 2004). Plant pesticides are generally called natural, botanical, phyto and bio pesticides (Ermel *et al.* 1991; EL-Nahal *et al.* 1994; Saxena *et al.* 1974; Ravikant *et al.* 2007).Arif *et al.* (2015) studied the toxicity of biosal, essential oil against *C. analis* by three methods Direct Application Methods, Glass Film Method and Filter Paper Impregnation Method after 24 hours of treatment. The toxicity of pesticide is estimated by using biochemical indicators i.e. Total protein, Glutamate Pyruvate transaminase and Glutamate oxaloacetate transaminase which have pre-described values. Enzyme activity can change due to the use of pesticides or chemicals and researchers worked on the activity of different enzymes. Saleem *et al.* (1998) observed enzymatic abnormalities in adult of *Tribolium castaneum* treated with cypermethrin. The effect of dimilin and neem extract nimolicine on enzyme activity of pulse beetle *Callosobruchus analis* was studied by (Tabassum *et al.* (2006). Tanveer *et al.* (2005) worked on *Sitophilus oryzae* L., treated with cypermethrin, *Acorus calamus*, Danitol, methoprene and neem extract and determined the effect on total protein contents. They also reported the reduction in total protein contents due to the environmental factors.

In this work, the enzymes and protein activity in *Hermolaus modestus* have been with the treatment of biosal, deltamethrin and lambda cyhalothrin. Tabassum *et al.* (1998) discussed the GOT (Glutamate oxaloacetate transaminase) and GPT (Glutamate pyruvate transaminase) activity in adults of *Alphitobus duaperinus* with neem formulation and Danitiol. Naqvi *et al.* (1990) studied the toxicity and residual effect of neem factor against adults of *Callosobruchus analis* and determined the LC_{50} value. In the present work LC_{50s} have been calculated for biosal, deltamethrin and lambda cyhalothrin against *Hermolaus modestus.*

Material and Methods

Treatment Process

The process of treatment performed by filter paper impregnation method. For this purpose eighteen sets of petridishes (one controlled in each set) were used and lined with same sized filter papers and then selected five suitable doses of each pesticides were pipette out on filter papers. Twenty adults of *H. modestus* of same size were released in all sets of petridishes. Mortalities were observed in all petridishes including control after 24 h of pesticides treatment.

The five different concentrations of biosal as 4.44, 5.55, 6.66, 7.77 and 8.88 μg/cm^2 showed 11.65, 25, 58, 73 and 93.3% mortalities of *H. modestus* respectively after 24 h of treatment. The LC_{50} of biosal was calculated as

6.45 μg/cm^2 by log probit graph paper. (Table: 1).
The five different concentrations of deltamethrin against *H. modestus* were 1.11x10^{-5}, 2.22 x10^{-5}, 4.99 x10^{-5}, 9.99 x10^{-5} and 2.053 x10^{-4} μg/cm^2 and average per cent mortalities were showed as 1.66, 16.66, 33.3, 61.65 and 71.65% respectively. The value of LC_{50} of deltamethrin was calculated as 0.00004 μg/cm^2 by log probit graph paper. (Table: 1).
The average per cent mortalities of *H. modestus* was observed as 30, 36.66, 65, 66 and 73.3% at the concentrations of 0.3188, 0.2777, 0.4166, 0.555 and 0.6944 μg/cm^2 of lambda cyhalothrin pesticide. The LC_{50} of lambda cyhalothrin was found as 0.349 μg/cm^2 by log probit graph paper. (Table: 1).

Biochemical Estimation

The estimation of GOT, GPT and Total protein contents after pesticides LC_{50} treatment in *H. modestus* was conducted. For this purpose 0.5g of treated and untreated insects were used separately.

Formation of Homogenate

0.5 g of untreated (control) and treated insects was taken by measuring with Sartorius model CP 224S. The measured insects crushed separately in 4 ML of cooled distilled water and then homogenized in homogenizer, model JANKE $ KUNKEL (ULTRA – TURRAX – T25), then centrifuged at 3000 rpm for 25 minutes by model (Heraeus) Multifuge 3 S-R and temperature was 4 C°. The obtained supernatants were kept in separate tubes and labelled them by the pesticides and insect name. This supernatants were kept in ice at 4 °C approximately during the biochemical experiments.

Method of Biochemical Estimation

A) Estimation of Total Protein Contents

This estimation was carried out by Roche/Hitachi Cobas® analyzer Modular P analyzer: ACN, 402. Reaction occurred between benzethonium chloride and protein in a basic medium to produce a turbidity that is more stable and evenly distributed, than observed with the TCA (trichloroacetic acid) methodology.

Test Principle

The sample was preincubated in an alkaline solution containing EDTA, which denatured the protein and eliminated interference from magnesium ions and then benzethonium chloride was added, producing a turbidity that was read at 505nm. Reagents: reagent used were R_1 – Sodium hydroxide: 530 mmol/L; EDTA – Na: 74 mmol/L and reagent R_2 – Benzethonium chloride: 32 mmol/L. The analyzer automatically calculates the analyte concentration of each sample.

B) Estimation of GOT (Glutamate oxaloacetate transaminase)

This biochemical test was performed with Roche/Hitachi 912 analyzer: ACN 143. The composition of reagents was R_3: TRIS buffer: 100 mmol/L, PH 7.8; L-aspartate: 300 mmol/L; NADH (yeast): 0.23 mmol/L; MDH ≥ 0.53 U/mL (8.83 µKat/L); LDH ≥ 0.75 U/mL (12.5 µKat/L); preservative and reagent R_4: α – Ketoglutarate: 75 mmol/L; preservative.

Test principle

Test is according to standardized method
- Sample and addition of reagent R_3
- Addition of reagent R_4 and start of reaction.

Glutamate oxaloacetate transaminase catalyzes the interconversion of amino acids and α – ketoacids by transfer of amino groups.

$$\Rightarrow \alpha - \text{Ketoglutarate} + \text{L} - \text{aspartate} \overset{\text{AST}}{\leftrightharpoons} \text{L} - \text{glutamate} + \text{oxaloacetate}$$

The enzyme AST catalyzes the equilibrium reaction.

$$\Rightarrow \text{Oxaloacetate} + \text{NADH} + \text{H}^+ \overset{\text{LDH}}{\leftrightharpoons} \text{L} - \text{malate} + \text{NAD}^+$$

NADH is oxidized to NAD^+.

C) Estimation of GPT (Glutamate pyruvate transaminase)

The estimation of GPT was performed with Roche/Hitachi 912 analyzer: CAN – 098. Reagent R_5: TRIS buffer: 125 mmol/L, PH 7.3; L-alanine: 625 mmol/L; NADH (yeast): 0.23 mmol/L; LDH ≥ 1.5 U/mL (25.0 µKat/L); preservative and reagent R_6: α – Ketoglutarate: 94 mmol/L; preservative. Glutamate Pyruvate transaminase which catalyze the conversion of amino acids to the corresponding α – Keto acids via the transfer of amino groups; they also catalyzer the reverse process. Wroblewski and LaDue (1956) described the first kinetic determination of GPT. The International Federation of Clinical Chemistry (IFCC) described and confirmed

standardization method for the determination of GPT (2002). In this method reagent R_5 mixed with sample and added reagent R_6.

$$\Rightarrow \alpha - \text{Ketoglutarate} + \text{L} - \text{alanine} \overset{\text{AST}}{\leftrightharpoons} \text{L} - \text{glutamate} + \text{Pyruvate}$$

ALT is the enzyme which catalyze the following equilibrium reaction. The pyruvate is catalyzed by lactate dehydrogenase in second reaction.

$$\Rightarrow \text{Pyruvate} + \text{NADH} + \text{H}^+ \overset{\text{LDH}}{\leftrightharpoons} \text{L} - \text{lactate} + \text{NAD}^+$$

Statistical Analysis

Standard error (S.E) and Confidence limit at 95% were calculated.

Results and Discussion

Muller and Kley (1982), Saito and Miyata (1988) and Trevan (1927) described the importance of LC_{50}. Arif *et al*. (2011) carried out the toxicity of deltamethrin and phytopesticides against *Callosobruchus analis* and determined the values of LC_{50} of treated compounds. In present work the LC_{50} of biosal, deltamethrin and lambda cyhalothrin against *Hermolaus modestus* were found 6.45 μ g/cm^2, 0.00004 μ g/cm^2 and 0.349 μ g/cm^2 respectively. (Table: 1).

Ahsan *et al.* (2005) studied the estimation of total protein contents during comparative study of cypermethrin, *Acorus calamus* with Danitol, methoprene and neem extract against *Sitophilus oryzae* (rice weevil). In the present finding the inhibition of total protein contents in adults of *H. modestus* after 24 h of treatment of biosal, deltamethrin and lambda cyhalothrin were 68.941, 95.392 and 75.426%. The mean g/dL values for total protein contents were 0.182, 0.027, and 0.144 g/dL, respectively. The maximum decline observed as 95.392% by deltamethrin. The total protein contents activity in *H. modestus* after the treatment of compounds were 31.059, 4.607, and 24.573%. (Table. 2; Fig. 1).

Enzyme GOT inhibition against adult of *H. modestus* after 24 h treatment of biosal, deltamethrin and lambda cyhalothrin were observed 1.325, 66.044 and 16.381% at LC_{50} doses of 6.45μg/cm^2, 0.00004 μg/cm^2 and 0.349 μg/cm^2, respectively. The mean U/L values of GOT were 694.53, 239.0 and 588.56 for biosal, deltamethrin and lambda cyhalothrin, respectively. 66.044% inhibition was the maximum decreased by deltamethrin as compare to biosal and lambda cyhalothrin. The activity of enzyme GOT as 98.67, 33.95, and 83.61% in *H. modestus*, treated with biosal, deltamethrin and lambda cyhalothrin, respectively. (Table. 3; Fig. 2).

Enzyme GPT inhibition against adults of *H. modestus* after 24 h treatment of biosal, deltamethrin and lambda cyhalothrin was observed 10.74%, 26.93% and 52.42%, respectively and mean U/L values were 72.16, 47.61 and 31.00 for biosal, deltamethrin and lambda cyhalothrin. The maximum decline showed by lambda cyhalothrin as 52.42%. The activity of enzyme GPT for biosal, deltamethrin and lambda cyhalothrin in treated insects, were 89.26, 73.07, and 47.58%. (Table. 4; Fig. 3).

In this study we estimated the effectiveness of pesticides (biosal, deltamethrin, lambda cyhalothrin) in *H. modestus* that how much the significant change occurred in total protein contents, GOT and GPT enzymes. The result showed the significance of pesticides in term of their activity decreased or increased.

Yasmin *et al*, (2010) studied the role of biosal and cypermethrin in the larvae of *Papilio demoleus* L. and showed the activity of protein contents and cholinesterase enzyme. Total protein contents decreased up to 31.28% with biosal and 36.44% with cypermethrin. They found biosal was much safer than cypermethrin. In the present study the decline in total protein contents was observed 68.941, 95.392 and 75.426% in *H. modestus* for biosal, deltamethrin and lambda cyhalothrin respectively. The effectiveness of pesticides on protein contents can be show by, Deltamethrin> Lambda cyhalothrin> Biosal. This result changed with the study of Yasmeen *et al*, (2010) due to the different test insect.

Tabassum *et al*, (2006) used NC and Nfc neem compounds (phytopesticides) and dimilin as a pyrethroid by filter paper impregnation method after 24 h of treatment against *C. analis*. The activity of GOT, GPT, ALP (Alkaline phosphatase) and ChE (Cholinesterase) was observed 84.85%, 88.14%, 71.60% and 38.05%, respectively for NC treated insects. 67.50%, 51.71%, 87.96% and 87.64% activity showed for Nfc treated *C. analis.* Pesticide dimilin treated insects showed activity of ALP, ChE, GOT and GPT as 73.41, 55.62, 90.68 and 91.11%, respectively. In the present studies, the activity of GOT and GPT were observed 98.67, 89.26% for biosal respectively, 33.95 and 73.07% for deltamethrin and 83.61, 47.58% for lambda cyhalothrin. The different results of present study due to the different insect and pesticides but the method was same. The activity of GOT enzyme was maximum as 98.67% with the treatment of biosal and was minimum as 33.95% with deltamethrin. The GPT activity was observed high as 89.26% for biosal and low as 47.58% for lambda cyhalothrin.

Table 1. - LC_{50} of insecticides studied.

S. No	Pesticides	LC_{50} ($\mu g/cm^2$)
1	Biosal	6.45
2	Deltamethrin	0.00004
3	Lambda Cyhalothrin	0.349

Table 2. Inhibition of Total protein contents in adult of *H. modestus* after 24 hrs of treatment of Biosal, Deltamethrin and Lambda Cyhalothrin at 95% of confidence limit by FIM.

S. No	Pesticides	mean g/dL	S.E. (±)	Confidence limit at 95%	Inhibition
1	Control	0.586	0.0293	0.5286 – 0.6434	00
2	Biosal	0.182	0.0012	0.155 – 0.205	68.941
3	Deltamethrin	0.027	0.0051	0.0168 – 0.0371	95.392
4	Lambda Cyhalothrin	0.144	0.0107	0.1230 – 0.1649	75.426

Table 3. Inhibition of enzyme GOT in adult of H. modestus after 24 hrs of treatment of Biosal, Deltamethrin and Lambda Cyhalothrin at 95% of confidence limit by FIM.

S. No	Pesticides	mean U/L	S.E. (±)	Confidence limit at 95%	Inhibition
1	Control	703.86	472.2	221.65 – 1629.37	00
2	Biosal	694.53	2.762	689.11 – 699.94	1.325
3	Deltamethrin	239.0	0.258	238.4 – 239.5	66.044
4	Lambda Cyhalothrin	588.56	0.856	246.4 – 249.77	16.381

Table 4. Inhibition of enzyme GPT in adult of H. modestus after 24 hrs of treatment of Biosal, Deltamethrin and Lambda Cyhalothrin at 95% of confidence limit by FIM.

S. No	Pesticides	Mean U/L	S.E. (±)	Confidence limit at 95%	Inhibition
1	Control	65.16	1.241	62.728 – 67.59	00
2	Biosal	72.16	1.137	69.931– 74.38	10.742
3	Deltamethrin	47.61	1.552	44.56– 50.65	26.933
4	Lambda Cyhalothrin	31.0	1.114	28.81 – 33.18	52.424

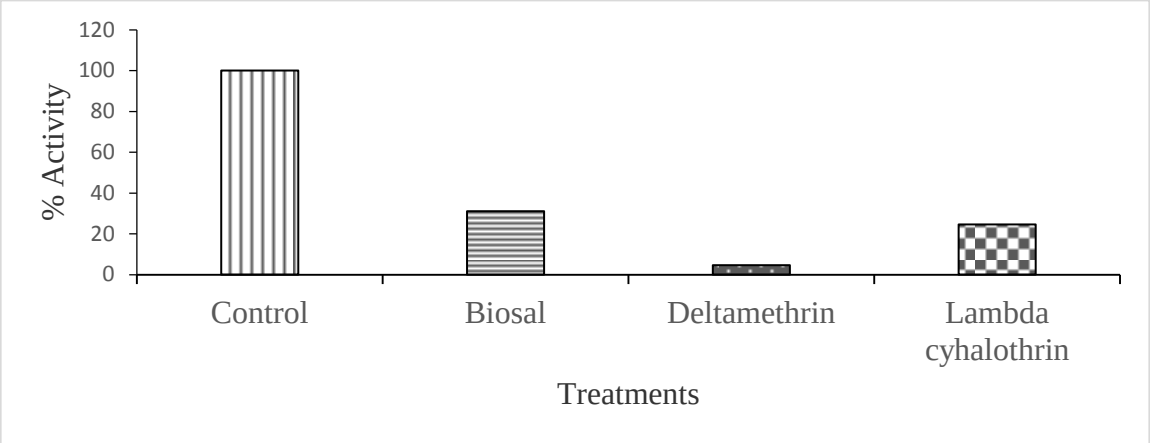

Fig. 1. *Per cent* Activity of Total protein contents in adults of *H. modestus* after LC_{50} treatment with different pesticides at 95% confidence limit.

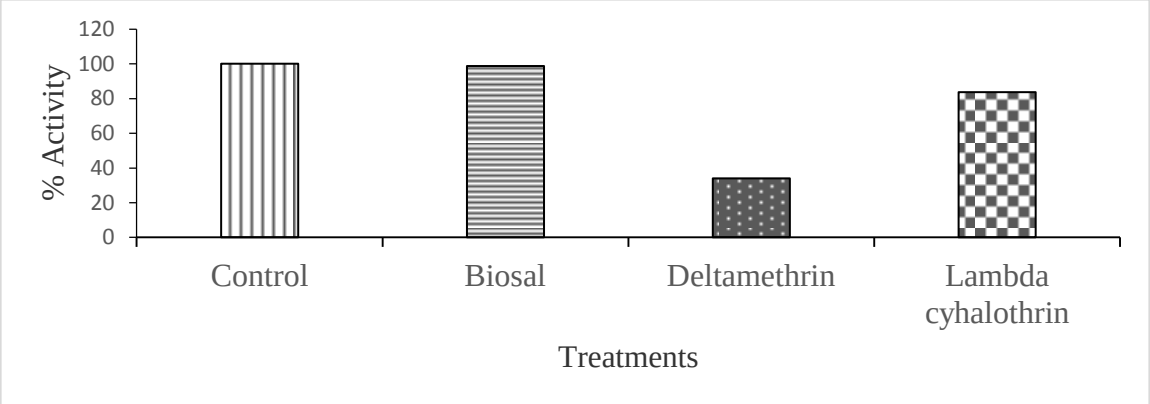

Fig. 2. *Per cent* Activity of GOT in adults of *H. modestus* after LC_{50} treatment with pesticides at 95% confidence limit.

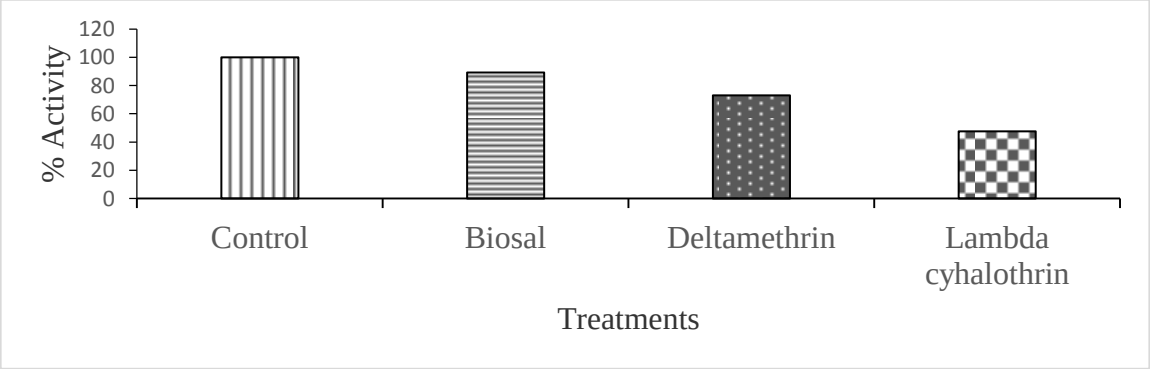

Fig. 3. *Per cent* Activity of GPT in adults of *H. modestus* after LC_{50} treatment with pesticides at 95% confidence limit.

References

Ahsan, T., Ahmad. I., Yasmeen. N., Tabassum, T., Azmi, M. A. and Shoaib, M. (2005). Effectiveness of cypermethrin 10. E. C. and *Acorus calamus* extract in comparison with Danitol, Methoprene and neem extract and their effect on total protein contents of *Sitophilus oryzae 1*. *.INT. J. BIO. BIOTECH*. 2(4): 951-954.

Arif, S., Naqvi, S. N. H., Raza, A., Tabassum, S. and Fatima, N. (2015). Fecundity effect of Biosal against *Callosobruchus analis* (Fabricius, 1781). *International Journal of Fauna and Biological Studies.*2 (2): 34-37.

Arif, S., Naqvi, S. N. H., Azmi, M. A., Younus, M. F., Aslam, M. and Siddiqui, T. F. (2011). Toxicity of two phytopesticides the acorus calamus oil and biosal (neem pesticide) as compared to deltamethrin by glass film method. *Pak. J. Entomol. Karachi* 26 (2): 135-140.

Ballantine, I. G. (1992). An overview of the U.S. pesticide registration guideline. *Agric. Newsletter*., 3(2): 1-6.

El-Nahal, A. K. M., Schmidt, G. H. and Risha, E. M. (1994). Influence of vapours of *Acorus calamus* L. oil on the reproductivity of some stored product Coleoptera. *Pak. J. Entomol. Karachi.* 9(1):21-27.

Ermel, K., Kalinowski, H. O., Schmuiterer, H. (1991). Isolation and characterization of marrangin, anew insect growth regulating (IGR) substance from seed Kernels of the marrayngo tree, *Azadirachta excestsal* (jack). *J. Appl. Entomol*., 112(5): 512-519.

Johri, P. K., Maurya, R., Singh, D., Tiwari. D. and Johri, R. (2004). Comparative toxicity of seven indigenous botanical extracts against the infestive stage of three insects pest of agricultural importance. *Journal of Applied Zoological Researches*. 15(2): 202-204.

Kaur, A., Thakur, S. S., Sabita-Raja, S. (1989) *Crysanthemum indicum* an effective growth and development inhibitor of *Dysdercus similes*. *J. Environ. Biol*., 10(4): 373-377.

Muller, H. and Kley, H. P. (1982). Retrospective study on the reliability of an approximate LD_{50} determined with a small number of animals. *Arch. Toxicol*. 51: 189-196.

Naqvi, S. N. H., Tabassum, R., Zia, N., Nurulain, S. M. (1990). Toxicity and residual effect of neem extract (Factor C) against stored grain pest *Callosobruchus analis.* Pakistan. J. Zool. 22(3): 271-278.

Naqvi, S. N. H., Tabassum, R. and Khan, M. Z. (1996). Toxicity determination neem compounds (Nfc and Nc) and dimilin by two methods against *Callosobruchus analis* PARC, Islamabad, Pakistan, p. 29-30.

Ravikant, U., Jaiswal, G. and Xadav, N. (2007). Toxicity repellency and oviposition inhibiting activity of some essential oils against *Callosobruchus chinensis*. *Journal of Applied Bioscience.* 33(1): 23-28.

Saito, T. and Miyata, T. (1988). Tropical and injection toxicities of some pyrethroids in the housefly *Musca domestica. J. Sanit. Zool.* 36 (1): 31-38.

Saleem, M. A., Shakoori, A. R. and Mantlr, D. (1998). Macromolecular and enzymatic abnormalities induced by adults beetles of stored grain pests *Tribolium castaneum* (Herbest) (Coleoptera: Tenebrionidae) Archives of insects Biochemistry and Physiology. 39(4): 144-154.

Schumann, G. *et al*. (2002). IFCC Primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 ˚C – part 4. Reference procedure for the measurement of catalytic activity concentrations of alanine aminotransferase. Clin Chem Lab Med. 40 (7): 718-724.

Sexena, B. P. E., Rohden, B. D., Verig, B. (1974). Morphological changes in the *Thermobia domestica* under the influence of *Acorus calamus* oil vapours. *Separatum Experimentia.*, 30: 1298.

Tabassum, R., Naqvi, S. N. H., Jahan, M. and Nurulain, S. M. (1998). Determination of the toxicities of Fenpropathrin (pyrethroid) and Neem Formulation (RB-a+POB+TX-100) against *Alphitobus diaperinus* adults and their effects of Transaminase *Tr. J. of Zoology*. 22: 319-322.

Tabassum, R., Naqvi, S. N. H. and Ahmad, I. (2006). Effect of neem extract nimolicine and dimilin on the enzymatic activities of pulse beetle *Callosobruchus analis F. J. Exp Zool. India.* 9(1): 197-202.

Tahir, S. (2000). *Pesticide effect on human health in Pakistan. Policy and strategy for rational use of pesticide*, Pakistan. 99/002/FAO, pp 57.

Tanveer AJ, Ahmed N, Yasmeen R, Tabassum A, Azmi, Shoaib M. (2005). Effectiveness of cypermethrin 10. EC and *Acorus calamus* extract in comparison with Danitol, methoprene and neem extract and their effect on total protein contents of *Sitophilus oryzae* L. *Intl. J. Biol and Biotech*. 2(4): 951-954.

Trevan, J. W. 1927. The error of deltamethrin of toxicity. In: Proc. *R. Soc. London.* 101B: 483-514.

Wroblewski, F., LaDue, J. S. (1956). Serum glutamic pyruvic transaminase in cardiac with hepatic disease. *Proc Soc Exp Bio Med*., 91(4): 569-571.

Yasmin, N., Naqvi, S. N. H., Khan, M. F., Arshad, Munir, and Tariq, R. M. (2010). Role of neem product (Biosal) and its impact on cholinesterase and protein contents in larvae of *Papilio demoleus* L. in comparison with cypermethrin. *Journal of Experimental Zoology, India*. Vol. 13 No. 2pp. 541-544.