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Efficacy of Diatomaceous Earth and Deltamethrin Alone and in Combinations on Mortality and Energy Reserves of Insecticide Resistant Strains of Stored Grain Pest, *Trogoderma granarium*

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NK performed experimental work, statistically analyzed the data and prepared first draft of the manuscript. TR improved the article. AF formatted article. FRS and ARS designed and supervised the work and also reviewed the entire manuscript.

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Synergism, Antagonism, Deltamethrin-DE combination, 4th Instar larvae, Grain protectants

ABSTRACT

The present study was conducted to determine the LC_{50} of deltamethrin and diatomaceous earth (DE) alone and in different combinations i.e., 0.5:01, 01:0.5 and 1:1 against 4th instars of insecticide resistant populations of *Trogoderma granarium* collected from godowns of Gujranwala (GUW), D.G. khan (DGK), Okara (OKR), Lahore (LAB-S) and Layyah. The LC_{50} values of deltamethrin against 4th instar larvae of GUW, DGK, OKR, Layyah and LAB-S were 514, 353, 303, 189 and 98 ppm respectively while for the DE these values were 3606, 2684, 2407, 2192 and 2065 ppm, respectively. Based on the values of LC_{50} deltamethrin was found to be more effective than DE. Among the tested populations, GUW population was most resistant while LAB-S population was least resistant. Efficacy of deltamethrin and DE in various combinations was also tested against the same larval stage of different strains of pest populations and the mixture 0.5DM+1DE was found to be most effective among all mixtures on the basis of LC_{50} values and relative toxic unit. The toxicity of this mixture was analyzed on glucose, glycogen, trehalose, total proteins, soluble proteins, free amino acids and total lipids contents of 4th instars larva of most resistant GUW and least resistant LAB-S populations at exposure to LC_{20}. Among the biochemical parameters glucose, soluble proteins and FAA contents were found to be elevated significantly whereas the concentration of total proteins, glycogen, trehalose and total lipids was found to be decreased in both populations. Depletion of energy reserves is most probably the cause of mortality of the pest.

INTRODUCTION

The most widely grown crop wheat (*Triticum aestivum* Lam) has been considered as the basic staple food and one of the first domesticated food crops of major civilizations of Europe and of Pakistan from previous 10,000 years. All over the world as well as in Pakistan, a huge damage to stored grain products is caused by stored grain insect pests among which *Trogoderma granarium* (Everts) is causing a remarkable loss to wheat, cereals, grains and other stored products in Asia and Africa (Burges, 2008; Phillips and Throne, 2010; Nadur *et al.*, 2015; Masolkar *et al.*, 2018). Post-harvest storage losses may range from 7-10% of total production from field to purchasers and 4-5% at marketing (Yadav *et al.*, 2018). *T. granarium* pass through five to six larval stages before developing into pupae and adult beetle. It completes its life cycle in 45-50 days (Kulkarni *et al.*, 2015; Hafiz *et al.*, 2017). Larvae of this beetle play a major role to damage the stored grain from 30-70%, thus are considered best for the control strategies as they can pass through starvation for years (through diapause), developed resistance against different insecticides, feed on low moisture content and hiding in crevices and cracks (Ahmedani *et al.*, 2009; Athanassiou *et al.*, 2015).

Various pesticides like organophosphates, pyrethroids and carbamates have been recommended to lessen insect pest infestation (Hargreaves *et al.*, 2000). The pyrethroids possessed different level of toxicity. Deltamethrin is most widely used in storage facilities to control the insect pest infestation. It acts by causing rapid paralysis of the nervous system by a sudden knockdown and disable insects feeding. It also causes poisoning through cuticular penetration or through oral uptake that led to permanent

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nervous breakdown (Leahey, 1985; Hayes and Laws, 1990). It is a Type 11 a-cyano synthetic pyrethroid (Casida and Durkin, 2013) and is graded as acute hazard in class 2 of WHO insecticide toxicity classification (WHO, 2009). It considered three times more toxic as compared to other synthetic insecticides (Gilbert, 2014). Synthetic pesticides posed various types of ecological problems, high mammalian toxicity and prone to emergence of insecticide resistance (Chintzoglou *et al*., 2008). Therefore, the researchers shifted their efforts towards biopesticide to reduce and ultimately eliminate the use of synthetic pesticides on which smallholder farmers currently rely on due to lack of effective alternatives (Vassilakos *et al*., 2015). In this context DE is a bio insecticide derived from fossilized deposits of siliceous marine or fresh water unicellular algae and mainly composed of silicon dioxide (Shah and Khan, 2014). It causes disruption of the lipid layer, physical abrasion and insects die due to dryness. Various formulations of DE are effective against many pest species like *Tribolium castaneum* Herbst and *Tribolium confusum* Duval (Athanassiou and Steenberg, 2007). The available literature also pointed out that the required DE dosage to effectively control insect pests is about 1000 ppm/tonne (Athanassiou and Korunic, 2007; Athanassiou *et al*., 2013) is very high for safety of workers as well it possessed toxic effect on density and quality of grains (Vayias *et al*., 2009; Korunic, 2013).

Due to the above-mentioned disadvantages of DE many researchers have recommended low dosage of DE (Korunic and Rozman, 2010; Almasi *et al*., 2013). The toxicity of such low doses could be enhanced by synergistic combinations of DEs with different additives as mentioned by (Athanassiou and Korunic, 2007; Korunic and Rozman, 2010; Almasi *et al*., 2013). The success rate of these combinations is dependent on various factors like humidity, temperature, insect species, insect sources and characteristics of the particular DE (Athanassiou *et al*., 2005, 2008). So, by using these combination of selective biological insecticides, emergence of resistance can be delayed that can provide very effective extended grain protection. It is the most favorable managing approach under different life histories of insect. Moreover, in mixture, various pesticides may develop synergism at much lower concentrations (O'Connor–Marer, 2000; Athanssiou *et al*., 2005; Cloyd, 2011; Machekano *et al*., 2017). These combinations may be cost-effective due less quantity of individual insecticides used, less number of applications required for a short period and comparatively less toxicty to famers and environment (Das, 2014). Deltamethrin and DE are often used in mixture strategy. Due to combined action, the concentrations of DE and various other pesticides concentrations in the mixtures are used in smaller amount than used alone. This combination acts at different modes of action, desiccation and chemical toxicity (Vayias *et al*., 2009; Korunic, 2013, 2016; Machekano *et al*., 2017; Delgarm *et al*., 2020).

Due to the rising resistance problem against conventional pesticides, environmental hazards, shortcoming related to DE and to reduce the deltamethrin residue in grains as well as considering the importance of synergism, the present study was planned to find out (1) toxicity of DE and deltamethrin when applied alone (2) toxicity of various combinations of DE and deltamethrin (3) to determine the toxic effect of the most effective mixture on energy reserves of the 4th instar larvae of *T. granarium.*

MATERIALS AND METHODS

Collection and rearing of insects

In current study four populations of *T. granarium* were collected from wheat storing godowns of Pakistan Agricultural Storage and Service Corporation Ltd in Okara (OKR), Gujranwala (GUW), Layyah and Dera Ghazi Khan (DGK) in Punjab, Pakistan. The wheat stored in godowns is being treated with deltamethrin for more than 38 years to manage the invasion of notorious insect pest especially the *T. granarium*. Lahore population of khapra beetle (LAB-S) reared in the insectary for more than 18 years without any exposure to fumigant or insecticides was used to grade the level of resistance in field collected deltamethrin resistant populations.

Different larval stages i.e. 1st, 2^{nd,} 3rd, 4th, 5th, 6th and adult beetles were maintained in sterilized jam jars (300ml) separately in a culture room at 30±1°C and 65±5% relative humidity (R.H) according to Riaz *et al.* (2014, 2018). To culture the *T. granarium* wheat grain, were used as a culture media. In each plastic jar containing 250g sterilized wheat grain, 24h old adult unmated beetles (100) with no sex discrimination were released. Each jar was covered with muslin cloth to prevent the escape of insects and from the attack of any other pest. Beetles were allowed to lay eggs and then separated from the flour. The flour containing eggs was poured back into the jars. After 32±2 days of developmental period, hundred newly emerged adults were resealed again in another set of jars containing flour for egg laying. This process was repeated 4-5 times to attain homogenous populations (Riaz *et al*., 2018). From this homogenous culture, after 15±2 days of egg hatching, 4th instar larvae were collected for estimation of LC_{50} and other toxicological studies.

Insecticides used

In this study technical grades of synthetic pyrethroid, deltamethrin ($C_{22}H_{19}Br_2NO_3$) [(S)-Cyano-(3-phenoxyphenyl)-methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate) (1.5% EC, w/v) was purchased from Agrochemicals (Private) Limited, Wahdat Road, Lahore. Diatomaceous Earth (SiO_2 90% + inert ingredient 10%) was obtained in wettable powdered (WP) from The Planters (Pvt) Ltd (TPL Services) Islamabad. For the preparation of different concentration of insecticides, absolute acetone was used as a solvent.

Preparation of stock and working solutions of deltamethrin and diatomaceous earth

The stock solutions of 4000 ppm of deltamethrin (1.5% EC) and 1.5% WP of DE were used for preparation of working solutions. The working solutions were mixed in different proportions at room temperature as shown in Table I. Final concentration of each combination was kept 4000 ppm in both sets in 10 ml total volume.

Table I. Composition of deltamethrin and diatomaceous earth combinations used for the estimation of LC_{50} values against populations of *T. granarium*.

Mixture	Ratios deltamethrin: DE	Concentration and quantity of component insecticides mixed (volume/volume)	
		Deltamethrin (4000 ppm)	Diatomaceous earth (4000 ppm)
1	0.5:1	5ml (2000 ppm)	5ml (2000 ppm)
2	1:0.5	2.5ml (1000 ppm)	7.5 (3000 ppm)
3	1:1	7.5 (3000 ppm)	2.5ml (1000 ppm)

Dose bioassay

For estimation of LC_{50} of deltamethrin, DE and those combinations of deltamethrin and DE mentioned in Table I, various concentrations such as 200-4000 ppm (on a scale of 200 ppm) of each insecticide were prepared in absolute acetone. Each concentration of deltamethrin, DE and their combinations was administered to 4th instar larvae of OKR, DGK, GUW, Layyah and LAB-S populations according to the residual film method (Busvine, 1971; Saleem and Shakoori, 1990; Shakoori *et al*., 2004). Aliquot of 1ml from each concentration of each insecticide was applied in respective labelled petri dishes (dia. 4.5cm) to form a uniform coating. The petri plates were air dried for 5 min to evaporate the solvent and formation of a thin film of insecticide was observed. Absolute acetone (1ml) was applied in control petri dishes and it was evaporated. Ten healthy 4th instar larvae (with no sex discrimination) from OKR, DGK, GUW, Layyah and LAB-S strains of *T. granarium* were transferred to these respective petri dishes at 30±1°C and 65±5% r.h for 48 h. The entire procedure was replicated three times. After the subsequent pesticide exposure period, mortality was observed for every treatment as described by Lloyd (1969). By using Minitab 16 software the mortality data was subjected to Probit analysis described by Finney (1971) for estimation of LC_{50} and LC_{20} at 95% fiducial limit and values of the slope. The level of resistance in 4th instar larvae of field collected populations of khapra beetle against each treatment were determined at LC_{50} level by comparing with LAB-S strain using resistance ratio (RR) by following the criteria described by Mazzarri and Georghiou (1995).

Synergistic interactions

Synergistic, antagonistic or additive interactions between the constituent insecticides for all the mixtures were calculated at LC_{50} value in terms of relative toxic unit (RTU) (Otitoloju, 2001).

$$\text{Relative Toxic Unit} = \frac{\text{Theoretical LC50of mixture}}{\text{Observed LC50 of mixture}}$$

Theoretical LC_{50} of mixture= (LC_{50} of insecticide A alone × Percentage of A in Mixture) + (LC_{50} of insecticide B alone× Percentage of B in Mixture).

Mixture exhibited synergism if the calculated relative toxic unit is higher than 1 and antagonism if these values are less than 1.

Administration of LC_{20}

Based on RR value, GUW strain were considered most resistant and LAB-S was considered least resistant strains of khapra beetle. The 4th instar larvae (1000) of these populations were exposed to LC_{20} of mixture 2 (0.5 deltamethrin: 1 DE) following the procedure as mentioned in the section of "Dose bioassay". The mortality is very low at LC_{20} value so, this treatment provided an insight about the toxic effects of mixture on energy reserves of *T. granarium*.

Biochemical assay

To evaluate the toxic effects of mixture on various energy reserves, twenty larvae of *T. granarium* from GUW and LAB-S populations were weighed and homogenized in 2ml of saline solution (0.89%) in a Teflon glass homogenizer at 4°C. The homogenate was centrifuged at 13500 rpm for 15 min at 4°C. In order to evaluate the toxic effects exerted by the mixture, untreated larvae of these populations were also proceeded in this way. The supernatants were used for the estimation of trehalose through anthrone method described by Carroll *et al*. (1956), soluble proteins according to Lowry *et al*. (1951) and glucose contents by O-toluidine method of Hartel *et al*. (1969). The method adopted for the estimation of lipids, was of Zollner and Kirsch (1962) and free amino acid contents was quantified according to Moore and Stein (1954) by using ethanolic extract of larvae. Anthrone methodology of Consolazio and Lacono (1963) was followed for glycogen contents and homogenate was prepared in 30% potassium hydroxide solution and extract for total protein contents was prepared in NaOH and estimation was done according to Lowry *et al*. (1951). For the effects of sub-lethal doses of mixture, unpaired "t" test at 95% confidence limit was used.

RESULTS AND DISCUSSION

Table II indicates the comparison of LC_{50} of deltamethrin, DE and in mixtures against 4th instar larvae of LAB-S and four deltamethrin tolerant strains of *T. granarium.* No mortality was observed in control petri plates for the all the sets of replicates.

Toxicity of individual insecticides

The LC_{50} of deltamethrin for 4th instar larvae of Layyah, OKR, GUW, DGK and LAB-S populations was 353, 189, 514, 303 and 98 ppm, respectively while LC_{50} values of DE was 3606, 2684, 2407, 2192 and 2065 ppm, respectively. Deltamethrin was found to be the most effective while DE was the least effective insecticide when administered alone. From all the tested populations, larvae of GUW population were the most resistant (RR 5.24) while the larvae of OKR population were the least resistant to deltamethrin (RR 2.02) in comparison to LC_{50} value of LAB-S population. All populations have very

Table II. LC_{50} of deltamethrin and DE and comparison of their efficacies in combinations against 4th instar larvae of one susceptible and three deltamethrin tolerant strains of *T. granarium.*

Insecticides/ mixture used	Ratio	Locality of population	LC_{50} (ppm) ±SEM*	95 % Fiducial limits	Slope±SE	χ^2	P**	RR***	RTU****
Deltamethrin	1:0	GUW	513.80±0.12	473.80–553.80	9.23±2.15	0.83	0.11	5.24	------
		Layyah	353.17±0.18	303.17–403.18	0.01±0.02	2.59	0.98	3.60	------
		DGK	303.07±0.14	283.08–323.07	7.93±1.33	4.92	0.89	3.10	------
		OKR	188.86±0.05	163.84–213.90	7.50 ± 1.35	4.65	0.91	2.02	------
		LAB-S	97.92±0.09	67.92–127.89	4.47±0.77	3.76	1.05	-----	------
Diatomaceous Earth	1:0	GUW	3605.87±0.17	3205.40–4005.27	13.04 ± 2.74	0.89	0.26	1.74	------
		Layyah	2684.31±0.021	2384.11–2984.51	22.81±4.17	3.12	0.85	1.30	------
		DGK	2406.56±0.31	2200.56–2612.30	15.11±2.41	2.12	0.89	1.16	------
		OKR	2191.95±0.48	1840.40–2543.40	19.04±3.60	7.40	0.59	1.06	------
		LAB-S	2064.55±0.09	1624.33–2484.77	24.03±5.76	1.59	0.99	-----	------
Mixture 1 deltamethrin:	0.5:1	GUW	663.58±0.31	563.28–763.81	3.62±2.65	0.15	0.92	3.42	5.82
		Layyah	562.71±0.21	356.41–769.01	1.69±0.79	0.02	0.99	2.90	5.08
		DGK	402.51±0.32	199.21–605.81	1.73±0.73	0.26	0.88	2.08	6.35
		OKR	213.015±0.17	163.60–263.51	2.21±0.78	2.46	0.29	1.10	10.73
		LAB-S	194.36±0.23	144.15–244.55	2.69±1.27	1.08	0.58	------	10.87
DE mixture 2 deltamethrin: DE	1:0.5	GUW	793.44±0.42	690.28–896.60	3.62±2.65	0.15	0.93	2.62	2.91
		Layyah	663.59±0.18	423.18–903.98	2.56±1.19	0.36	0.83	2.19	2.55
		DGK	501.89±0.24	399.19– 604.69	1.99±0.80	0.13	0.93	1.66	3.00
		OKR	322.38±0.61	272.17 –377.57	2.85±0.83	2.82	0.42	1.06	3.98
		LAB-S	303.40±0.04	197.18 –409.22	2.47±0.83	1.22	0.54	------	3.72
Mixture 3 deltamethrin: DE	1:1	GUW	1723.03±0.24	1520.20–1926.07	4.13±1.08	0.77	0.99	2.80	1.34
		Layyah	1471.99±0.27	1520.20–1926.07	5.58±1.20	0.40	0.99	2.37	1.15
		DGK	1070.56±0.07	768.20–1372.86	3.74±0.71	9.43	0.31	1.73	1.41
		OKR	748.91±0.16	698.41–799.41	2.69±0.52	8.12	0.42	1.21	1.71
		LAB-S	619.68±0.27	469.50–769.86	3.67±0.64	8.57	0.37	-----	1.82

*Mean ± standard error of mean; ** Degree of freedom for all experiments = 19; *** Resistance ratio (RR): Resistance level High (RR > 10) Moderate (5 < RR < 10) Low (RR< 5); ****Relative toxic unit: (RTU) 1 (additive action), < 1 (antagonism), or >1 (synergism).

low RR value in case of DE administration (Table II). Our findings revealed that the field collected populations are gradually developing resistance against deltamethrin due to its inappropriate application in stored product facility (Rossi *et al*., 2010; Wakil *et al*., 2018). Feroz *et al*. (2020) reported deltamethrin resistance in 4th and 6th instar larvae of *T. granarium*. Delgram *et al*. (2020) documented tolerance against deltamethrin in *T. confusum* and *R. dominica*. Likewise, Lorini and Galley (2000) reported deltamethrin resistance in different strains of *R. dominica*. Moreover, the results indicated that low mortality was observed when DE was used alone against all the tested populations. These findings are in accordance with Machekano *et al*. (2017) who documented low efficacy of DE alone against *T. castaneum, Prostephanus truncates* and *S. zeamais*. The low efficacy of DE when administered alone may be due to various factors like RH (Stathers *et al*., 2004), source of insect pest (Vayias *et al*., 2009), temperature (Athanassiou *et al*., 2005) and properties of DE (Korunic, 2013; Nwaubani *et al*., 2014).

Synergistic effect of insecticides

Based on values of RTU, the mixture 1 (0.5DM:1DE) of deltamethrin and DE showed strongest synergistic relationship in 4th instar larvae of all populations. This mixture showed highest synergism (10.87 and 10.73 RTU values) for LAB-S and OKR population respectively. For Mixture 2 (1DM+0.5DE) a strong synergistic association (RTU 2.91, 2.55, 3.00 3.98 and 3.72) was observed in 4th instar larvae of GUW, Layyah, DGK, OKR and LAB-S populations respectively. In the mixture 3 (1DM:1DE) a weaker synergistic relationship (RTU 1.34, 1.15, 1.41, 1.71 and 1.82) was observed in GUW, Layyah, DGK, OKR and LAB-S populations respectively. The mixture 1 containing higher concentration of DE and lower concentration of DM was found to be more effective than the other two mixtures against all populations. Synergistic or antagonistic relationship is based on the type of insecticide, their mode of action, concentrations used in mixture and the class of insect pest as proposed by Khan *et al*. (2013). Our findings are in accordance to Ceruti and Lazzari (2005) who reported the control of *S. zeamais* by combination of deltamethrin and DE. Similarly, Korunic and Rozman (2010) also documented that combination of deltamethrin and DE is more effective as compared to individual insecticide. They used various combinations of deltamethrin and DE to control *S. zeamais*, *R.dominica* and *T. casteneum*. Our results are also in accordance to Machekano *et al*. (2017) who documented synergistic effect of deltamethrin and DE against *S. zeamais. T. castaneum* and *P. truncates*. Vayias *et al*. (2009) findings are in agreement to the current results. They reported higher efficacy of mixture of deltamethrin and DE against *T. confusum* as compared to treatment with individual insecticide. It was proposed that combination of deltamethrin and DE with different mode of action like toxicity induced by deltamethrin and physical disruption of epicuticle by DE caused a negative effect on insect pest (Cook *et al*., 2008). The desiccated insect pests had reduced activities like mating, feeding and oviposition due to physiological stress of DE (Arthur, 2000; Korunic, 2013, 2016) and they became prone to deltamethrin toxicity (Korunic and Rozman, 2010; Machekano *et al*., 2017). The synergistic interaction among tested mixtures in current study indicated that there is a complementary efficacy between deltamethrin and DE combinations which can be used in field as an IPM strategy in stored grain facilities to suppress the development of resistance in postharvest system. Machekano *et al*. (2017) documented deltamethrin and DE combination is effective where *T. cataneum* is the target species and DE-spinosad combination is effective where *P. truncates* is the target insect pest while both combinations are found effective against *S. zeamais*. The deltamethrin and DE combination is also effective against *T. granarium* as revealed in current studies. So, there is a dire need to test such combinations against other insect pests of stored grain commodities to achieve a proper control against stored grain insect pests. The choice of combinations by farmers and grain handlers also depends on the cost and availability of the combinations (Machekano *et al*., 2017).

Effect on energy reserves

Table III shows the effect of mixture 1 (0.5DM+1DE) at LC_{20} concentration on energy reserves of 4th instar larvae of GUW and LAB-S populations of *T. granarium*. Change in percent concentration of these metabolites in exposed group with reference to unexposed group of the similar population was shown in (Fig. 1). Among the biochemical parameters glucose, soluble proteins and FAA contents were found to be up regulated significantly in response to insecticidal treatment. Whereas the concentrations of total proteins, glycogen, trehalose and total lipids in treated groups of 4th instar larvae were down regulated with reference to the untreated groups.

The present investigation was also designed to find out the biochemical basis of effects of mixture 1 against resistant and susceptible populations of *T. granarium*. Invasion of khapra beetle is treated with various types of pesticides during storage process in godowns that effect different biomolecules having crucial role in behavioral, physiological and biochemical responses of an organism (Yazdani *et al*., 2013). The increased levels of glucose and decreased level of glycogen and trehalose in these populations after 48h contact to the most effective combination showed that larvae required more energy to deal with the stress conditions. This elevated level of glucose may propose that glycolysis process was started to deal with in the strain induced by combination of deltamethrin and DE in which glycogen and trehalose are converted into glucose that is noticeable from increased level of glucose and decrease in glycogen and trehalose level. Hafiz *et al.* (2017) also reported similar findings when treated *T. granarium* with deltamethrin. Shakoori *et al.* (2018a and b) also revealed similar exposure results with the mixture of esfenvalerate, phopshine and λ-Cyhalothrin respectively on *T. granarium.*

Table III. Effect of mixture 1 (0.5DM+1DE) administrated at LC_{20} for 48h on energy related molecules and other metabolites of 4th instar larvae of *T. granarium*.

Metabolites	Populations	Untreated group (µg/mg)**	Treated group (µg/mg)
Glucose	GUW	41.43±.20*	71.39±0.27
	LAB-S	26.32±41	42.51±0.20
Glycogen	GUW	487.01±0.40	135.39±0.08
	LAB-S	285.00±0.35	59.88±0.30
Trehalose	GUW	6787.87±0.14	2626.26±0.10
	LAB-S	5268.08±0.27	1808.08±0.51
Total proteins	GUW	104.75±0.46	45.01±0.47
	LAB-S	98.34±0.23	30.82±0.43
Soluble proteins	GUW	52.69±0.20	80.16±0.30
	LAB-S	43.34±0.41	58.55±0.50
Free amino acids	GUW	112.21±0.35	149.32±0.52
	LAB-S	78.33±0.44	123.23±0.46
Total lipids	GUW	15485.32±0.33	6481.41±0.24
	LAB-S	25971.01±0.20	9434.24±0.37

*, Mean± standard error of mean; **, n=3 (no. of replicates in each experiments).

Significantly increased level of soluble proteins and free amino acids was also observed after 48 hours exposure of the most effective mixture of deltamethrin and DE that is related to increase in production of protein due to enzyme stimulation to control the toxic results of insecticide while decrease in total protein level was considered to be related to decrease in the transaminases activity as shown by Shakoori *et al.* (1994) and Hafiz *et al.* (2017) after exposure to the Sumicidan Super, a synthetic pyrethroid to larvae of *T. castaneum* and deltamethrin to larvae of *T. granarium.* Similarly, Bizhannia *et al.* (2005) also supported these changes in protein that are degraded to amino acids to continue the metabolic process through citric acid cycle. Ali *et al.* (2011) and Hussain *et al.* (2012) also revealed the elevation in FAA contents in *T. castaneum* with exposed to abamectin.

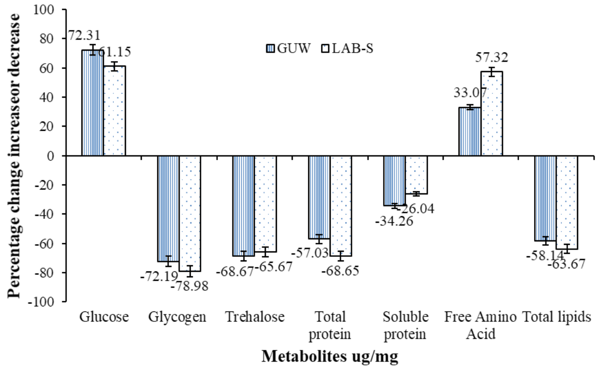

Fig. 1. Effect of mixture 1 (0.5DM+1DE) administrated at LC_{20} for 48 h on the percent increase (+) or decrease (-) in metabolites of 4th instar larvae of *T. granarium* of GUW and LAB-S population.

Lipid contents were found to be decreased in current study due to conversion of lipid to protein to provide the energy source under the condition of stress produced by the pesticide mixture. Similar effect on lipid and glycogen contents induced by various insecticides were observed by Omar *et al.* (2005), Ali *et al.* (2007), Shoba *et al.* (2011), Ali *et al.* (2011), Shaurub and Aziz (2015), Shakoori *et al.* (2016) and Hafiz *et al.* (2017). These findings are in partial agreement to Riaz *et al.* (2019) who reported similar effects in biomolecules after exposure to abamectin:emamectin and abametin:spinosad combinations in 4th and 6th instar larvae of *T. granarium.*

CONCLUSION

The emergence of deltamethrin resistance is increasing in godowns. The use of DE alone is not effective in ware houses of Pakistan but the combinations of deltamethrin and DE was found to be effective in control measures of *T. granarium*. There is also an opportunity to discover the toxic effect of these combinations with addition of some other insecticides (which are already reported in literature) as a single combination at much less concentration to possibly counter the tolerance induced against either of the components when applied alone. The use of these potential effective combination treatments could help to delay the development of insect pest resistance against individual insecticides while promoting safer food in Pakistan. Moreover, the abnormalities induced in the key biochemical components of khapra beetle by LC_{20} of binary combination showed that it is highly susceptible to the mixtures of insecticides. So, the use of this synergistic method can effectively control this insect pest in godowns.

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Statement of conflict of interest

The authors have declared no conflicts of interest.

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