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Biochemical response in phosphine resistant and susceptible adult beetles of *Trogoderma granarium* to the sublethal dose

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Abstract

Different pesticides and fumigation are used for stored grain pests because they are causing huge damage to stored grains world especially in tropical regions. In the present study, effect of phosphine is being evaluated on Khapra bettle (*Trogoderma granarium*) which is a very serious pest in stored grain godowns of Pakistan. For this purpose, the LC_{50} of phosphine against adults of two different strains of *T. granarium* collected from different cities of Punjab like Lahore and Faqeerwali were determined by FAO method. On the basis of LC_{50} Lahore strain was considered as resistant strain whereas Faqeerwali strain as susceptible strain. Resistant and susceptible adults of these two strains of *T. granarium* were exposed to sub-lethal dose (LC_{30}) for 20 hours to evaluate different enzymes such as AcP, AkP, amylase, ChE, trehalase, ALAT, ASAT, ICDH, LDH and metabolites viz., glucose, glycogen, free amino acids, protein and total lipids. In susceptible populations all enzymes activities were enhanced after treatment with phosphine except for ASAT and ChE while resistant beetles significantly raised (with respect to their controls) the activities of AcP, Akp and amylase. Phosphine treatment on resistant adult beetles caused depletion of glycogen and trehalose and glucose contents increased by 54%. Total lipids, protein and FAA increased in both populations. Carbohydrates increased in phophine the induction of the major enzymes under the present experimental condition.

Keywords: Phosphine resistance, biochemical contents, Khapra beetles, sublethal dose

Introduction

Pakistan is facing grain storage problems at large scale throughout the country, due to short of commercial grain storages and their management, on commercial level, warehouses, godowns, bunker and tower silos are used and protect them from hidden enemies like birds, rats and insect pests. The storage losses due to stored grain pests go upto 10-18% app. in Pakistan [60, 61]. The khapra beetle, *Trogoderma granarium* Everts, is one of the world's most feared stored-product pests. In fact, it has been described as one of the 100 worst invasive species worldwide [1]. Its discovery in California in 1953 led to a massive control and eradication effort [2]. But difficult to control because its larvae has ability to live without food for long periods of time and to survive on foods of low moisture content. These beetles tend to crawl into tiny cracks and crevices and remain there for long periods, making them relatively tolerant to many surface insecticides and fumigants [3]. Khapra beetle is the major threat to stored wheat in Pakistan also due to its high infestation potential, temperature, light, moisture, season, and host species. Khapra beetle may have one to nine or more generations per year as a result of high humidity causes serious damages [4]. The grain consumed by male is 15.5 to 18 mg in 24 to 28 days while the female consumed 18 to 24 mg in 27.5 to 31 days approximately. For infestations of whole premises stored in large amount, fumigation is most suitable to maintain food hygiene and will continue to have an important role for many years to come [5]. Each fumigant has its own limitations. Methyl Bromide (MB) is facing a phase-out in developed countries since 2005 and worldwide by the year 2020 under the terms of the Montreal Protocol [6]. So the world has no way out except to depend upon phosphine which is now the only important and easily available fumigant on commercial scale. [7]. A major drawback of phosphine is that it is a comparatively slow-acting poison. The commodity must be fumigated in an enclosure that is sufficiently gas-tight that insect pests are exposed to a lethal dose for a sufficient period. These standards are difficult or impossible to attain in many circumstances [8].

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So repeated applications of phosphine in poorly sealed structure, resulting in under-dosing have been cited, cause of the development of strong resistance in stored grain pests [9, 10, 11]. The problem of phosphine resistance in insects was first detected in the 1970s in a laboratory-based assessment of insects collected during a world-wide pesticide resistance survey. In1982, reports of field resistance to phosphine were verified in Bangladesh and later in other countries including Pakistan and India, and also in Africa and in Southeast Asia [12]. Many researchers have carried out investigation on the causes and mechanism of phosphine resistance in insect pests but the exact mechanism of phosphine toxicity is yet to be unknown [13].

The main objective of the present study is to investigate the biochemical and macromolecular responses in phosphine resistant and susceptible larvae of Trogoderma granarium to sub lethal dose, that may be helpful to know the proper mechanism of development of phosphine resistance in stored grain pests.

Materials and methods
Two strains of *T. granarium* Everts were used in this study. Crushed wheat was used as a supporting medium. Wheat was initially fumigated with Phosphine to kill the insects if any present. Following fumigation, wheat was spread in fresh air for 4-5 h. The wheat was placed in oven overnight at 60°C, and then it was shifted into sterilized jars for culture rearing. The jars were filled 1/4th with wheat and 50 beetles were added inside it. The jars were covered with muslin cloth to prevent escape of beetles and entry of any other small organisms. The beetles were transferred to next jars after 2 days, to maintain the age of larvae for experimental purposes. Wheat containing eggs was placed back in the same jars, in which the adult obtained after 5±1 days of hatching, were used in the present study. The adults were used for toxicological studies.

The susceptible strain of *T. granarium* was developed in the Biochemistry laboratory of the University of Punjab, Lahore. The insects were collected from godowns where farmers had never been exposed to any kind of pesticides and fumigant to protect them from pests. However, these were reared and bred up to 22 generations at 30±1 °C, 65±5% R.H. to get an exact susceptible strain treated as control.

Toxicants Used
Generic Name of this chemical is Phosphine while hydrogen phosphide and phosphorus trihydride are the common names of Phosphine gas.
The EPA Chemical Code of this insecticide is 066500. It belongs to Inorganic Phosphine Family.

Chemical Characteristics
It is a Colourless gas, its density is 1.379 g/l, gas (25 °C), melting point, - 132.8 °C boiling point, - 87.7 °C, solubility in water 31.2 mg/100 ml (17 °C) and viscosity 1.1 x 10^{-5} Pa s
Empirical Formula: PH_3 (CAS #: 7803-51-2)

Procedure
The first thing to do for LC_{50} determination was the generation of Phosphine gas, which was done according to the technique given in FAO method [14].
Phosphine was generated from aluminium phosphide tablets, collected over acidified water. Three glass vials, containing thirty healthy adult of *T. granarium* in each, were placed in the desiccators. Gas was injected into desiccators with micro

syringe through a rubber septum fitted on the dessicator lid. The desiccators were kept in the lab at 30±1 °C and 65±5% R.H. for 20 h after which observations on mortality were made. The percentage of killed percentage was corrected by Abbott's formula [15].

The criterion for mortality was that described by Lloyd [16]. Data was analyzed by the method outlined by Busvine [17] and described by Finny [18]. Each treatment was repeated four times.

Then the mortality data was subjected to logit analysis using POLO-PC [19] to estimate different lethal concentrations up to LC_{90} and confidence limit and regression lines (in ppm Phosphine) for adult of *T. granarium*.

Biochemical Analysis
About 90 adults were homogenized in 0.89% saline with a help of motor driven glass homogenizer under cold conditions. The homogenate was centrifuged at 4200g for 45 min.

The supernatant thus obtained was used for the estimation of various enzyme activities like acid phosphates (AcP; orthophoshoric monoester phosphohydrolase, acid optimum, EC:3.1.3.2) [20]; alkaline phosphates (AkP; orthophosphoric monoester phosphohydrolase alkaline optimum EC: 3.1.3.1) activity [21]; lactate dehydrogenase (LDH; L-lactate NAD: oxidoreductase; EC: 1.1.1.27) activity [22]; isocitrate dehydrogenase (ICDH); Threo-Ds-isocitrate: NADP: oxidoreductase, EC: 1.1.1.42) activity [23]; Aspartate aminotransferase (ASAT: L-aspartate: 2-oxoglutrate aminotransferase, EC: 2.6.1.1 and alanine aminotransferase E: 2.6.1.2) and alanine aminotransferase (EC: 2.6.1.2) activities [24]; cholinesterase ChE: acetylcholine acetylhydrolase, EC: 3.1.1.7) cholinesterase ChE: acetylcholine activity [25]; amylase (1,4-D glucan, glucanhydrolase, EC: 3.2.1.1) activity [26]; trehalase activity [27].

The supernatant was also analyzed for protein contents [28], Glucose content [29] and trehalose content [30-32]. Total lipids and FAA contents were estimated from ethanol extract of treated and control adult beetles following centrifugation at 2500 rpm [33, 34] and Glycogen contents were extracted by crushing the whole adult beetles in KOH [35].

Results
Toxicity of Phosphine
Treatment with phosphine revealed LC_{50} 4.7 ppm for resistant population and 3.2 ppm for susceptible population.
Table I shows the effects of sublethal dose of phosphine administered to resistant and susceptible adults for 20 hours on different enzymes such as AcP, AkP, amylase, ChE, trehalase, ALAT, ASAT, ICDH, LDH and metabolites viz., glucose, glycogen, free amino acids, protein and total lipids. Figure 1 shows % increase (+) or decrease (-) in the enzymatic activities as well as concentrations of various metabolites in the two populations.

Effect on resistant beetles
Enzyme activities
Sublethal dose of phosphine administered for 20 hours to adult resistant beetles significantly raised (with respect to their controls) the activities of AcP, AkP and amylase by 38%, 6% and 27% respectively. Other enzyme activities which showed significant decrease were ASAT (11%), ICDH (77%), ChE (16%), and LDH (1%) (Fig.1). Significant decrease observed in ICDH.

Carbohydrates and total lipids

Phosphine treatment on adult beetles caused depletion of glycogen and trehalose decreased by 47% and 12%, respectively. Glucose contents increased by 54%. Total lipids increased by 38% after exposing the adult beetles to the fumigant (Fig.1).

Proteins and FAA.

After exposure of adult beetles to the phosphine for 20 hours, FAA and proteins increased by 30% and 75%, respectively. (Fig.1).

Effects on susceptible beetles
Enzyme activities

All enzymes activities were enhanced after treatment with phosphine except for ASAT and trehalase which was declined by 30% and 2% respectively while amylase activity remained almost unaltered. There was a significant increase by 65%, 24%, 20% and 24% in ICDH, AcP, AkP, and ALAT activities, respectively, whereas the non-significant rise in activity of amylase was also noticed (Fig.1).

Carbohydrates and total lipids phosphine exposure to adult beetles produced significant increase (40%) (with reference to control) in total lipids. Glucose, Glycogen and trehalose content increased by 6%, 54% and 19% as compared to control (Fig. 1).

Proteins and FAA.

Fumigant treatment caused increase in FAA (9%) and protein (86%) (Fig.1).

Table I: Effects of phosphine on some enzyme activities and macromolecules of adult beetles of resistant and susceptible populations of *T. granarium.*

Parameters	Control	Resistant population	Control	Susceptible population
	(n=4)	30 ppm (n=4)	(n=4)	30 ppm (n=4)
AcP (IU/mg)	$0.701 \pm 0.01*^c$	0.980 ± 0.01^a	$0.72 \pm 0.021*^c$	0.89 ± 0.0097
AkP (IU/mg)	2.120 ± 0.112^c	2.240 ± 0.119^a	2.15 ± 0.090^d	2.59 ± 0.094
Amylase (SU/mg)	0.480 ± 0.001^c	0.610 ± 0.002^a	0.444 ± 0.002^d	0.449 ± 0.002
ALAT (IU/mg)	1.036 ± 0.035^b	1.060 ± 0.033^a	0.98 ± 0.029^b	1.22 ± 0.0314
ASAT (IU/mg)	1.180 ± 0.112^b	1.050 ± 0.119^a	1.22 ± 0.90^a	0.851 ± 0.094
LDH (IU/mg)	30.52 ± 0.452^b	30.33 ± 0.518^c	35.58 ± 1.191^c	36.92 ± 0.1786
ICDH (SU/mg)	53.41 ± 0.6^a	12.24 ± 0.7^d	49.98 ± 0.2^d	82.3 ± 0.0002^a
ChE (SU/mg)	3.38 ± 0.0179^a	3.120 ± 0.006^b	3.83 ± 0.0368	3.68 ± 0.0225
Trehalase (IU/mg)	0.580 ± 0.010^a	0.490 ± 0.011^b	0.589 ± 0.008^b	0.575 ± 0.0093
Glucose mg/g	48.21 ± 1.88	74.29 ± 1.88	55.62 ± 2.01	58.88 ± 2.48
Glycogen mg/g	1.15 ± 0.013	0.58 ± 0.01	1.21 ± 0.01	0.57 ± 0.01
Trehalose ug/mg	2.78 ± 0.056	1.48 ± 0.051	2.53 ± 0.05	2.05 ± 0.051
Lipid Mg/g	59.19 ± 1.06	81.61 ± 0.94	5.63 ± 0.8	80.95 ± 0.88
Protein ug/mg	21.7 ± 0.61	38.08 ± 0.86	26.37 ± 0.72	49.11 ± 1.28
FAA ug/mg	100.00 ± 1	130.0 ± 1	99.00 ± 1.0	108.00 ± 1.0

Abbreviations used: AcP, acid phosphatase; AkP, alkaline phosphatase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate, pyruvate transaminase; ICDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; IU, International unit; mSU, milli Somogyi unit, FAA, free amino acids * Mean ± SEM, For abbreviations see Table I; The values in a row having no common superscript (ab) are significantly different at 0.05 significant level according to DRMD

Definitions of enzyme units: IU, international unit, the amount of enzyme, which under defined assay conditions, will catalyze the conversion of 1μ mol of substrate per minute; mSU, the amount of enzyme digesting 5000 mg of starch in the experimental conditions used here.
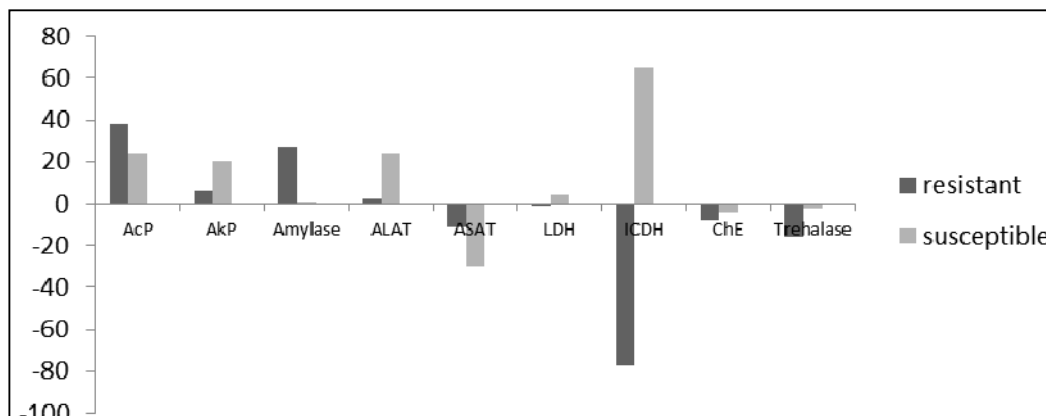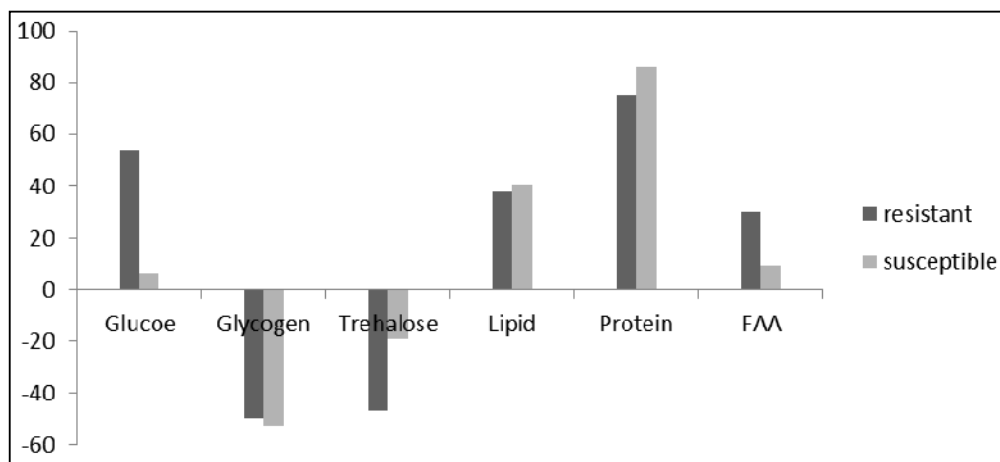
Fig 1: Percent increase (+) and decrease (-) in various enzymatic activities and Concentration of various enzymatic activities and metabolites of resistant and susceptible Adults of *T. granarium* following phosphine treatment with reference to control.

Discussion

The reduced levels of glucose, glycogen and trehalose contents recorded in the present study may suggest that energy production through glycolysis was switched on and accelerated to cope with the insecticidal stress [36]. Vyjayanthi and Subramanyam [37] also reported enhanced trehalase activity in the midgut of silkworms treated with insecticides. Saleem and Shakoori [38] also reported elevation of LDH and ICDH when *Tribolium castaneum* larvae were treated with other pyrethroids such as cypermethrin and permethrin. Saleem and Shakoori [39] related raised activity of LDH to its higher production and consequently accumulation of lactic acid from its substrate i.e., pyruvic acid in the tissues. This might have an important role in the development of resistance in the resistant population of *T. granarium*. Transaminases (ALAT and ASAT) were reduced after 20 hour of phosphine application. Reduction in ALAT and ASAT activities can be related to inhibition of transamination and thus blocking the additional energy production. Likewise, depleted level of transaminases [40] in *Tribolium castaneum* larvae after exposure to sublethal doses of Sumicidin Super (esfenvalerate). This reduced transamination can be related to increase in soluble protein and FAA. Reduction in the total protein maybe due to its breakdown as a result of chemical stress. It is also reported by Etebari and Matindoost [41] who has suggested that different stresses can decrease the amount of total protein in silkworm haemolymph. This could be due to the breakdown of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a keto acid, they will help to supply energy for the insect and play a role in compensatory mechanisms under fumigation stress, to provide intermediates to the Krebs cycle, by retaining free amino acid content in haemolymph [42].

In the present study ChE greatly inhibited in the resistant population which are in contrast to the report of Saleem and Shakoori [43-45a,b] that the detoxication enzymes are always induced after insecticide treatment, just as it happened in case of sensitive population. Other researchers, Sudderuddin and Lim [46] have also reported the inhibition of esterases by the synthetic pyrethroids in stored grain pests. In case of both populations of *T. granarium* AkP and AcP activity was increased in contrast to Shakoori [47]. These enzymes are involved in dephosphorylation and energy transfer. Phosphine treatment developed lipemia in both populations. Downer [48] explained that Lipid turnover in insects is regulated by neuroendocrine-controlled feedback loops. Mulye and Gordon [49] have shown that lipid synthesis and catabolism in the fat body was severely impaired in juvenile hormone analogue treated budworms. It also similar with the study of Shakoori [50] and Saleem [51] while looking into the toxicity of Talstar on susceptible and resistant strains of *T. castaneum*. In contrast to resistant population, phosphine application caused induction of all other enzyme activities in susceptible population except ASAT and ChE. Raised activity of LDH activity was also reported by Tufail [52] while studying the biochemical changes in larvae of *T. castaneum* after bifenthrin treatment. Shakoori *et al.* (1998) also reported similar results after treating the adults of *T. castaneum* with Cymbush (cypermethrin 10 EC). Contrarily, reported somewhat similar effects of the sublethal doses of bifenthrin in adults of OP-resistant T. Castaneum [53]. The elevated activities of various enzymes after insecticide poisoning have also been reported from other laboratories [54-56]. The increase level of endogenous enzymes is responsible for the condition of stress developed by the phosphine toxicity. Induction of all enzymes in this susceptible population could assist in defence mechanism by utilization of body reserves. It is evident from decrease in glycogen and trehalose contents could also be inferred from this result that this beetle is utilizing all body reserves (glycogen and trehalose) in addition to glucose as primary source of energy production and the respiration has perhaps enhanced to cope with the environmental stress [57,58]. Decrease in ASAT in susceptible population indicates malfunctioning of hepatic caeca caused by this fumigant.

Conclusion

It can therefore, be summed up that phosphine caused major enzymatic and macromolecular abnormalities in both the populations of *T. granarium*. This data can help to understand the mechanism of development of resistance for the effective control of these populations of stored grain pest.

References

1. Lowe S, Browne M, Boudjelas S, DePoorter M. 100 of the world's worst invasive alien species: A selection from the Global Invasive Species Database. Invasive Species Specialist Group, World Conservation Union, (IUCN), 2000, 2015.
2. Kerr JA. Khapra beetle returns. Pest Control. 1984; 49(12):24-25.
3. Lindgren DL, Vincent LE, Krohne HE. The khapra beetle, *Trogoderma granarium* Everts. Hilgardia. 1955;

24:1-36.

4. Ramzan M, Chahal BS. Effect of interspecific competition on the population build-up of some storage insects. Ind. J. Ecol 1986; 13:313-317.

5. Bell CH. Fumigation in the 21st century. Crop Protec. 2000; 19:563-569.

6. UNEP. United Nations Environment Programme, Montreal Protocol on Substances that Deplete the Ozone Layer, 1998: Assessment of Alternatives to Methyl Bromide. Methyl Bromide Alternatives Committee. Nairobi, Kenya, 1998.

7. Donahaye EJ. Current status of non-residual control methods against stored product pests. Crop Protec. 2000; 19(8-10):563-569.

8. Collins PJ, Daglish GJ, Pavic H, Kopittke RA. Response of mixed-age cultures of phosphine-resistant and susceptible strains of lesser grain borer, *Rhyzopertha dominica*, to phosphine at a range of concentrations and exposure periods. J Stored Prod. Res. 2005; 41(4):373-385.

9. Friendship CAR, Halliday D, Harris AH. Factors causing resistance to phosphine in insect pests of stored produce. In: V. Howe, Editor, Proceedings of GASGA Seminar on Fumigation Technology in Developing Countries, Trop. Develop. Res. Inst., Lond. 1986, 141-149.

10. Mills KA. Phosphine dosages for control of resistant strains of insects. In: V. Howe, Editor, Proceedings of GASGA Seminar on Fumigation Technology in Developing Countries, Trop. Develop. & Res. Inst., Lond, 1986, 119-131.

11. Zeng L. Development and countermeasures of phosphine resistance in stored grain insects in Guangdong of China. In: Z. Jin, Q. Liang, Y. Liang, X. Tan and L. Guan, Editors, Stored Products Protection. Proceedings of the Seventh International Working Conference on Stored-product Protection. 14-19 October 1998,Beijing, China, Sichuan Publishing House of Science and Technology, Chengdu, People's Republic of China. 1999, 642-647.

12. Taylor R. The use of Phosphine as a MB Alternative in Post-Harvest Treatments. In: regional Workshop on Methyl bromide alternatives for post-harvest treatments in Eastern and Central Europe, UNEP. Bulgaria, Sofia. 28-30 May 2002, 15-20.

13. Chaudhry MQ. Phosphine Resistance. Pesticide Outlook 2000; 11(3):88-91.

14. FAO. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative method for adult of some major pest species of stored cereals with methyl bromide and phosphine. FAO Method No. 6. FAO Plant Prot. Bull. 1975; 23:15-35.

15. Abbott WS. A method for computing the effectiveness of insecticides. J. Econ. Entomol. 1925; 18:265-7.

16. Lloyd CJ. Study on the related compounds of a pyr *Sitophilus granaries* L. Cole stored Prod. Res 1969; 5:337-356.

17. Busvine JR. A Critical Review of the Techniques for Testing Insecticides. CAB, London, 1971.

18. Finney DJ. Probit analysis, 3rd. ed., Cambridge University Press London, 1971.

19. Software Leora. POLO-PC: A User's Guide to Probit or Logit Analysis (LeOra Software, Berkeley, CA). 1987.

20. Andersch MA, Szcypinski AJ. A colorimetric method for determination of acid phosphatase from serum. Am. J Clin. Pathol. 1947; 17:571.

21. Bessey OA, Lowry OH, Brock MJ. A method for the rapid determination of alkalin phosphatase with 5cc of serum. J Biol. Chem. 1946; 164:321-329.

22. Cabaud PG, Wroblewski F. Colorimetri measurement of lactate dehydrogenase activity of body fluids. Am. J Clin. Pathol. 1958; 30:234-236.

23. Bell JL, Baron DN. A colorimetric method for determination of isocitric dehydrogenase. Clin. Chem. Acta. 1960; 5:740-747.

24. Reitmann S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetate and glutamate pyruvate transaminase. Am. J Clin. Path. 1957; 28:56-63.

25. Rappaport F, Fischl J, Pintos N. An improved method for the determination of cholinesterase activity in serum. Clin. Chem. Acta. 1959; 4:227-230.

26. Wootton IDP, Freeman H. Microanalysis in medical biochemistry, Churchill Livingstone, London, 1982, 91-114.

27. Dahlqvist A. In: methods in enzymology - complex carbohydrates (eds. F.F. Neufeld and V. Guinburg), vol. 8, Academic Press, New York, 1966, 584-591.

28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol. Chem. 1951; 193:265-275.

29. Hartel A, Helger R, Lang H. A method for determination of glucose. Z. Klin. Chem. Klin. Biochem. 1969; 7:183-184.

30. Carroll NV, Longley RW, Roe JH. The determination of glycogen in liver and muscle by use of anthrone reagent. J. Biol. Chem. 1956; 220:586-593.

31. Roe JH, Dailey RE. Determination of glycogen with anthrone reagent. An. Biochem. 1966; 15:78245-250.

32. Steele JE, Paul T. Corpus cardiacum stimulated trehalose efflux from cockroach (*Periplaneta americana*) fat body: control bycalcium. Can. J Zool. 1985; 63:63-66.

33. Zöllner N, Kirsch K. Microdetermination of lipids bythe sulfo-phosphovanillin reaction. Z. Gec. Exp. Med. 1962; 135:545-561.

34. Moore S, Stein WH. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. 1954; 211:907-913.

35. Consolazio CF, Iacono JM. Carbohydrates. In: newer methods for nutritional biochemistry with applications and interpretations (ed. A.A. Albanese), Academic Press, New York. 1963; (1):317-367.

36. Tufail N, Saleem MA, Shakoori AR. Biochemical changes in sixth instar larvae of Pak and FSS-II strain of red flour beetle *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) following administration of sublethal doses of a synthetic pyrethroid, bifenthrin. Pakistan J Zool. 1994; 26:197-206.

37. Vyjayanthi N, Subramanyam MV. Effect of fenvalerate-20EC on sericigenous insects. II. Digestive enzymes in the nutritive physiology of silkworm, Bombyx mori L., Ecotoxicol. environ. Saf. 2002; 53:212-220.

38. Saleem MA, Shakoori AR. Biochemical studies in Talcord 10EC. 1. Effect on some enzyme activities and macromolecules of 6th instar larvae of *Tribolium castaneum*. Pakistan J Zool. 1996; 28:75-83.

39. Saleem MA, Shakoori AR. Effects of permethrin and Deltamethrin on some biochemical components of *Tribolium castaneum* larvae. Pakistan J Zool. 1985; 17:321-328.

40. Shakoori AR, Agha S, Malik MZ, Saleem MA, Ali SS. Biochemical abnormalities produced by sublethal doses of a synthetic pyrethroid, Sumicidan Super, on the 6th instar larvae of red flour beetle, *Tribolium castaneum*. Pakistan J. Ent. Karachi. 1994c; 9:5-20.

41. Etebari K, Matindoost L. The study on effects of larval age and starvation stress on biochemical macromolecules abundance of hemolymph in silkworm Bombyx mori. In: proc. Sixteenth Iranian Plant Prot. Congr., General Entomology Symposium, August 28–September 1, University of Tabriz, Iran, 2004, 435.

42. Nath BS, Suresh A, Mahendra VB, Kumar RP. Changes in protein metabolism in haemolymph and fat body of the silkworm, *Bombyx mori* L., in response to organophosphorus insecticides toxicity. Ecotoxicol. Environ. Saf. 1997; 36:169-173.

43. Saleem MA, Shakoori AR. Biochemical effects of sublethal doses of cypermethrin on the sixth--instar larvae of *Tribolium castaneum* (Herbst.).Arch. Insect Biochem. Physiol. 1986; 3:447-455.

44. Saleem MA, Shakoori AR. Permethrin and Malathion-induced macromolecular abnormalities in adult *Tribolium castaneum* (Herbst.). Arch. Insect Biochem. Physiol. 1987a; 5:45-55.

45. Saleem MA, Shakoori AR. Joint effects of Dimilin and Ambush on enzyme activities of *Tribolium castaneum* larvae. Pestic. Biochem. Physiol. 1987b; 29:127-137.

46. Shakoori AR, Malik MZ, Saleem MA. Toxicity of cyhalothrin (Karate) to sixth instar larvae of Pakistani strain of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Pakistan J Zool. 1994a; 26:57-64.

47. Downer RGH. Lipid metabolism. In: comprehensive insect physiology, Biochemistry, and pharmacology (eds. G.A. Kerkut and L.I. Gilbert) vol. 10, Pergamon Press, Oxford, 1985, 75-114.

48. Mulye H, Gordon R. Effects of two juvenile hormone analogs on haemolymph and fat-body metabolites of the eastern spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae). Can. J Zool. 1993; 71:1169-1174.

49. Shakoori AR, Tufail N, Saleem MA. Response of Malathion resistant and susceptible strains of *Tribolium castaneum* (Herbst.) to bifenthrin toxicity. Pakistan J Zool. 1994b; 26:169-178.

50. Saleem MA. Toxicological studies on synthetic pyrethroids against red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). Ph.D. thesis, University of the Punjab, Lahore, Pakistan. 1990.

51. Tufail N, Saleem MA, Shakoori AR. Biochemical changes in sixth instar larvae of Pak and FSS-II strain of red flour beetle *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) following administration of sublethal doses of a synthetic pyrethroid, bifenthrin. Pakistan J Zool. 1994; 26:197-206.

52. Shakoori AR, Saleem MA, Mantle D. Some macromolecular abnormalities induced by a sublethal dose of Cymbush 10EC in adult beetles of *Tribolium castaneum*. Pakistan J. Zool. 1998; 30:83-90.

53. Shakoori AR, Tufail N, Saleem MA. Response of Malathion resistant and susceptible strains of *Tribolium castaneum* (Herbst.) to bifenthrin toxicity. Pakistan J Zool. 1994b; 26:169-178.

54. Shakoori AR, Agha S, Malik MZ, Saleem MA, Ali SS. Biochemical abnormalities produced by sublethal doses of a synthetic pyrethroid, Sumicidan Super, on the 6th instar larvae of red flour beetle, *Tribolium castaneum*. Pakistan J. Ent. Karachi. 1994c; 9:5-20.

55. Orr GL, Downer GH. Effect of lindane (gamma-hexachlorocyclohexane) on carbohydrate and lipid reserves in the American cockroach, *Periplaneta americana* L. Pestic. Biochem. Physiol. 1982; 17:89-95.

56. Shakoori AR, Malik MZ, Saleem MA. Toxicity of cyhalothrin (Karate) to sixth instar larvae of Pakistani strain of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Pakistan J Zool. 1994a; 26:57-64.

57. Ahmad F, Zaidi SWN. Comparative efficacy of three synthetic igrs (bruce, runner and falcon) on the development of trogoderma granarium (everts) (coleoptera: dermestidae). J Glob. Innov. Agric. Soc. Sci. 2014; 2(3):116-119.

58. Hassan M, Sagheer M, Ranjha MH, Ali Q, Hanif CMS, Anwar H. Evaluation of some plant essential oils as repellent and toxicant against trogoderma granarium (everts) (coleoptera: dermestidae). J Glob. Innov. Agric. Soc. Sci. 2014; 2(2):65-69.