Pakistan J. Zool., vol. 50(6), pp 2185-2192, 2018. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2018.50.6.2185.2192

Toxicological Effect of Esfenvalerate on Carbohydrate Metabolizing Enzymes and Macromolecules of a Stored Grain Pest, *Trogoderma granarium*

Farah Rauf Shakoori[1,*], Tanzeela Riaz[2], Uzma Ramzan[1], Anum Feroz[1] and Abdul Rauf Shakoori[2,3,*]

[1]*Department of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore*
[2]*Faculty of Life Sciences, University of Central Punjab, Lahore*
[3]*School of Biological Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore*

Article Information
Received 12 March 2018
Revised 25 May 2018
Accepted 04 June 2018
Available online 21 September 2018

Authors' Contribution
FRS and ARS designed and supervised the study. TR, AF and UR conducted the experimental work. FRS contributed reagents and analytical tools. ARS, FRS and TR analyzed the data and wrote the manuscript.

Key words
***Trogoderma granarium*, Esfenvalerate, Metabolites, Carbohydrate metabolizing enzyme, Pyrethroids.**

ABSTRACT

The aim of present research was to evaluate the toxicity of esfenvalerate on carbohydrate metabolism and macromolecular concentrations of 4th and 6th instar larvae of a stored grain pest, *Trogoderma granarium*. The LC_{50} values of esfenvalerate for 4th and 6th instar larvae of Phosphine and deltamethrin-susceptible population was 34.29 and 28.05ppm, respectively, while 39.30 and 32.67ppm were for the 4th and 6th instar larvae of Phosphine and deltamethrin-tolerant population, respectively. The sub lethal dose of esfenvalerate (LC_{20}) significantly decreased the contents of glycogen, glucose, trehalose and free amino acid while total protein and total lipid contents, were significantly increased with reference to their control (untreated group). Among carbohydrate metabolizing enzymes, the activities of trehalase, amylase and invertase were significantly reduced after treatment with sub lethal dose of esfenvalerate as compared to control. The metabolic derangements induced by sub-lethal dose of esfenvalerate suggest that infestation caused by *T. granarium* in godowns could be overcome by calculating lethal dose of esfenvalerate.

INTRODUCTION

Insect pests are considered a real threat to stored grain products and they are responsible for huge loss (10-20%) to wheat during storage. About 20 billion dollar loss has been estimated during storage of food for 6-8 months of storage at farm, market, godowns and other storages facilities (Bengston *et al*., 2005). The Khapra beetle, *Trogoderma granarium* (Everts) is the most notorious and quarantine pest of stored wheat (Szinicz, 2005; Burges, 2008; Castalanelli *et al*., 2010). Larvae of Khapra beetle are most destructive, they not only attack the grain but also reduce it nutritive value (Ahmedani *et al*., 2009). During infestation, Khapra beetle contaminates wheat with setae and skin which leads to gastrointestinal irritation, dermatitis and allergic reactions (Jood *et al*., 1996). Temperature 35°C is its optimum temperature but if the temperature is below 25°C for a longer period or if insect population become more populated which cause diapause (Riaz *et al*., 2014). The diapausing larvae act as a reservoirof population and they are more tolerant to insecticides. Moreover, the Khapra beetle larvae can easily be transferred through trade and in this way they are introduced in various countries of the world and considered as invasive species globally. Previous reports have already pointed out that Khapra beetle has the potential to establish in different geographical regions of the world (Viljoen, 1990) and its distribution is cosmopolitan (Burges, 2008; Mark *et al*., 2010).

Various classes of insecticides like organochlorines, organophospahtes and carbamates are used to control Khapra beetle (Riaz *et al*., 2017) but various insecticides like deltamethrin, malathion, diclorvos and primiphos methyl *etc*. that are effective against other major stored grain insect species are found ineffective against Khapra beetle. Various studies have also reported that *T. granarium* has also become resistant to Phosphine that was a major fumigant to control Khapra beetle (Riaz *et al*., 2017). Pyrethroids are third generation and new group of insecticides. Their chemical characteristics are due to the active ingredients of natural pyrethrum (Hargreaves *et al*., 2000). They are less toxic to non- target animals as compared to organochlorines and organophosphate but they found very effective against a variety of stored product

* Corresponding authors: farah.shakoori@yahoo.com; arshakoori.sbs@pu.edu.pk
0030-9923/2018/0006-2185 $ 9.00/0

insect pests. Esfenvalerate belongs to Type II pyrethroids that are characterized by higher insect mortality and mortality increased with increase in temperature (Coats, 1990; Mokry and Hoagland, 1990; Ware, 2002).

The main objective of present study was to find the biochemical alterations, particularly in carbohydrate metabolism in susceptible and resistant larvae after exposure to sub-lethal concentration of esfenvelarate because insecticides at sub-lethal concentrations produced significant metabolic changes by directing a distinctive secondary target in susceptible and tolerant populations.

MATERIALS AND METHODS

Rearing and maintenance of insect larvae

In current investigation two populations of Khapra beetle *i.e.* phopshine and deltamethrin-susceptible population (Lahore population that has never been exposed to any type of insecticide or fumigant since sixteen years) and phopshine and deltamethrin-tolerant population (Okara population that has a fifteen years history of phosphine fumigation followed by deltamethrin exposure in PASSCO godowns of Okara) were used. Both cultures were maintained and reared on wheat flour and crushed wheat in clean glass jars of 300ml capacity at 35±2°C and 60±5 relative humidity according to Riaz *et al.* (2014). The 4th and 6th larval instars were collected during propagation of insect culture from egg to adult (Riaz *et al.*, 2016) and homogeneous stock was maintained for further experiments.

Insecticide used

Technical grades of esfenvalerate [(S)-α-cyano-3-phenoxybenzyl(S)-2-(-4-chlorophenyl)-3 methylbutyrate] 0.5%EC were purchsed from the Agricultural Chemical Group of FMC Corporation Lahore, Pakistan.

Determination of LC_{50}

For the determination of LC_{50} of esfenvalerate serial dilutions (50, 45, 40, 35, 30, 25, 20, 15, 10 and 5ppm) were prepared in acetone according to recommendations of WHO (2012). Esfenvalerate dilution (1.0ml/plate) were applied separately in the center of glass Petri plate (9cm^2) by residual film method as recommended by FAO (1971). Petri plate was rotated manually to distribute the insecticide uniformly and acetone was allowed to evaporate at room temperature. Control petri plate had no insecticide but containing the acetone only and three replicate for each concentration was prepared simultaneously.

Ten healthy insects (each 4th and 6th instar larvae) of susceptible and tolerant populations were introduced in different Petri plates for 20 h and plates were incubated at 35±2°C and 60±5% relative humidity in continuous darkness (Hafiz *et al.*, 2017). After exposure to insecticide, larvae were ventilated by transferring them to their respective labeled glass jars containing 2/3rd of sterilized crushed wheat grain. After 48h, mortality was recorded according to Lloyd (1969). The data obtained was analyzed by Probit analysis (Finny, 1971) for the determination of LC_{50}. Corrected mortality of 4th and 6th instar larvae was determined by using Abbot Formula (Abbot, 1925).

Effect of LC_{20} on metabolites

After determination of LC_{50} for each population separately, the sub-lethal dose of esfenvalerate (LC_{20}) was selected for determining toxic effects of insecticides because mortality was low at sublethal dose, so biochemical responses produced are more enough to unvail the mode of action. Approximately 400 larvae of (4th and 6th instar) of susceptible and tolerant populations of Khapra were exposed separately to LC_{20} along with their controls (unexposed group) for 24 h, at 35±2°C and 60±5% relative humidity. After exposure, biochemical analysis of alive 4th and 6th larval instars (treated and control) were performed.

Biochemical analysis

Thirty larvae for each (treated and control) of both populations were weighed and then macerated in 1.5ml saline (0.89%) in motor-driven Teflon glass homogenizer at 4°C for each test followed by centrifugation at 3000×g for 30 minutes at 4°C. Clear supernatant were used for the estimation of glucose, trehalose, trehalase, amylase and invertase. Glucose and trehalose contents were estimated according to Hartel *et al.* (1969) and Roe and Dailey (1966), respectively. Trehalase, amylase and invertase activities were estimated by the procedure described by Dahlqvist (1966), Wootton *et al.* (1982) and Ishaya and Swiriski (1976), respectively. Glycogen contents were extracted by crushing the whole 4th and 6th instar larvae in and 30% potassium hydroxide solution (KOH) and estimated by the anthrone method of Consolazio and Lacono (1963). Total protein and free amino acid contents in extract were estimated according to Lowry *et al.* (1951) and Moore and Stein (1954), respectively. Total lipid contents were calculated according to Zollner and Kirsch (1962).

Statistical analysis

Statistical analysis was performed in Minitab 16 and data was presented as mean ± standard error of mean. Toxic effects of LC_{20} of esfenvalerate on concentration of macromolecules and various enzymes was calculated by "paired t" test at 95% confident limit. The significance level was non-significant ($p>0.05$) and significant ($p\leq0.05$).

RESULTS

LC_{50} of esfenvalerate

The LC_{50} of esfenvalerate against 4th instar larvae of susceptible and tolerant populations were 34.29 and 39.30ppm, respectively, while LC_{50} for 6th instar larvae were 28.05 and 32.67ppm, respectively (Table I).

Effect of esfenvalerate on metabolites of T. granarium

The toxic effect of sub-lethal concentration of esfenvalerate (LC_{20}) on metabolites of 4th and 6th instar larvae of susceptible and tolerant populations were shown in Table II. Percent change in the metabolites of 4th and 6th instar larvae of both populations was calculated with respect to their controls (untreated group) was shown in Figure 1A, B.

Glycogen, glucose, trehalose and free amino acid contents

Glycogen, Glucose and Trehalose contents were significantly decreased in 4th and 6th instar larvae of both populations when compared with their control groups. The glycogen contents in 4th instar larvae were significantly decreased (36.23 and 26.59%) and in 6th instar larvae significant decrease was recorded (23.21 and 26.59%) of both susceptible and tolerant populations, respectively. Glucose contents of 4th instar larvae were significantly decreased (34.01 and 19.77%) and in 6th instar larvae significant decline (32.19 and 16.71%) was observed in susceptible and tolerant populations, respectively. Likewise, trehalose contents of 4th instar larvae were significantly depleted (10.79 and 15.73%) and in 6th instar larvae significant depletion was (12.58 and 21.94%) in susceptible and tolerant populations, respectively. In contrast, free amino acid contents of 4th instar larvae were significantly increased (177.88 and 154.01%) and in 6th instar larvae significant increased was (335.0 and 127.61%) in susceptible and tolerant populations, respectively (Fig. 1A, B).

Table I.- Probit analysis showing LC_{50} of 4th and 6th instar larvae of susceptible and tolerant populations of *T. granarium*.

Populations of *T. granarium*	LC_{50} (ppm) at 95% fudicial limit			
	Estimated	Lower	Upper	Slope ± SEM
4th instar larvae				
Susceptible	34.29	30.42	39.15	34.84±2.14
Tolerant	39.30	35.06	45.16	34.55±2.45
6th instar larvae				
Susceptible	28.05	25.12	31.12	25.86±1.49
Tolerant	32.67	29.78	35.92	24.89±1.53

Table II.- Toxic effects of LC_{20} of esfenvalerate on various metabolites and enzyme activities of 4th and 6th instar larvae of *T. granarium*.

Parameters		4th instar larvae		6th instar larvae	
		Untreated	Treated	Untreated	Treated
Glycogen (µg/mg)	Susceptible	0.53±0.02[a]	0.34±0.01*	0.95±0.02[a]	0.73±0.01*
	Tolerant	0.72±0.01	0.53±0.02*	0.72±0.01	0.53±0.02*
Glucose (µg/mg)	Susceptible	29.52±1.00	19.48±0.99*	45.69±1.05	30.98±1.04*
	Tolerant	49.52±1.00	39.73 ±0.56*	63.78 ±1.51	53.12 ±1.27*
Trehalose (µg/mg)	Susceptible	20.79±0.12	18.55±0.22*	24.12±0.11	21.08±0.23*
	Tolerant	45.43±0.43	38.28±0.53*	51.98± 0.71	40.58±0.41*
Trehalase (I.U/mg)	Susceptible	0.55±0.01	0.35±0.01*	0.61±0.01	0.46±0.01*
	Tolerant	0.92±0.01	0.74±0.01*	0.85±0.01	0.65±0.01*
Amylase (Somogyi unit/mg)	Susceptible	12.43±0.13	10.51±0.05*	16.73±0.09	14.64±0.17*
	Tolerant	16.40±0.18	13.51±0.21*	20.42 ± 0.17	18.63±0.17*
Invertase (IU/mg)	Susceptible	20.79±0.12	18.55±0.22*	24.12±0.11	21.08±0.23*
	Tolerant	8.05±0.29	4.39±0.14*	2.99±0.03	1.97±0.07*
Total proteins (µg/mg)	Susceptible	62.28±1.84	31.17±1.05*	33.21±0.22	18.49±0.39*
	Tolerant	13.38±1.88	6.84±0.61*	51.64±0.76	44.32±0.93*
Free amino acids (µg/mg)	Susceptible	5.20±0.24	14.45±0.15*	2.72±0.78	4.35±0.11*
	Tolerant	6.98±0.25	17.73±0.33*	4.20±0.44	9.56±0.05*
Total lipids (µg/mg)	Susceptible	0.06 ±0.01	0.05 ±0.01*	0.05 ±0.01	0.03±0.01*
	Tolerant	0.09 ±0.01	0.07 ±0.01*	0.08±0.01	0.06±0.01*

[a]Mean ± Standard error of mean; n = 5 (number of replicates used in each experiment and each replicate contain twenty beetles); *, Significant ($p \leq 0.05$).

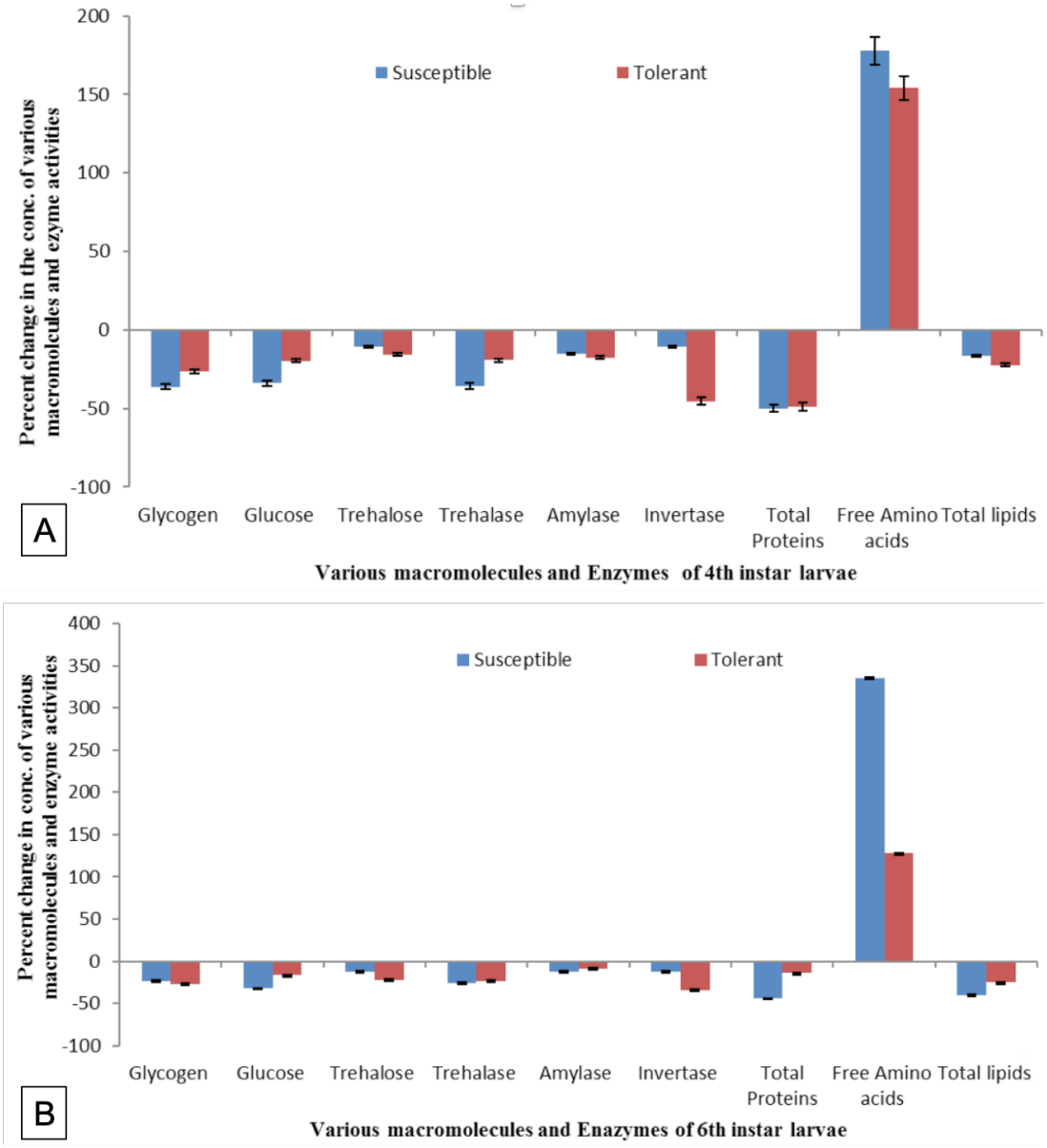

Fig. 1. Percent (%) change in concentrations of various macromolecules and enzyme activities of 4th (A) and 6th (B) instar larvae of *T. granarium* with reference to control.

Trehalase, amylase and invertase activities

Trehalase, amylase and invertase activities were significantly decreased in 4th and 6th instar larvae of both populations. Trehalase activities of 4th instar larvae were significantly diminished (35.66 and 19.51%) and in 6th instar larvae significant diminishing was (25.63 and 23.40%) in both susceptible and tolerant populations, respectively. In the same way, amylase activities in 4th instar larvae were significantly reduced (15.48 and 17.63%) and in 6th instar larvae significant reduction was (12.45 and 8.77%) in susceptible and tolerant populations, respectively. Similarly, invertase activities in 4th instar larvae were significantly reduced (10.79 and 45.45%) and in 6th instar larvae significant reduction was (12.58 and 34.09%) in both susceptible and tolerant populations, respectively (Fig. 1A, B).

Total protein and lipid contents

Total protein and lipid contents were significantly decreased in 4th and 6th instar larvae of both populations.

Protein contents of 4th instar larvae were significantly decreased (49.95 and 48.87%) and in 6th instar larvae significant decrease was observed (44.32 and 14.17%) in susceptible and tolerant populations, respectively. Similarly, lipid contents of 4th instar larvae were significantly depleted (16.66 and 22.22%) and in 6th instar larvae significant depletion was observed (40 and 25%) in both susceptible and tolerant populations, respectively (Fig. 1A, B).

DISCUSSION

In the present study, toxic effects of esfenvalerate on carbohydrate metabolism and macromolecular concentrations of 4th and 6th instar larvae of stored grain pest *T. granarium* was studied. The 4th and 6th instar larvae possessed resistance ratio RR (1.14 and 1.16) against Esfenvalerate, respectively. Very small value of resistance ratio revealed that *T. granarium* is susceptible to esfenvalerate exposure and possessed no tolerance in the godowns against esfenvalerate. The LC_{50} value of 4th and 6th larval instars of both populations of Khapra beetle showed different level of susceptibility to esfenvalerate at 35±2°C and 60±5% relative humidity. The 4th instar larvae were more tolerant than 6th instar larvae against esfenvalerate. Shakoori et al. (2018) found that 4th instar larvae of *T. granarium* were more tolerant than 6th instar larvae against lambda-Cyhalothrin. Riaz *et al*. (2016) also reported 4th instar larvae of *T. granarium* more tolerant to Phosphine than other stages. Hafiz *et al*. (2017) working on *T. granarium* also documented same kind of results against deltamethrin. Mujeeb and Shakoori (2007) found that different developmental stages of three strains of *T. castaneum* behaved differently against a pyrethroid with trade mark fury. Although, free glycogen is present in insect's haemolymph but in order to maintain continuous supply of glucose during stress condition that may cause the release of corticosteroids, glucagon and catecholamine which accelerate the glycogenolysis and glucose is released from glycogen broken down to cope with energy demand (Dezwann and Zandee, 1972; Shoba *et al*., 2011).

Reduction in lipids, glycogen, trehalose and glucose contents were noticed in 4th and 6th instar larvae of both populations of *T. granarium*. Decrease in lipid contents uncovered that pesticide introduction may make the transformation of lipids to proteins in order to produce feasible supplementary energy to cope the insecticidal pressure. Normally free glycogen floating in insect heamolymph is present but in order to balance glucose level in blood, glycogen is broken down and glucose is released under stress conditions. Such changes give significant stimulus for glycogenolysis in insect tissues and glycogen units production increased in response to stress condition induced by insecticidal exposure which leads to the release of glucagon, corticosteroids and catecholamines stimulating glucose formation from the breakdown of glycogen to diminish energy demand (Dezwann and Zandee, 1972; Shoba *et al*., 2011). Shakoori *et al*. (2016, 2018) reported decrease in glycogen and lipids contents but elevation in glucose level in *T. granarium* after exposure to sub lethal dose of Phosphine and lambda-Cyhalothrin. Hafiz *et al*. (2017) also reported depletion in glycogen and lipid contents but elevation in glucose level in various developmental stages *T. granarium* after exposure to deltamethrin. The results about glycogen and lipids contents reported by Mulye and Gordon (1993), Shakoori *et al*. (1994), Omar et al. (2005) and Ali et al. (2007) are in favor of current investigation but are in contrast to cuurent study in case of glucose level. Despite the continuous conversion of glycogen to glucose, the level of glucose was consistently decreased after exposure to esfenvalerate which indicated that esfenvalerate actively caused stress in insect by decreasing its energy reserve and ultimately leading to death of insect.

Increased levels of free amino acid contents but reduction in total protein contents were seen among 4th and 6th larval instars of the both populations. The increased level in FAA may be attributed with reduced activities of transaminases which may cause elevation in free unsaturated fat contents as documented by Shakoori *et al*. (1994) in larvae of *T. castaneum* after exposure to esfenvalerate. Depletion in total protein contents may be justified by the suggestions of Nath *et al*. (1997) that total protein contents be changed to amino acids and these amino acids may enter to kreb cycle as keto acids to supply energy under stress conditions. Thus, reduction in total protein contents might be a compensatory tool during stress phase to supply intermediates of kreb cycle by keeping free amino acids contents in the insects. Hafiz *et al*. (2017) reported an increase in FFA in various developmental stages of *T. granarium* after exposure to deltamethrin. Hussain *et al*. (2012) and Ali et al. (2011) also noticed elevation in FAA in *T. castaneum* and *R. dominica* after exposure to abamectin and melathion, respectively.

The activities of enzyme such as trehalase, amylase and invertase which are primarily concerned with energy production were decreased which was probably due to either decreased synthesis or enzyme inhibition. Mehrabadi *et al*. (2011) reported decrease in amylase activity in *T. granarium* after exposure to medicinal plant extracts. Vyjayanthi and Subramanyam (2002) reported reduced amylase activity in silkworm following exposure to fenvalerate and Shekari *et al*. (2008) also reported diminished amylase action over *Xanthogaleruca luteola*

after treatment with *Artemisia annua* extricate. Amylase plays a vital role for generating a variety of oligosaccharide units for energy through carbohydrate metabolism while invertase is the glycosidehydrolases that catalyzes the cleavage of sucrose into its monosaccharide fructose and glucose units as suggested by Naumoff and Livshits (2001). Saleem *et al.* (1988) observed a decline in invertase activity in adult beetles *T. Castaneum* after 24 h treatment of synteetic pyrethroid called cypermethrin. Saleem and Shakoori (1987) reported significantly reduced levels of trehalase activity in *T. Castaneum* adults after treating them with sublethal doses of Malathion. Moreover, Saleem and Shakoori (1987) recommended the pyrethroids at sub-lethal concentrations had shown a decreased gut amylase activity in *T.castaneum* larvae. Current results revealed that esfenvelarate reduced the activities of invertase, trehalse and amylase at sub-lethal concentration and it may find its way as an important pest control strategy by disturbing carbohydrate metabolism.

CONCLUSION

Resistance ratio and laboratory bioassays indicated that Khapra beetle collected from godowns of Okara are sensitive to esfenvelarate. The decrease in concentrations of various macromolecules enzyme activities due to sub lethal doses reveal more effective against Khapra beetle because it induced stress in insect which cannot be coped even at sublethal doses. So, esfenvelarate can be effectively used to control this insect pest in godowns.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. econ. Ent.,* **18:** 265-267.

Ahmedani, M.S., Haque, M.I., Afzal, S.N., Aslam, M. and Naz, S., 2009. Varietal changes in nutritional composition of wheat kernel (*Triticum aestivum* L.) caused by Khapra beetle infestation. *Pak. J. Bot.*, **41**: 1511-1519.

Ali, N.S., Munir, M., Ali, S.S. and Shakoori, A.R., 2007. Efficacy of mixtures of an organophosphate, malathion and a synthetic pyrethroid, deltamethrin against lesser grain borer, *Rhyzopertha dominica*. *Pakistan J. Zool.*, **39**: 179-184.

Ali, N.S., Ali, S.S. and Shakoori, A.R., 2011. Effects of sublethal doses of talstar on biochemical components of malathion-resistant and susceptible adults of Rhyzopertha dominica. *Pakistan J. Zool.,* **43**: 879-887.

Ali, N.S., Ali, S.S. and Shakoori, A.R., 2013. effects of sublethal doses of malathion on biochemical components of malathion-resistant and -susceptible adults of *Rhyzopertha dominica*. *Pakistan J. Zool*., **45**: 203-212.

Bengtsson, J., Ahnström, J. and Weibull, A.C., 2005. The effects of organic agriculture on biodiversity and abundance: A meta analysis. *J. appl. Ecol.*, **42**: 261-269. https://doi.org/10.1111/j.1365-2664.2005.01005.x

Burges, H.D., 2008. Development of the Khapra beetle, *Trogoderma granarium,* in the lower part of its temperature range. *J. Stored Prod. Res.*, **44**: 32-35. https://doi.org/10.1016/j.jspr.2005.12.003

Castalanelli, M.A., Severtson, D.L., Brumley, C.J., Szito, A., Foottit, R.G., Grimm, M. and Groth, D.M., 2010. A rapid non-destructive DNA extraction method for insects and other arthropods. *J. Asia-Pacific Ent.*, **13**: 243-248. https://doi.org/10.1016/j.aspen.2010.04.003

Coats, J.R., 1990. Mechanisms of toxic action and structure-activity relationships for organochlorine and synthetic pyrethroid insecticides. *Environ. Hlth. Perspect.*, **87**: 255. https://doi.org/10.1289/ehp.9087255

Consolazio, C.F. and Iacono, J.M., 1963. Carbohydrates. In: *Newer methods for nutritional biochemistry with applications and interpretations* (ed. A.A. Albanese). Academic Press, New York, USA, pp. 317-367. https://doi.org/10.1016/B978-0-12-048001-2.50012-0

Dahlqvist, A., 1966. In: *Methods in enzymology - Complex carbohydrates*, Vol. 8. Academic Press, New York, pp. 584-591. https://doi.org/10.1016/0076-6879(66)08105-9

Dezwann, A. and Zandee, D.I., 1972. The utilization of glycogen and accumulation of some intermediate during anaerobiosis in *Mytilusedulis*. *Comp. Biochem. Physiol*., **43**: 47-54.

FAO, 1971. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides-tentative method for larvae of cattle ticks, *Boophilusmicroplus* spp. FAO Method No. 7. *FAO Pl. Protec. Bull.*, **19**: 15-18.

FAO, 1974. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative methods for adults of some major beetle pests of stored cereals with Malathion or lindane. *FAO Pl. Protec. Bull*., **22**: 127-137.

Finney, D.J., 1971. *Probit analysis*. 3rd Ed. Cambridge

University Press London., pp. 333.

Hafiz, A., Riaz, T. and Shakoori F.R., 2017. Metabolic profile of a stored grain pest *Trogoderma granarium* exposed to deltamethrin. *Pakistan J. Zool*., **49**: 183-188. https://doi.org/10.17582/journal.pjz/2017.49.1.183.188

Hargreaves, K., Koekemoer, L.L., Brooke, B.D., Hunt, R.H., Mthembu, J. and Coetzee, M., 2000. *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Med. Vet. Ent.*, **14**: 181-189. https://doi.org/10.1046/j.1365-2915.2000.00234.x

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

Hussain, R., Ashfaq, M. and Saleem, M.A., 2012. Effect of abamectin on body protein content and activity of selected enzymes in adults of insecticide-resistant and-susceptible strains of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Pakistan J. Zool*., **44**: 1159-1163.

Ishaya, I. and Swirski, E., 1976. Trehalase, invertase and amylase activities in the larvae of Egyptian cotton worm, *Spodoptera littoralis* (L.). *J. Insect Physiol.*, **17**: 945-953. https://doi.org/10.1016/0022-1910(71)90110-7

Jood, S., Kapoor, A.C. and Singh, R., 1996. Effect of insect infestation and storage on lipids of cereal grains. *J. Agric. Fd. Chem.*, **44**: 1502-1506. https://doi.org/10.1021/jf950270e

Lloyd, C.J., 1969. Study on the cross tolerance to DDT related compounds of a pyrethrin-resistant strain of *Sitophilus granarius* L. (Coleoptera: Curculionidae). *J. Stored Prod. Res.,* **5**: 337-356. https://doi.org/10.1016/0022-474X(69)90008-3

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. biol. Chem*., **193**: 265-275.

Mark, A.C., Severtson, D.L., Brumley, C.J., Szito, A., Foottit, R.G., Grimm, M., Munyard K. and Groth, D.M., 2010. A rapid nondestructive DNA extraction method for insects and other arthropods. *J. Asia-Pacific Ent.,* **13**: 243-248. https://doi.org/10.1016/j.aspen.2010.04.003

Mehrabadi, M., Bandani, A.R., Saadati, F. and Mahmudvand, M., 2011. Amylase activity of stored products insects and its inhibition by medicinal plant extracts. *J. Agric. Sci. Technol.*, **13**: 1173-1182.

Mokry, L.E. and Hoagland, K.D., 1990. Acute toxicities of five synthetic pyrethroid insecticides to *Daphnia magna* and *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.,* **9**: 1045-1051. https://doi.org/10.1897/1552-8618(1990)9[1045:ATOFSP]2.0.CO;2

Moore, S. and Stein, W.H., 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. biol. Chem*., **211**: 907-913.

Mujeeb, K.A. and Shakoori, A.R., 2007. Toxicity of synthetic pyrethroid, fury, against different developmental stages of three strains of *Tribolium castaneum* (Herbst.). *Pakistan. J. Zool.*, **39**: 361-366.

Mulye, H. and Gordon, R., 1993. 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*., **71**: 1169-1174. https://doi.org/10.1139/z93-159

Nath, B.S., Suresh, A., Mahendra, V.B. and Kumar, R.P., 1997. Changes in protein metabolism in haemolymph and fat body of the silkworm, *Bombyxmori* L., in response to organophosphorus insecticides toxicity. *Ecotoxicol. environ. Safe*., **36**: 169-173. https://doi.org/10.1006/eesa.1996.1504

Naumoff D.G. and Livshits V.A., 2001 Molecular structure of the *Lactobacillus plantarum* sucrose utilization locus: Comparison with *Pediococcus pentosaceus*. *Mol. Biol.,* **35**: 15. https://doi.org/10.1023/A:1004890415516

Omar N.A.M., Mousa, A., El-Husseini, M.M. and El-Bishry, M.H., 2005. Changes in lipid contents due to infection with *Bacillus thuringiensis* kurstaki in larvae of the greater wax moth *Galleria mellonella* L., (Lepidoptera: Galleridae). *Egypt. J. Biol. Pest Contr.,* **15**: 41-44.

Riaz, T., Shakoori, F.R. and Ali, S.S., 2014. Effect of temperature on the development, survival, fecundity and longevity of stored grain pest, *Trogoderma granarium. Pakistan J. Zool.,* **46**: 1485-1489.

Riaz, T., Shakoori, F.R. and Ali, S.S., 2016. Toxicity of phosphine against tolerant and susceptible populations of *Trogoderma granarium* collected from Punjab, Pakistan. *Punjab Univ. J. Zool*., **31**: 2530.

Riaz, T., Shakoori, F.R. and Ali, S.S., 2017. Effect of phosphine on esterases of larvae and adult beetles of phosphine-exposed populations of stored grain pest, *Trogoderma granarium* collected from different godowns of Punjab. *Pakistan J. Zool.*, **49**: 819-824. https://doi.org/10.17582/journal.pjz/2017.49.3.819.824

Roe, J.H. and Dailey, R.E., 1966. Determination of glycogen with the anthrone reagent. *Anal. Biochem.*, **15**: 245-250. https://doi.org/10.1016/0003-

2697(66)90028-5

Saleem, M.A. and Shakoori, A.R., 1987. Joint effects of dimilin and ambush on enzyme activities of *Tribolium castaneum* larvae. *Pestic. Biochem. Physiol.*, **29**: 127-137. https://doi.org/10.1016/0048-3575(87)90070-8

Shakoori, A.R., Agha, S., Malik, M.Z., Saleem, M.A. and Ali, S.S., 1994. 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.,* **9**: 5-20.

Shakoori, F.R., Feroze, A. and Riaz, T., 2016. Effect of sub-lethal doses of phosphine on macromolecular concentrations and metabolites of adult beetles of stored grain pest, *Trogoderma granarium*, previously exposed to phosphine. *Pakistan J. Zool.,* **48**: 583-588.

Shakoori, F. R., Feroze, A., Gondal, A., Akram, S. and Riaz, T., 2018. Impact of Lambda-Cyhalothrin on carbohydrate metabolizing enzymes and macromolecules of a stored grain pest, *Trogoderma granarium*. *Pakistan J., Zool.,* **50**: 1467-1474.

Shekari, M., Sendi, J.J., Etebari, K., Zibaee, A. and Shadparvar, A., 2008. Effects of *Artemisia annua* L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomellidae). *Pestic. Biochem. Physiol.*, **91**: 66-74. https://doi.org/10.1016/j.pestbp.2008.01.003

Shoba, V., Elanchezhiyan, C., Hemalatha, S. and Selvisabanayakam, S., 2011. Sublethal effect of phytopesticide nimbecidine on biochemical changes in the adult male insect *Sphaerodema rusticum* (Heteroptera: Belostomatidae). *Int. J. Res. Pharm. Sci*., **2**: 12-17.

Szinicz, L., 2005. History of chemical and biological warfare agents. *Toxicology*, **214**: 167-181. https://doi.org/10.1016/j.tox.2005.06.011

Viljoen, J.H., 1990. The occurrence of Trogoderma (Coleoptera: Dermestidae) and related species in southern Africa with special reference to *T. granarium* and its potential to become established. *J. Stored Prod. Res*., **26**: 43-51. https://doi.org/10.1016/0022-474X(90)90036-R

Vyjayanthi, N. and Subramanyam, M.V.V., 2002. Effect of fenvalerate-20EC on sericigenous insects: I. Food utilization in the late-age larva of the silkworm, *Bombyx mori* L. *Ecotoxicol. environ. Safe.*, **53**: 206-211. https://doi.org/10.1006/eesa.2002.2228

Ware, G.W., 2002. *An introduction to insecticides*, 3rd edition. University of Minnesota. Available at: https:// pdfs.semanticscholar.org/56f5/46e7d246e2deb750e4fc0517c71879920490.pdf

WHO, 2012. *WHO specification and evaluations for public health pesticides*. WHO, Geneva.

Wootton, I.D.P. and Freeman, H., 1982. *Microanalysis in medical biochemistry*. Churchill Livingstone, London, pp. 91-114.

Zollner, N. and Kirsch, K., 1962. Micro determination of lipids by the sulfophospho vanillin reaction. *Z. Gec. Exp. Med.,* **135**: 545-561.