



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

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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 LC₅₀ 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 (LC₂₀) 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.

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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.

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 (Szincz, 2005; Burges, 2008; Castalaneli *et al.*, 2010). Larvae of Khapra beetle are most destructive, they not only attack the grain but also reduce its 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 reservoir of 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, organophosphates 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

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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 esfenvalerate 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 $35\pm 2^\circ\text{C}$ 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 purchased from the Agricultural Chemical Group of FMC Corporation Lahore, Pakistan.

Determination of LC₅₀

For the determination of LC₅₀ 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 (9cm²) 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 $35\pm 2^\circ\text{C}$ and $60\pm 5\%$ 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 LC₅₀. Corrected mortality of 4th and 6th instar larvae was determined by using Abbot Formula (Abbot, 1925).

Effect of LC₂₀ on metabolites

After determination of LC₅₀ for each population separately, the sub-lethal dose of esfenvalerate (LC₂₀) was selected for determining toxic effects of insecticides because mortality was low at sublethal dose, so biochemical responses produced are more enough to unveil the mode of action. Approximately 400 larvae of (4th and 6th instar) of susceptible and tolerant populations of Khapra were exposed separately to LC₂₀ along with their controls (unexposed group) for 24 h, at $35\pm 2^\circ\text{C}$ and $60\pm 5\%$ 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 $3000\times g$ 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 \pm standard error of mean. Toxic effects of LC₂₀ 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 ($p\leq 0.05$).

RESULTS

LC₅₀ of esfenvalerate

The LC₅₀ of esfenvalerate against 4th instar larvae of susceptible and tolerant populations were 34.29 and 39.30ppm, respectively, while LC₅₀ 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 (LC₂₀) 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, Trehalose and Free amino acid 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 a similar way, free amino acid contents of 4th instar larvae were significantly decreased (64.01 and 60.63%) and in 6th instar larvae significant decrease was (37.47 and 56.07%) in susceptible and tolerant populations, respectively (Fig. 1A, B).

Table I.- Probit analysis showing LC₅₀ of 4th and 6th instar larvae of susceptible and tolerant populations of *T. granarium*.

Populations of <i>T. granarium</i>	LC ₅₀ (ppm) at 95% fiducial 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 LC₂₀ of esfenvalerate on various metabolites and enzyme activities of 4th and 6th instar larvae of *T. granarium*.

Parameters		4 th instar larvae		6 th 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*

^aMean ± Standard error of mean; n = 5 (number of replicates used in each experiment and each replicate contain twenty beetles); *, Significant (p ≤ 0.05).

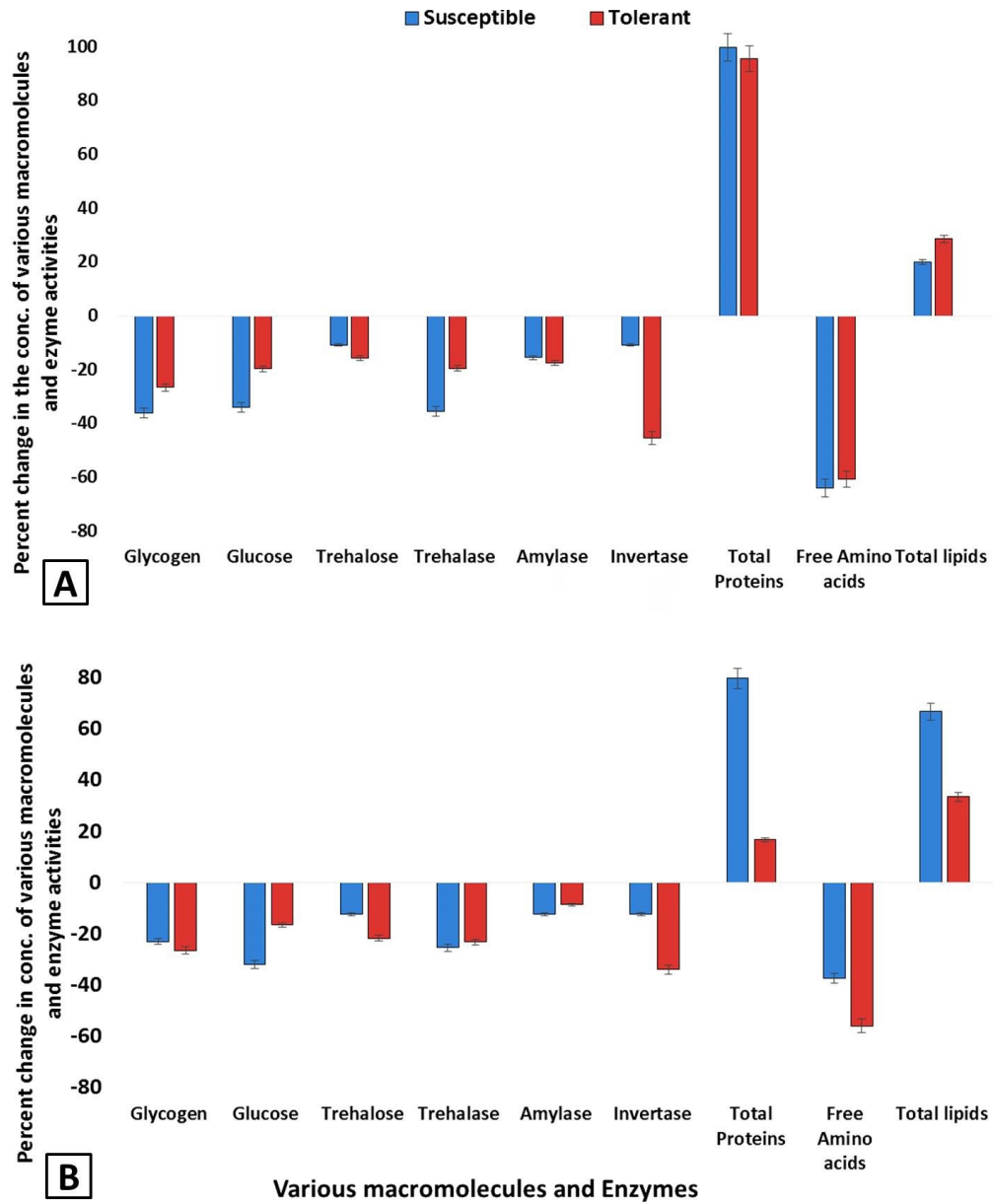


Fig. 1. Percent (%) change in concentrations of various macromolecules and enzyme activities of 4th (A) and 6th (B) instar larvae of *T. granarium* with reference to control.

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 increased in 4th and 6th instar larvae of both populations.

Protein contents of 4th instar larvae were significantly increased (99.81 and 95.61%) and in 6th instar larvae significant increase was observed (79.61 and 16.52%) in susceptible and tolerant populations, respectively. Similarly, lipid contents of 4th instar larvae were significantly risen (20.00 and 28.57%) and in 6th instar larvae significant rise was observed (66.67 and 33.33%) 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 LC₅₀ 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. Shakoory *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 Shakoory (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 haemolymph 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). Shakoory *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), Shakoory *et al.* (1994), Omar *et al.* (2005) and Ali *et al.* (2007) are in favor of current investigation but are in contrast to current 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 Shakoory *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* extract. 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 synthetic 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 sublethal concentrations had shown a decreased gut amylase activity in *T.castaneum* larvae. Current results revealed that esfenvalerate reduced the activities of invertase, trehalase 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 esfenvalerate. The decrease in concentrations of various macromolecules enzyme activities due to sublethal doses reveal more effective against Khapra beetle because it induced stress in insect which cannot be coped even at sublethal doses. So, esfenvalerate can be effectively used to control this insect pest in godowns.

Statement of conflict of interest

Authors have declared no conflict of interest.

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