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Metabolic Profile of a Stored Grain Pest *Trogoderma granarium* Exposed to Deltamethrin

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ABSTRACT

Effect of deltamethrin on metabolic profile of 4th, 6th instar larvae and adult beetles of deltamethrin-resistant populations of *T. granairum* collected from wheat godowns of Gujranwala, Okara and D.G khan has been studied in current investigation. The toxicological data generated from deltamethrin-resistant populations was compared with deltamethrin-susceptible population that has not been exposed to any insecticide since thirteen years. From analysis of metabolic profile, it was found that soluble proteins, glucose contents and free amino acids increased, whereas glycogen and lipid contents were reduced in all deltamethrin-resistant populations as compared to deltamethrin-susceptible population. Soluble Proteins were significantly elevated (79, 100 and 37%) in 4th and 6th instar larvae and adult beetles of Gujranwala, (14, 24 and 14%) in Okara and (14, 13 and 2%) in D.G Khan populations, respectively compared to susceptible population. Free amino acids content of Gujranwala (22, 42, 75%), Okara (18, 30 and 17%) and D.G Khan (4, 15 and 25%) also showed the same trend of elevation in the 4th and 6th instar larvae and adult beetles, respectively compared to susceptible population. Glucose contents were significantly enhanced in Gujranwala (25, 336 and 356%), Okara (36, 163 and 74%) and D.G khan populations (122, 250 and 311%) for 4th and 6th instar larvae and adult beetles, respectively. Glycogen level, on the other hand, was significantly reduced in Gujranwala (82, 74 and 83%), Okara (27, 42 and 51%) and D.G khan populations (52, 56 and 64%) for 4th and 6th instar larvae and adult beetles, respectively when compared with susceptible population. Lipid contents of 4th and 6th instar larvae and adult beetles also depleted in Gujranwala (54, 65 and 65%), Okara (16, 21 and 25%) and D.G khan (36, 35 and 50%) populations, respectively as compared to susceptible population. To deal with stressful condition of exposure to deltamethrin, the insect utilizes its energy reserves to meet the energy requirement of enhanced metabolic activity.

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PThis study is a part of Ph.D thesis of AH. FRS and TR designed and supervised the study. AH, TR and FRS executed the experimental work and wrote the article.

Key words
Stored grain pest, Trogoderma granarium, Deltamethrin, Metabolites.

INTRODUCTION

Wheat is though a major food cereal crop of Pakistan, its production is decreasing over the years. Amongst numerous factors that are responsible for this low yield, losses of stored grains due to insect pests ranges from 5-10% of the world grain production (Ahmad *et al*., 1991). The major insect species known to infest the stored wheat grains is Khapra beetle, *Trogoderma granarium* (Khattak *et al*., 2000; Atwal *et al*., 2005), which is considered to be one of the most destructive pests of stored wheat (Lowe *et al*., 2000; Pasek, 2004; Stibic, 2007; Burges, 2008; Mark *et al*., 2010). At present, large numbers of pesticides are being commonly used to eradicate this pest (Daglish *et al*., 2003; Nayak and Daglish, 2006). Excessive use of insecticides has caused development of resistance in insects (Price, 1984; Saleem *et al*., 2000; Fuentes-Contreras *et al*., 2007). Alam *et al*. (1999) have reported high level of resistance in *T. granarium* from Sindh and Punjab to different insecticides. Resistance to deltamethrin has been reported worldwide (Fragoso *et al*., 2003; Ribeiro *et al*., 2003). Little is known about the deltamethrin resistance in *T. granarium* in Pakistan.

The present study was therefore, aimed at evaluating the response of insecticide resistant and susceptible strains of *T. granarium* to exposure to deltamethrin. For this, various metabolites *i.e*., soluble proteins, total lipids, free amino acids, glucose and glycogen contents were evaluated in different developmental stages of *T. granarium* collected from different godowns of Punjab. The data obtained may be helpful in control strategy for this devastating pest.

MATERIALS AND METHODS

Four populations of stored grain pest, *T. granarium* were used in this study. Master cultures of three populations of *T. granarium* resistant to deltamethrin were collected from PASSCO godowns of Gujranwala, Okara and D.G khan. These godowns have more than 35 years history of deltamethrin exposure to wheat. One untreated

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population was taken from culture room of Department of Zoology, University of Punjab, Lahore and this population was never exposed to any pesticide/fumigant since thirteen years so it was termed as susceptible population.

Maintenance of culture

The master culture of susceptible and resistant populations of Khapra beetle was reared in the 300 ml sterilized jam jars covered with muslin cloth which were tightened with rubber band to avoid escape of beetles and intrusion of other insects, lizards and rodents etc. Homogenous culture of all populations was prepared by allowing newly emerged 100 adult beetles to mate and lay eggs in separate wheat flour containing jars for five days. After mating, adults were removed from the flour by sieving and the eggs in wheat flour were allowed to hatch and sterilized crushed wheat was added for newly emerging larvae (FAO, 1986). In this way, after successive 4-5 generations of age wise homogeneous culture was obtained. The culture was maintained at 35±2ºC with 60±5% relative humidity (Riaz *et al*., 2014). From age wise homogeneous stock of each population 4th, 6th instar larvae and adult beetles were used to record LC_{50} and other toxicological data.

Determination of LC_{50}

For determination of LC_{50}, dilutions of deltamethrin were prepared in aceton according to recommendations of WHO (2012) and insects (4th, 6th instar larvae and newly emerged adult beetles) were exposed to deltmethrin by filter paper impregnated method recommended by FAO. For determination of LC_{50}, different doses *i.e.,* 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 110, 120, 130, 140 and 150 ppm were used in triplicates. For preparation of doses, 1 ml of insecticide solution of each concentration was applied with the help of glass pipette on the centre of filter paper having size of 130 cm and was spread uniformly (Anonymous, 1969, 1974; Champ, 1968; Lorini and Galley, 1999). Control petri plates were prepared in the same way but filter papers were treated with aceton. Air dried filter papers were placed in petri plates and ten healthy insects were introduced in their respective labelled Petri plate and covered. After 48 h, mortality was recorded according to Lloyd (1969), according to whom larvae showing no movement after pressing with brush were considered dead. To calculate LC_{50} values of 4th, 6th instar larvae and adult beetles of *T. granarium* mortality data was subjected to Probit analysis by Minitab 16 software (Finney, 1971) and were expressed in ppm.

Biochemical analyses

From LC_{50} data of all populations, it was evident that Gujranwala, Okara and D.G. Khan populations were resistant to deltamethrin with reference to susceptible population. Twenty 4th, 6th instar and adult beetles from susceptible and resistant populations of khapra beetle were weighed and homogenize in 2 ml 0.89% saline solution with the help of motor-driven Teflon glass homogenizer at 4°C in five replicates. Homogenates were centrifuged at 3000 × g for 30 minutes in refrigerated centrifuge at 4°C. After centrifugation, supernatant obtained was used for the estimation of glucose and soluble proteins contents by *O*-toluidine method by Hartel *et al*. (1969) and Lowry *et al*. (1951), respectively. Lipid contents and FAA were estimated from ethanol extract of beetles. The total lipids were estimated by the method of Zollner and Kirsch (1962). Free amino acids were estimated according to Moore and Stein (1954) method. Glycogen contents were estimated in 30% potassium hydroxide solution (KOH) according to Anthrone method of Consolazio and Lacono (1963).

Statistical analysis

The data was subjected to one way ANOVA and Tukey's test to compare the significance difference between means of susceptible and resistant populations at $P< 0.05$.

RESULTS

The LC_{50} values of 4th and 6th instar larvae and adult beetles of Gujranwala, Okara and D.G Khan strains are shown in Table I.

Table I.- LC_{50} values of Gujranwala, Okara and D.G Khan population.

S No.	Populations	Developmental stages	LC_{50} (ppm)
1.	Susceptible	4th instar	77.01
		6th instar	59.03
		Adults	55.84
2.	Gujranwala	4th instar	119.11
		6th instar	93.83
		Adults	83.44
3.	Okara	4th instar	108.37
		6th instar	92.11
		Adults	76.25
4.	DG khan	4th instar	97.58
		6th instar	78.53
		Adults	71.33

The 4th and 6th instar larvae and adult beetles of deltamethrin susceptible and resistant populations (Gujranwala, Okara and D.G Khan) were analysed for various metabolites.

Soluble proteins

The soluble proteins were significantly increased in

deltamethrin-resistant populations of Gujranwala, Okara and D.G Khan compared to susceptible population. Among 4th instar larvae of Gujranwala, Okara and D.G Khan populations 79, 14 and 14% increase was recorded while in 6th instar larvae 100, 24 and 13% increase was calculated respectively, compared to susceptible population. In adult beetles of Gujranwala, Okara and D.G Khan populations 37, 14 and 2% increase in protein content were estimated with reference to susceptible population (Figs. 1, 2 and 3).

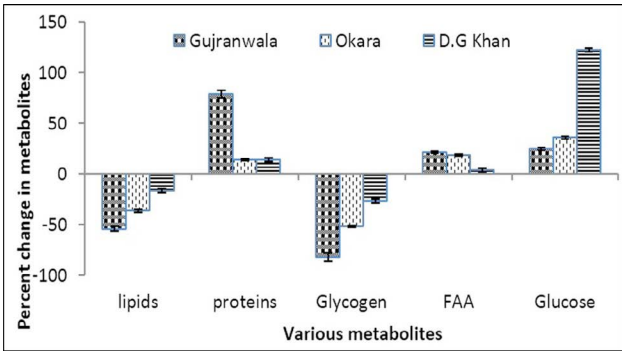

Fig. 1. Percent (%) change in various metabolites of 4th instar larvae of resistant population, compared with susceptible population.

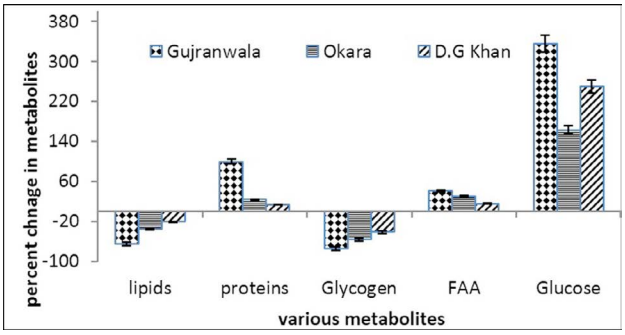

Fig. 2. Percent (%) change in various metabolites of 6th instar larvae of resistant population compared with susceptible population.

Glucose contents

The 4th, 6th instar and adult beetles of Gujranwala, Okara and D.G khan showed significant rise in glucose contents. The increase was 25, 35 and 122% in 4th while in 6th instar larvae, 336, 162 and 249% increase was noticed in Gujranwala, Okara and D.G Khan populations, respectively as compared to susceptible population. In case of adult beetles, 356, 74 and 311% increase was calculated in Gujranwala, Okara and D.G Khan populations, respectively with reference to susceptible population (Figs. 1, 2 and 3).

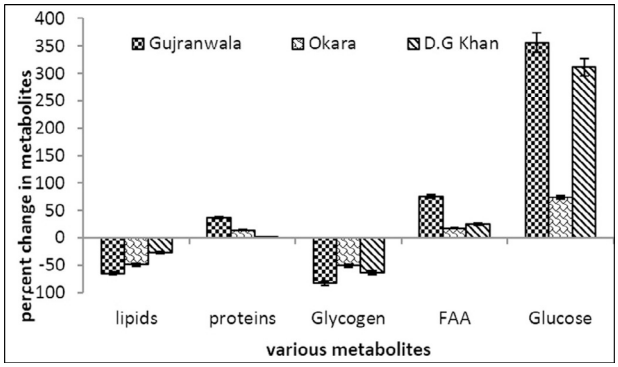

Fig. 3. Percent (%) change in various metabolites of adult beetles of resistant population compared with susceptible population.

Free amino acids

Free amino acid contents in resistant populations were observed to increase in 4th and 6th instar larvae and adult beetle compared to susceptible population. In 4th instar larvae of Gujranwala, Okara and D.G Khan populations the increase was 22, 18 and 4%, respectively as compared to susceptible population. In 6th instar larvae, there was significant increase of 42, 30 and 15%. In adult beetles of Gujranwala, Okara and D.G Khan populations, a significant rise of 75, 16 and 24% was found as compared to susceptible population (Figs. 1, 2 and 3).

Glycogen

In deltamethrin-resistant populations glycogen contents showed significant deplation in 4th, 6th instar larvae and adult beetles as compared to susceptible population. Among 4th instar larvae, 82, 27 and 52% increase was found in Gujranwala, Okara and D.G Khan populations as compared to susceptible population. Glycogen contents in 6th instar larvae were significantly decreased 74, 42 and 56% and adult beetles exhibited 83, 51 and 64% decline in resistant populations of Gujranwala, Okara and D.G Khan, respectively compared to susceptible population (Figs. 1, 2 and 3).

Lipid contents

In deltamethrin-resistant populations, lipid contents were significantly reduced with reference to susceptible population. In 4th instar larvae of Gujranwala, Okara and D.G Khan populations, the decrease was 53, 16 and 36% compared to susceptible population. Lipid contents in 6th instar larvae were significantly decreased 65, 21 and 35% and adult beetles showed 65, 25 and 50% decrease in resistant populations of Gujranwala, Okara and D.G Khan, respectively compared to susceptible population (Figs. 1, 2 and 3).

DISCUSSION

Deltramethrin-resistant populations *viz*., Gujranwala, Okara and D.G Khan showed significantly high value of LC_{50} as compared to deltramethrin-susceptible population of *T. granarium.* The LC_{50} values of Gujranwala, Okara and D.G Khan populations for 4th, 6th instar larvae and adults beetles were (118, 105 and 99 ppm), (79, 81, and 59 ppm) and (67, 58 and 54 ppm) respectively. The LC_{50} data revealed that Gujranwala population was the most resistant population as compared with susceptible population. Likewise, it was found that 4th instar larvae were most resistant and adult beetles were least resistant in all populations. Riaz *et al*. (2016) also reported that 4th instar larvae of *T. granarium* is most resistant among other developmental stages after exposure to phosphine. Ali *et al*. (2007) found adult beetles of *R. Dominica* more susceptible than other developmental stages after exposure to deltamethrin. Saleem *et al*. (2008) demonstrated similar type of result in *Spodoptera litura* after exposure to organochlorine, organophosphate, pyrethroid and carbamate.

In godowns infestation of khapra beetle is treated with deltamethrin so, effect of deltamethrin on metabolic profile of 4th, 6th instar larvae and adult beetles of Gujranwala, Okara and D.G Khan populations was investigated in this study. Results revealed that soluble proteins were increased significantly in 4th, 6th instar larvae and adult beetles of deltramethrin-resistant populations as compared to susceptible population. Shakoori *et al*. (2016) also reported an increase in soluble protein contents in adult beetles of *T. granarium* after 24 and 48 h exposure to phosphine but then soluble protein contents started to decrease. Ali *et al*. (2011) also reported an increase in protein contents in *R. dominica* after exposure to melathion. This increase may be due to increase protein biosynthesis as a result of enzyme induction to counter the toxic effect of insecticide (Ali *et al*., 2011).

Elevated levels of FAA were noticed among 4th and 6th instar larvae and adult beetles of all resistant populations as compared to susceptible population. This is due to presence of alternate energy sources at this stage. Shakoori *et al*. (2016) also found an increase in FFA level in adult beetles of *T. granarium* after exposure to phosphine. Hussain *et al*. (2012) and Ali *et al*. (2011) reported that FAA contents of adult beetles of *T. castaneum* were elevated after treatment with abamectin.

Glucose contents were significantly increased in 4th, 6th instar larvae and adult beetles of all deltamethrin-resistant populations. The elevated levels of glucose contents recorded in present study may suggest that glycolysis was switched on to survive in the stress conditions induced by insecticidal exposure (Dezwann and Zandee, 1972; Tufail *et al*., 1994). The data reported by Ali *et al*. (2014) with *T. castaneum* and Shakoori *et al*. (2016) on *T. granarium* after exposure to phosphine are also in accordance to present study.

Lipid and glycogen contents were decreased in 4th, 6th instar larvae and adult beetles of Gujranwala, Okara and D.G Khan populations with reference to susceptible population. It indicates that exposure of pesticide cause conversion of lipid to protein as possible supplementary energy source. Mulye and Gordon (1993) also reported that lipid synthesis and catabolism of the fat body was severely impaired in juvenile hormone analogue treated budworms. Reduction in glycogen contents may be due to utilization of glycogen as energy source in aerobic as well as anaerobic pathways under stress conditions. Omar *et al*. (2005), Mulye and Gordon (1993), Shakoori *et al*. (1994), Ali *et al*. (2007), Shoba *et al*. (2011) and Shakoori *et al*. (2016) has also reported similar effect of various insecticides on lipid and glycogen contents.

CONCLUSION

Metabolic profile of *Trogoderma granarium* demonstrated that this pest can be controlled by using toxic doses of deltamethrin. Metabolic abnormalities was induced by deltamethrin in all four populations of *T. granarium*. This insecticide can be used to control khapra beetle if the doses were calculated periodically as per need of the pest.

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