



Impact of λ -Cyhalothrin on Carbohydrate Metabolizing Enzymes and Macromolecules of a Stored Grain Pest, *Trogoderma granarium*

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ABSTRACT

The present study was aimed to evaluate the biochemical effects of λ -cyhalothrin on carbohydrate metabolism and macromolecular concentrations of 4th and 6th instar larvae of a stored product pest, *Trogoderma granarium*. The LC₅₀ values of λ -cyhalothrin for 4th and 6th instar larvae of Lahore population was 15.93 and 13.76ppm respectively, while 19.07 and 16.21ppm were for the 4th and 6th instar larvae of Gujranwala population, respectively. The resistance ratio RR (0.67 and 1.17) for 4th and 6th instar larvae, respectively, indicated very little or no tolerance in *T. granarium* against λ -cyhalothrin in the godowns. The sub lethal dose of λ -cyhalothrin (LC₂₀) significantly increased the contents of free amino acids and soluble proteins while contents of total proteins, lipids, glycogen and trehalose were significantly reduced with reference to their control (untreated group). Among carbohydrate metabolizing enzymes, the activities of amylase and invertase were significantly reduced while activity of trehalase were significantly increased after treatment with sub lethal dose of λ -cyhalothrin as compared to control. The metabolic derangements induced by sub-lethal dose of λ -cyhalothrin suggest that infestation caused by *T. granarium* in godowns could be overcome by calculating lethal dose of λ -cyhalothrin.

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Authors' Contribution

FRS and TR designed and supervised the research project. TR, AF, AG and SA conducted the experimental work and FRS contributed reagents and analytical tools. FRS and TR analyzed the data and wrote the manuscript.

Key words

Trogoderma granarium, Lambda-cyhalothrin, Metabolites, Carbohydrate metabolizing enzymes, Pyrethroids.

INTRODUCTION

Khapra beetle *Trogoderma granarium* (Everts) are considered to be the most destructive quarantine species of wheat grains (Pasek, 2004; Atwal *et al.*, 2005; OEPP/EPPO, 2007; Mark *et al.*, 2010). Apart from its importance as a pest that causes serious infestations on wheat, *T. granarium* is a very important quarantine species for several areas, such as Russia, US and Australia. Hence, the presence of these species in grain commodities during exports requires the immediate rejection of the infested cargo, and the concomitant consequences in exports of a country that is based on grain production and market, such as Pakistan. The larval stages may undergo diapauses under unfavorable conditions and these diapausing larvae are observed to be more tolerant to various insecticides and fumigants (Edde, 2012). Various botanical insecticides (Kestenholtz *et al.*, 2007), synthetic pesticides (Wang *et al.*, 2006) and fumigants (Walter, 2006) have been used to control khapra beetle. The unplanned use of pesticides

and fumigants have resulted in the development of resistance (Saleem *et al.*, 2000; Zettler and Arthur, 2000; Benhalima *et al.*, 2004; Assie *et al.*, 2007). There are many reports on the resistance of khapra beetle against phosphine which is mainly used now a days to control the khapra beetle. In this way development of resistance has become a global issue and control of pests have become difficult due to this phenomenon (Zettler and Arthur, 2000; Daghish, 2008; Pimentel *et al.*, 2009).

Despite the importance of khapra beetle, disproportionally there are very few studies about its control other than phosphine. In this context, energy reserves used for metabolism could be measured in terms of total lipids, sugars and protein contents. The energy consumption by living organism could be measured by its electron transport activity. The energy available to resistant populations for growth could be measured by measuring the differences between energy consumption and energy reserves (Guedes *et al.*, 2006) as nutrients are indeed an obligatory requirement for the survival of all living organisms (Salunke *et al.*, 2009). Therefore the aim of present of study was to shed light on the effect of sub-lethal concentration of Type II pyrethroid trademark λ -cyhalothrin on various metabolites and carbohydrate

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metabolizing enzymes of 4th and 6th instar larvae of *Trogoderma granarium*. Our goal was to contribute in developing effective control strategy for khapra beetle.

MATERIALS AND METHODS

Rearing and maintenance of insect larvae

Two populations (Lahore and Gujranwala) of *T. granarium* were used in this study. Both populations of insects were reared in culture room of Department of Zoology, University of the Punjab, Lahore at 35±2°C and 60±5% relative humidity in continuous darkness (Riaz *et al.*, 2014). Master culture of Gujranwala population was initially collected from PASCO godowns of Gujranwala where wheat is being exposed to phosphine fumigation for more than fifteen years. The culture of Lahore population was maintained in culture room of Department of Zoology, University of the Punjab, Lahore and has never been exposed to any type of insecticide or fumigant since 2001. Adult beetles were fed on sterilized wheat flour and broken wheat in sterilized glass jars of 300ml capacity. The culture was reared to collect age wise homogeneous stock of 4th and 6th instar larvae (Riaz *et al.*, 2016) and homogeneous stock was maintained for further experiments.

Insecticides used

Technical grades of λ -Cyhalothrin [(RS)-alpha-cyano-3-phenoxybenzyl3-(2-chloro-3,3,3trifluoropropenyl)-2, 2, -dimethylcyclopropanecarboxylate] 2.5% EC were obtained from the Agricultural Chemical Group of FMC Corporation Lahore, Pakistan.

Determination of LC₅₀

To determine the LC₅₀ value of λ -Cyhalothrin serial dilutions (25, 22.5, 20, 17.5, 15, 12.5, 10, 7.5, 5, 2.5 ppm) were prepared in acetone by using calculated amount of insecticide. Insecticidal dilution (1.0 ml/plate) were applied separately in the center of glass Petri plates (diameter, 9 cm) by residual film method as recommended by FAO (1971). Petri plates were rotated manually to distribute the insecticide uniformly and acetone was allowed to evaporate at room temperature. Control Petri plates had no insecticide but containing the acetone only and three replicates for each concentration was prepared simultaneously. Ten healthy insects (each 4th and 6th instar larvae) of Lahore and Gujranwala populations were introduced in different Petri plates for 20 h at 35±2°C and 60±5% relative humidity in continuous darkness. 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 48hrs, larvae were checked for mortality by using camel

hair brush. They were considered dead if they did not show any movement on touching with camel hair brush (Llyod, 1969). The data obtained was subjected to Probit analysis (Finny, 1971) for the determination of LC₅₀ and Abbot Formula (Abbot, 1925) were used to determine the corrected mortality for 4th and 6th instar larvae of each population.

Effect of LC₂₀ on metabolites

After determination of LC₅₀ for each population separately, the sub-lethal dose of λ -Cyhalothrin (LC₂₀) was selected for ascertaining toxic effects of insecticides as at these doses the mortality was low, though physiological/biochemical responses were significant enough to understand the mode of action. Approximately 400 larvae of each (4th and 6th instar) of Lahore and Gujranwala populations were exposed separately to their respective LC₂₀ along with their respective controls (untreated) for the duration of 24 h, at 35±2°C and 60±5% relative humidity. After exposure, alive 4th and 6th instar larvae (treated and control) were used immediately for biochemical analysis while dead larvae were discarded.

Biochemical analysis

Thirty larvae for each (treated and control) of both populations were weighed and macerated separately in 1.5ml saline (0.89%) with the help of motor-driven Teflon glass homogenizer at 4°C followed by centrifugation at 3000×g for 30 min in refrigerated centrifuge at 4°C. Thus, clear supernatant was used for the estimation of soluble proteins, glucose, trehalose, trehalase, amylase and invertase activities. Glucose contents of beetle extract were determined by the *o*-toluidine method described by Hartelet *et al.* (1969). Trehalose contents were estimated by the anthrone method of Carroll *et al.* (1956) as modified by Roe and Dailey (1966). For the estimation of trehalase, amylase and invertase activities, procedure of Dahlqvist (1966), Wootton and Freeman (1982) and Ishaya and Swiriski (1976) were adopted, respectively. Glycogen contents were extracted by crushing the whole 4th and 6th instar larvae in KOH and estimated by the anthrone method of Consolazio and Lacono (1963).

Total and soluble protein contents in the beetle extract were estimated according to Lowry *et al.* (1951). Tissue homogenates of 4th and 6th instar larvae for total proteins were prepared in 0.5N NaOH followed by heating at 70°C in water bath for 15 min. Homogenates were centrifuged at 3000×g for 30 min at 4°C and clear supernatants were used for estimation of total proteins. Free amino acids contents in beetle extract were determined by the method of Moore and Stein (1954). Tissue homogenates of 4th and 6th instar larvae were prepared in 80% ethanol followed by

centrifugation at $461 \times g$ for 10 min. Clear supernatants were used for the estimation of free amino acids contents. Total lipid contents of beetle extract were estimated according to Zollner and Kirsch (1962). Homogenates of 4th and 6th instar larvae were prepared in hot ethanol with the help of motor-driven glass homogenizer. Homogenate containing test tubes were covered with aluminum foil and these tubes were incubated in pre-heated incubator at 65°C for overnight. Homogenates were centrifuged at $461 \times g$ for 15 min. Clear supernatants were used for the analysis of total lipid contents.

Statistical analysis

Statistical analysis was carried out in Minitab 16. All the data was presented in the form of mean \pm standard error of mean (S.E.M.), while data pertaining to effects of sub-lethal dose of λ -Cyhalothrin (LC_{20}) on metabolites was preceded through "t" test paired observations at 95% confident limit and comparison of individual mean for the determination of statistical significance was done. The level of significance for each experiment was specified to be non-significant ($p > 0.05$) and significant ($p \leq 0.05$).

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

| Populations of <i>T. granarium</i> | LC_{50} (ppm) at 95% fudicial limit | | | | | | | |
|---------------------------------------|---------------------------------------|-------|-------|------------------|--------------------|-------|-------|------------------|
| | Lahore strains | | | | Gujranwala strains | | | |
| | Estimated | Lower | Upper | Slope \pm SEM | Estimated | Lower | Upper | Slope \pm SEM |
| 4 th instar larvae | 15.93 | 14.09 | 18.07 | 16.84 \pm 0.98 | 19.70 | 17.27 | 23.25 | 20.45 \pm 1.43 |
| 6 th instar larvae | 13.76 | 12.11 | 15.48 | 14.89 \pm 0.84 | 16.21 | 14.39 | 18.36 | 16.56 \pm 0.97 |

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

| Parameters | | Locality | | | |
|---|-------------------------------|-------------------|------------------------------|--------------------|-------------------|
| | | Lahore strains | | Gujranwala strains | |
| | | Untreated | Treated | Untreated | Treated |
| Glycogen (μ g/mg) | 4 th instar larvae | 5.14 \pm 0.02* | 3.74 \pm 3.74 ^a | 0.73 \pm 0.03* | 0.53 \pm 0.01 |
| | 6 th instar larvae | 18.59 \pm 5.14* | 5.14 \pm 0.02 | 2.30 \pm 0.04* | 1.13 \pm 0.03 |
| Trehalose (μ g/mg) | 4 th instar larvae | 0.86 \pm 0.01* | 0.70 \pm 0.01 | 39.70 \pm 0.12* | 38.19 \pm 0.38 |
| | 6 th instar larvae | 0.72 \pm 0.03* | 0.48 \pm 0.48 | 44.24 \pm 0.98* | 40.92 \pm 0.25 |
| Glucose (μ g/mg) | 4 th instar larvae | 41.11 \pm 1.26* | 44.86 \pm 0.37 | 25.13 \pm 1.02* | 43.98 \pm 1.13 |
| | 6 th instar larvae | 22.66 \pm 0.75* | 26.04 \pm 0.14 | 32.85 \pm 0.64* | 52.71 \pm 1.23 |
| Total proteins (μ g/mg) | 4 th instar larvae | 70.92 \pm 1.44 | 41.17 \pm 1.05* | 1.61 \pm 1.03 | 46.84 \pm 0.61* |
| | 6 th instar larvae | 33.52 \pm 1.12* | 23.49 \pm 0.39* | 44.44 \pm 0.72* | 40.32 \pm 0.93* |
| Total soluble proteins (μ g/mg) | 4 th instar larvae | 4.17 \pm 0.18 | 6.67 \pm 0.04* | 4.82 \pm 0.11 | 7.54 \pm 0.15* |
| | 6 th instar larvae | 1.41 \pm 0.03 | 2.19 \pm 0.08* | 1.83 \pm 0.03 | 2.61 \pm 0.08* |
| Free amino acids (μ g/mg) | 4 th instar larvae | 5.65 \pm 0.06 | 8.85 \pm 0.15* | 7.09 \pm 0.05 | 10.41 \pm 0.33* |
| | 6 th instar larvae | 3.92 \pm 0.07 | 5.49 \pm 0.11* | 4.44 \pm 0.22 | 7.43 \pm 0.05* |
| Total lipids (μ g/mg) | 4 th instar larvae | 0.07 \pm 0.01 | 0.04 \pm 0.01 | 0.08 \pm 0.01 | 0.05 \pm 0.01* |
| | 6 th instar larvae | 0.05 \pm 0.01 | 0.03 \pm 0.01* | 0.07 \pm 0.01 | 0.05 \pm 0.01* |
| Trehalase (I.U/mg) | 4 th instar larvae | 0.54 \pm 0.02 | 1.97 \pm 0.05* | 0.69 \pm 0.01 | 0.86 \pm 0.02 |
| | 6 th instar larvae | 0.60 \pm 0.01 | 1.69 \pm 0.06* | 0.93 \pm 0.03 | 1.40 \pm 0.06* |
| Amylase (Somogyi unit/mg) | 4 th instar larvae | 12.43 \pm 0.13 | 9.39 \pm 0.23 | 15.60 \pm 0.15 | 12.67 \pm 0.13* |
| | 6 th instar larvae | 16.72 \pm 0.09 | 12.37 \pm 0.09* | 18.01 \pm 0.20 | 13.72 \pm 0.13* |
| Invertase (IU/mg) | 4 th instar larvae | 3.60 \pm 0.12 | 2.26 \pm 0.08* | 8.67 \pm 0.32 | 7.28 \pm 0.09* |
| | 6 th instar larvae | 1.95 \pm 0.06 | 1.46 \pm 0.06* | 3.61 \pm 0.18 | 2.78 \pm 0.07* |

^a. Mean \pm Standard error of mean; n = 5 (No. of replicates used in each experiment and each replicate contain twenty beetles); *, Significant ($p \leq 0.05$).

RESULTS

LC_{50}

The LC_{50} λ -Cyhalothrin against 4th instar larvae of Lahore and Gujranwala populations were 15.93 and 19.07ppm, respectively, while LC_{50} for 6th instar larvae were 13.76 and 16.21ppm, respectively (Table I).

Metabolites

Table II shows the toxic effect of sub-lethal concentration of λ -Cyhalothrin (LC_{20}) on metabolites of 4th and 6th instar larvae of Lahore and Gujranwala populations. Figure 1 shows the percent change in the metabolites of 4th and 6th instar larvae of both populations was calculated with respect to their controls (untreated).

Carbohydrates (glycogen, trehalose and glucose)

Glycogen and trehalose contents were significantly decreased in of 4th and 6th instar larvae of both populations. The glycogen contents were significantly decreased (35.78 and 37.70%) in 4th instar larvae and (261.67 and 109.09%) decreased in 6th instar larvae of Lahore and Gujranwala populations, respectively. The trehalose contents of 4th instar larvae were significantly decreased (23.42 and 3.95%) and in 6th instar larvae significant decrease was (12.58 and 21.94%) in Lahore and Gujranwala populations, respectively (Fig. 1).

Glucose contents, on the other hand, were significantly increased in larval instars of both populations. The glucose contents were significantly increased (8.36 and 42.88%) in 4th instar larvae and (32.19 and 16.71%) increase in 6th instar larvae of Lahore and Gujranwala populations, respectively (Fig. 1).

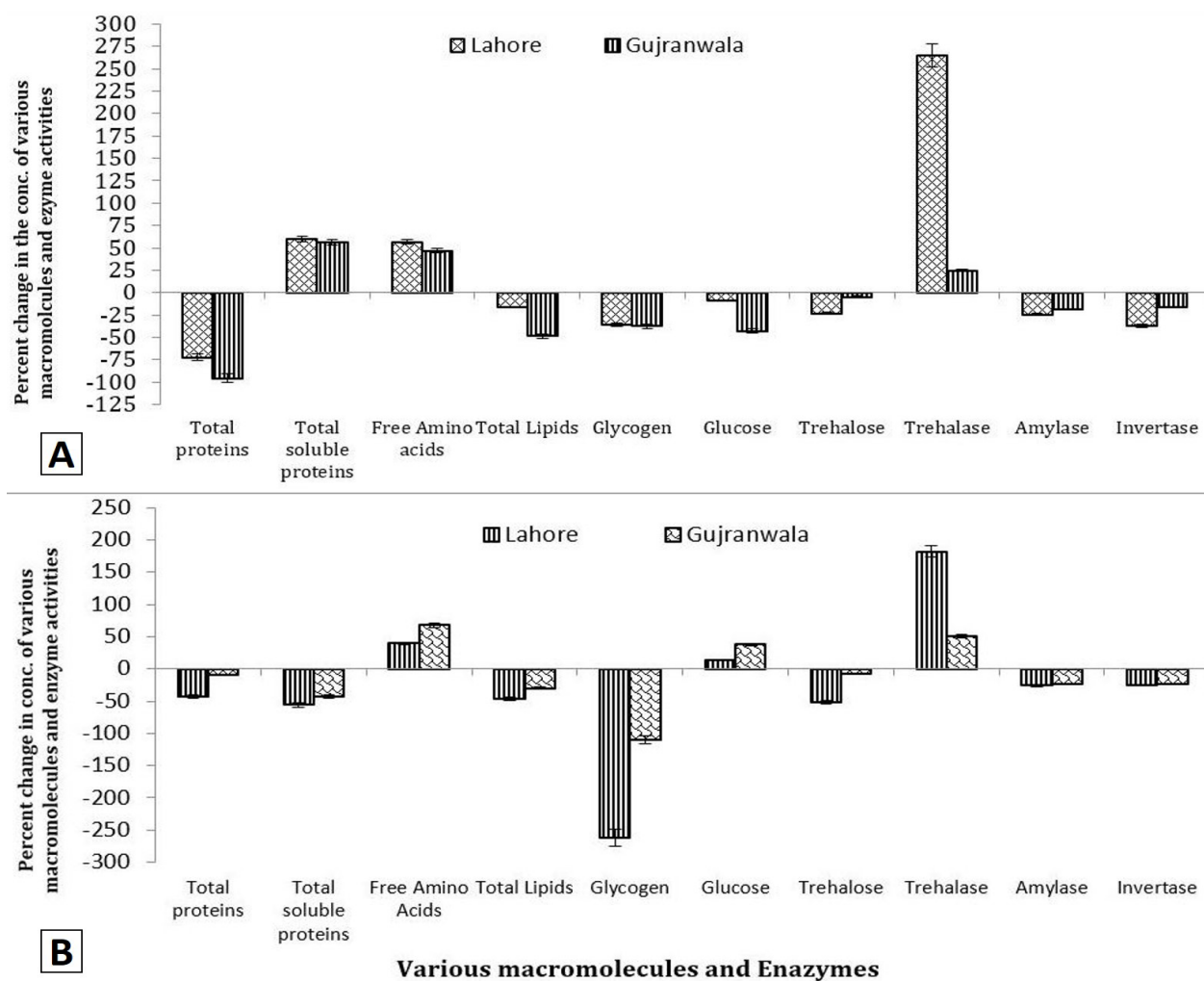


Fig. 1. Percent (%) change in concentrations of various macromolecules and enzyme activities of 4th (A) and 6th (B) instar larvae of *Trogoderma granarium* with reference to control.

Total protein, soluble proteins and free amino acid contents

The total protein contents were significantly decreased in 4th and 6th instar larvae of both populations. The 4th instar larvae possessed (72.26 and 95.62%) and 6th instar exhibited (42.69 and 10.19%) decrease in total protein contents in Lahore and Gujranwala populations, respectively (Fig. 1).

The contents of free amino acids and soluble proteins were significantly increased in 4th and 6th instar larvae of Lahore and Gujranwala populations. The 4th and 6th instar larvae possessed (59.937 and 56.517%) increase in soluble protein contents and (56.657 and 46.869%) significant increase in free amino acids in Lahore and Gujranwala populations, respectively (Fig. 1).

Total lipid contents

Similarly, lipid contents of 4th instar larvae of Lahore and Gujranwala populations were significantly depleted (16.514 and 48.379%) and in 6th instar depletion was 46 and 29.824%, respectively (Fig. 1).

Carbohydrases (amylase, invertase and trehalase activities)

Amylase and invertase activities in both larval instars of both populations were depleted significantly. The amylase activities were significantly decreased (24.45 and 18.76%) in 4th instar larvae and (26.01 and 23.82%) decrease in 6th instar larvae of Lahore and Gujranwala populations, respectively. Similarly invertase activities of 4th instar larvae were significantly decreased (37.22 and 16.03%) and in 6th instar larvae significant decrease was 25.58.58 and 22.99% in Lahore and Gujranwala populations, respectively (Fig. 1).

Trehalase activities in 4th and 6th instar larvae of both populations were significantly increased. The 4th instar larvae possessed (264.83 and 24.63%) significant increase while 6th instar larvae possessed (25.63 and 23.40%) significant increase in trehalase activities in Lahore and Gujranwala populations, respectively (Fig. 1).

DISCUSSION

In current investigation, toxic effects of λ -Cyhalothrin on biochemical parameters of 4th and 6th instar larvae of a stored grain pest *T. granarium* was investigated. The resistance ratio RR (0.67 and 1.17) for 4th and 6th instar larvae, respectively, indicated very little or no tolerance in *T. granarium* against λ cyhalothrin in the godowns. The 4th and 6th instar larvae of Lahore and Gujranwala populations of *T. granarium* possessed different levels of susceptibility to various concentrations of λ -Cyhalothrin at 35 \pm 2°C and 60 \pm 5% relative humidity. Mujeeb and Shakoori (2007) found that different developmental stages of 3 strains of *T.*

castaneum behaved differently against a pyrethroid with trade mark fury.

Elevated levels of FAA and soluble proteins while reduction in total protein contents were noticed among 4th and 6th instar larvae of both populations as compared to their respective controls. The elevation in FFA and soluble protein contents may be due to decreased activities of transaminases and this reduction in transaminase may lead to the elevation of soluble proteins and free fatty acid contents as suggested by Shakoori *et al.* (1994) in larvae of *T. castaneum* after treatment with esfenvalerate. Hafiz *et al.* (2017) noticed an increase in FFA and soluble protein contents in 4th, 6th instar larvae and adult beetles of *T. granarium* after exposure to sub lethal dose of deltamethrin. Similarly, in adult beetles of *T. granarium* Shakoori *et al.* (2016) reported an elevation in FFA and soluble protein contents after exposure to sub lethal dose of phosphine. Hussain *et al.* (2012) and Ali *et al.* (2011) also reported significant increase in FAA and soluble protein contents in adult beetles of *T. castaneum* after treatment with abamectin and *R. dominica* after treatment with melathion, respectively. Reduction in total protein contents can be related to the investigations of Etebari and Matindoost (2004) who reported a significant decrease in total protein contents in silkworm after exposure to various insecticidal stresses. Nath *et al.* (1997) suggested that total protein may be converted to amino acids and these amino acids may enter the citric acid cycle as keto acids to supply energy under stress conditions. So, reduction in total protein contents may be a compensatory mechanism during stress phase to supply intermediates of citric acid cycle by keeping free amino acids contents in the insects.

Depletion in lipids, glycogen and trehalose contents were noticed in 4th and 6th instar larvae of both populations. 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 (de Zwann and Zandee, 1972; Shoba *et al.*, 2011). Reduction in lipid contents revealed that pesticide exposure may cause the conversion of lipids to proteins to generate possible supplementary energy to cope with insecticidal stress. Shakoori *et al.* (2016) reported depletion in lipid and glycogen contents and elevation in glucose level after exposure to sub lethal dose of phosphine and Hafiz *et al.* (2017) also noticed significant decrease in lipid and glycogen contents and increase in glucose level in *T. granarium* after treatment with deltamethrin. These results are in accordance to the investigations reported by Mulye and Gordon (1993), Shakoori *et al.* (1994), Omar *et al.*

(2005) and Ali *et al.* (2007).

The induction in trehalase activity may suggest a defense mechanism to cope with insecticidal stress through utilization of energy reserves which was affirmed by decrease in glycogen, trehalose and total protein contents. Ali *et al.* (2011) reported increased activity of trehalase in malathion-resistant and susceptible adult of *R. dominica* after treatment with pyrethroid. Vyjayanthi and Subramanyam (2002) also reported enhanced trehalase activity in the midgut of silkworms treated with fenvalerate.

Amylase plays an important role in producing 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 (Naumoff 2001). Despite of particularly important enzyme, very limited studies have been found about the activities of invertase in stored grain pests. Saleem *et al.* (1998) observed a decrease in invertase activity in adult beetles of *T. castaneum* after 24 h treatment of synthetic pyrethroid cypermethrin. Present results revealed that λ -Cyhalothrin inhibited the activity of amylase and trehalase at sub-lethal dose and it may find its way as an important pest control strategy by disturbing carbohydrate metabolism. Mehrabadi *et al.* (2011) reported decrease in amylase activity in *T. granarium* after treatment with medicinal plant extracts. Similarly, Vyjayanthi and Subramanyam (2002) reported decreased amylase activity in silkworm after exposure to fenvalerate and Shekari *et al.* (2008) also reported decreased amylase activity in *Xanthogaleruca luteola* after treatment with *Artemisia annua* extract.

CONCLUSION

Trogoderma granarium collected from godowns showed no resistance against λ -Cyhalothrin in laboratory bioassays. The metabolic and enzymatic abnormalities induced by its sub lethal doses indicate that pest is sensitive to λ -Cyhalothrin and can be effectively controlled by using proper dosage and application of this pesticide.

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Ethical standard

This manuscript does not contain any studies with human participants or animals performed by any of the authors.

Statement of conflict of interest

The authors stated no conflicts of interest.

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