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Response of Some Biochemical Components in Phosphine Susceptible and Resistant Populations of 4th Instar Larvae of Trogoderma Granarium (Coleoptera: Dermestidae)

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Abstract

Stored grain pests are controlled by a number different pesticides and fumigants. This study, investigated the effect of phosphine on khapra beetle *(Trogoderma granarium)* which is a notorious pest in stored grain godowns in Pakistan and a significant trade pest around the world. For this purpose, the LC50 of phosphine against 4th instar larvae of two different strains of *T. granarium* (collected from different cities of Punjab, Khaniwal (Khw) and Chishtian (Chi) were determined. The LC50 values shown by these strains were 3.8 and 7.0 ppm respectively. On the basis of LC50 the Chishtian strain was considered as resistant to phosphine, whereas Khaniwal strain was regarded as a susceptible strain. The effect of sub lethal doses (LC10, LC20, and LC30) on the larval stages of two *T. granarium* strains were evaluated. The toxic effect of phosphine was observed on glucose, glycogen, total lipid, FAA, protein and trehalose of the strains after 24 hours of exposure. The treatment showed significant increase in glucose content in Khaniwal (susceptible) and decrease in resistant strains throughout the treatment. Lipid content showed a highly significant increase for all doses of phosphine in both strains. Glycogen, Trehalose, protein and FAA contents depicted highly significantly increases in the resistant strain at LC_{10}, LC_{20}, and LC_{30}.

Keywords: khapra beetle; phosphine resistance; fumigant.

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1. Introduction

Pakistan is encountering significant problems with insect pest development due to agro-ecological conditions of country [1]. A socioeconomic survey in Pakistan in 2016-17 confirmed that insect infestation was the most significant cause of loss of stored grains during storage. Stored grain pests cause significant losses in quality and quantity of stored products and pose a major problem for the agriculture sector and food industry by deteriorating the stored product [2]. One of the pests causing tremendous loss to stored wheat in Indo-Pak is the khapra beetle [3,4,5,6]. In tropical countries where conditions are hot, humid and storage facilities are improper and inadequate, it is estimated that losses may reach up to 50% as compared to 5-10% of world's grain losses. Initially control was conducted by insecticides, but excessive use if these chemicals generated resistant populations; then some fumigants gave control at high dosages and penetrated into all cracks and crevices re-establishing control. However, owing to misuse of fumigants on a daily basis high tolerance of phosphine soon developed among larvae of khapra beetle, especially those concealed in crevices for 2-3 months before fumigation at 20°C [7,8,9]. Khapra beetle in Sind and Punjab areas of Pakistan showed high levels of resistance against different insecticides and fumigants [10,11,12, 13]. The objective of the present study is to evaluate resistance against insecticide with different biochemical parameters levels and to assess the effect of phosphine on khapra (*Trogoderma granarium* Everts). It is expected that this work will help to better understand the chemical control mechanisms of stored grain pests.

2. Materials and Methods

Fresh cultures were collected from wheat go downs of Chishtian (Chi) and Khaniwal (Khw) city located in Punjab Province of Pakistan. As a result of poor management, Lahore farm houses had never been properly fumigated with phosphine resulting in the deterioration of the stored products by the pests. Another sample was collected from Khaniwal godowns where phosphine was seldom used. Wheat samples containing *T. granarium* were collected in sterilized plastic bags and brought to laboratory for study.

2.1 Maintenance of culture

The master cultures of *T. granarium* (two populations) were maintained in a temperature and humidity controlled room at 35±1°C and 65±5%RH [14,15]. A pure homogeneous stock of each population was developed in the culture room of Biochemistry and Toxicology Laboratory of the Zoology Department, University of Punjab, Pakistan. Crushed wheat was used as a supporting medium. Wheat was initially fumigated with phosphine to kill the insects if any present. Following fumigation, wheat was spread in fresh air for 4-5 h. The wheat was placed in an oven overnight at 60°C, and then shifted into sterilized jars for culture rearing. The 300ml glass jam jars were filled $1/4^{th}$ with wheat and 50 adult beetles of *T. granarium* were added inside it. The jars were covered with muslin cloth to prevent escape of beetles and entry other small organisms. Adult beetles were left in the culture medium for 5-6 days to ensure egg laying. By using a separating sieve and camel hair brush dead beetles were discarded and flour containing eggs was separated. The eggs developed into adult beetles via larval and pupal stages. These adult beetles were again transferred to jars for continuity of the culture and a homogeneous stock was maintained. For this study, homogeneous stock of 4^{th} instar larvae from

each population were obtained after 42±1 days and LC_{50} and other toxicological data recorded.

2.2 Toxicant used

Generic name of this chemical is phosphine while hydrogen phosphide and phosphorus trihydride are the common names of phosphine gas. The EPA chemical code of this insecticide is 066500. It belongs to Inorganic Phosphine Family. Empirical Formula: PH3 (CAS #: 7803-51-2). For farm use, pellets of aluminium phosphide, calcium phosphide, or zinc phosphide release Phosphine upon contact with atmospheric water. These pellets also contain agents to reduce the potential for ignition or explosion of the released phosphine. Phosphine is the only widely used, cost-effective, rapidly acting fumigant that does not leave significant residues on the stored product.

2.3 Procedure adopted

For determination of LC_{50} against *T. granarium*, phosphine was generated from aluminium phosphide in the laboratory. Commercially available aluminium phosphide (AlP) pellets containing (approximately0.2g) are recommended as the most suitable source of phosphine (PH_3).Phosphine was generated in the laboratory according to the technique given in FAO Plant Protection Bulletin (1975). All procedure for phosphine generation was carried out in a fume hood.

2.4 Administration of phosphine

Glass vacuum desiccators were used for phosphine administration to insects. The volume of desiccators was measured to evaluate the dose volume of phosphine. The lid of the desiccators was covered with rubber sheet. A thin layer of grease was applied on the edge of the lid to a air tight. Saturated solution of sodium nitrite in a Petri dish was placed inside the desiccator to maintain the RH at 65±5%. A ceramic plate with holes was placed over the bottom narrow compartment of desiccators onto which the insect vials with holed lids were placed. Three glass vials (5gm), containing 20 healthy larvae of 4^{th} instar of *T. granarium* in each, were placed in the desiccators. Gas was injected into desiccators with Hamilton microsyringe through a rubber septum fitted on the desiccator lid. The PYREX desiccators were kept in the lab at 30±1 °C and 65±5 % R.H. for 24 hrs after which observations on mortality were made.

2.5 Mortality assessment

After 24hours, the desiccators were opened and insect vials taken out. The 4^{th} instar larvae were transferred to separate crushed wheat medium and maintained at 30±1°C and 60±5% RH for 24 hours after which mortality was assessed. According to Lloyd (1969). The %mortality was corrected by Abbot's formula (Abbot, 1925). Data were analyzed by the method outlined by Busvine (1971) and described by Finny (1971). Each treatment was repeated four times. Then the mortality data was subjected to logit analysis using POLO-PC (LeOra Software, 1987) to estimate different lethal concentrations up to LC_{90} and confidence limit and regression lines (in ppm Phosphine) for 4^{th} instar larvae of *T. granarium*. Mortality at different concentrations, used to estimate the concentration-mortality curves.

3. Results

3.1 Lethal Concentration (LC_{50})

The calculated LC_{50} Values of Khw 3.8 ppm and Chi 7.0 ppm clearly indicated that Chi is a resistant population while Khw is a susceptible population.

Table 4

Populations	Developmental Stages	LC50 (ppm)	Regression Equation
Chi	4th instar larvae	7.0	y = 9.165x - 17.01 $R^2 = 0.966$
Khw	4th instar larvae	3.8	y = 10.5x + 1.66 $R^2 = 0.974$

3.2 Biochemical Estimation

The effect of Phosphine resistance on glucose, glycogen, lipid, Trehalose, protein and free amino acid contents of 4th instar larvae of *T. granarium* and these effects was determined after 24 at LC_{10}, LC_{20}, and LC_{30}. (Table I-III). Biochemical Analysis

Table 1: Percent increase or decrease (-) in biochemical components

Biochemical components	Population	Phosphine treatment (ppm)					
		10	20	30	10 vs. 20	10 vs. 30	20 vs. 30
Glucose	Chi*	-15	-24	-35	-10	-24	-15
	Khw**	13	43	65	27	47	15
Glycogen	Chi*	33	40	28	5	-3	-8
	Khw**	-22	-28	-33	-7	-14	-8
Lipids	Chi*	29	59	33	23	3	-16
	Khw**	20	53	57	28	31	2
Trehalose	Chi*	5	7	11	2	6	4
	Khw**	-13	-13	-8	0	6	5
Protein	Chi*	2	14	26	11	23	11
	Khw**	-12	-24	-4	-14	8	26
FAA	Chi*	12	42	69	27	51	19
	Khw**	-5	-24	-15	-20	-10	13

* = Resistant populations

** = Susceptible population

Table 2: Effect of phosphine on some biochemical components of 4th instar larvae of Chi population of T. *granarium*

Parameters	Control (n=4)	Phosphine treatment		
		10 ppm (n=4)	20 ppm (n=4)	30 ppm (n=4)
Glucose (mg/g)	*23.94 ±0.82[a]	20.31 ±0.67[b]	18.31 ±0.82[c]	15.49 ±0.82[d]
Glycogen (mg/g)	4.72 ±0.05[d]	6.26 ±0.06[b]	6.6 ±0.05[a]	6.04 ±0.066[c]
Lipids (mg/g)	48.69 ±1.42[c]	62.96 ±1.56[b]	77.34 ±1.66[a]	64.79 ±1.20[b]
Trehalose (µg/mg)	1.54 ±0.018[b]	1.62 ±0.018[a]	1.650 ±0.02[a]	1.718 ±0.024[c]
Protein (µg/mg)	15.85 ±1.08[b]	16.23 ±0.40[a]	18.02 ±0.46[c]	20.01 ±0.24[d]
FAA (µg/mg)	23.12 ±1.00[a]	26.00 ±0.400[b]	33.00 ±0.30[a]	39.230 ±0.40[c]

* Mean ± SEM

For abbreviations see Table. 1.1

The values in a row having no common superscript (ab) are significantly different at 0.05 significance level according to DRMD

Table 3: Effect of phosphine on some biochemical components of 4th instar larvae of Khw population of *T. granarium*.

Parameters	Control (n=4)	Phosphine treatment		
		10 ppm (n=4)	20 ppm (n=4)	30 ppm (n=4)
Glucose (mg/g)	19.54 ±0.54	22.03 ±0.53	28.03 ±0.51	32.27 ±0.72
Glycogen (mg/g)	3.71±0.03	2.89 ±0.03	2.68 ±0.06	2.47 ±0.05
Lipids (mg/g)	50.72 ±1.01	60.79 ±0.80	77.73 ±0.95	79.39 ±1.09
Trehalose (µg/mg)	1.63 ±0.03	1.41 ±0.03	1.41 ±0.02	1.490 ±0.025
Protein (µg/mg)	11.19 ±0.301	9.89 ±0.302	8.51 ±0.301	10.69 ±0.301
FAA (µg/mg)	18.13 ±0.500	17.11 ±0.50	13.35 ±1.00	15.00 ±0.50

* Mean ± SEM

For abbreviations see Table. 1.1

The values in a row having no common superscript (ab) are significantly different at 0.05 significance level according to DRMD

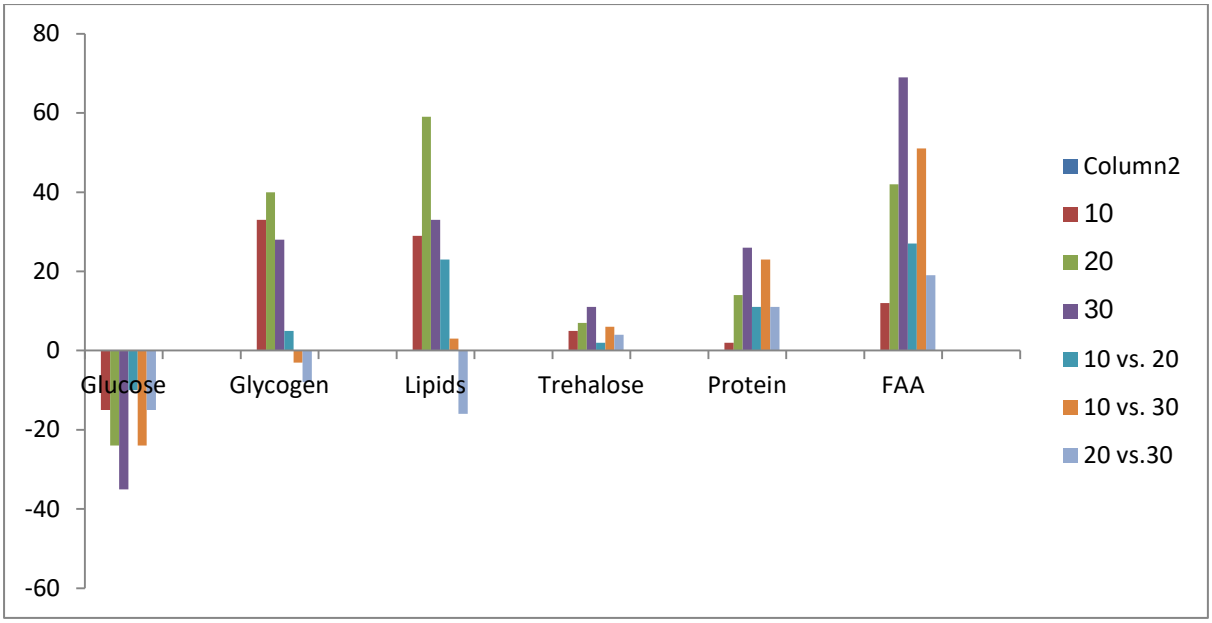

Figure 1: Response of biochemical components in phosphine resistant populations of *Trogoderma granarium*

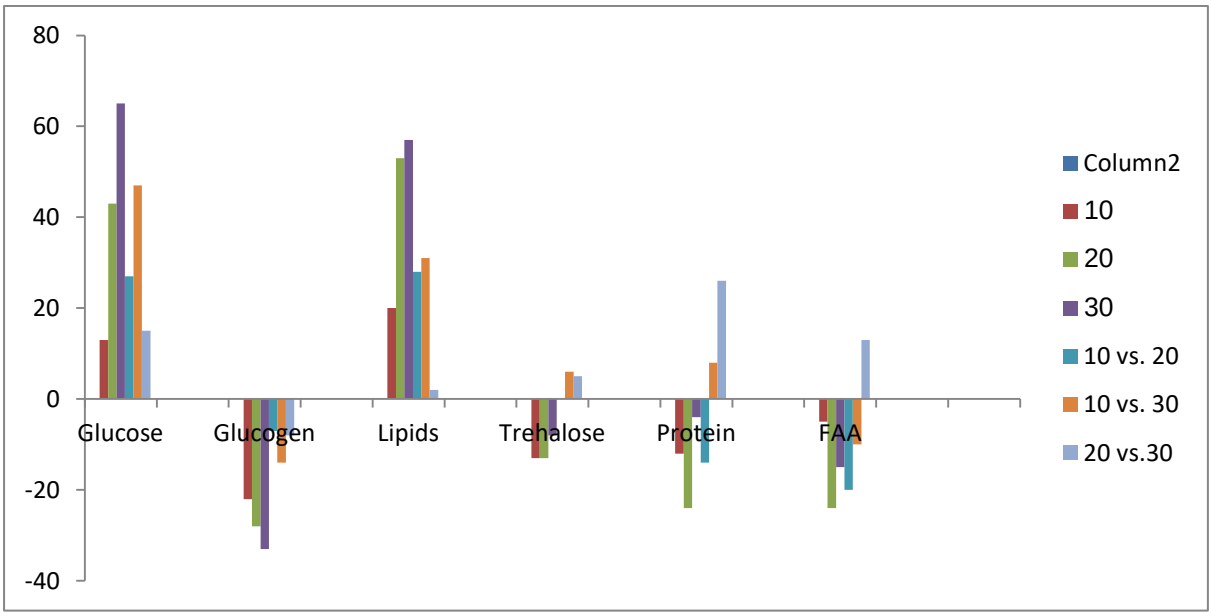

Figure 2: Response of biochemical components in phosphine susceptible populations of *Trogoderma granarium*

After 24 hours, control glucose contents of the susceptible Khw strain of *T. granarium* at LC_{10} was 22.03 ± 0.53, whereas the resistant Chi strain showed 20.31 ± 0.07 respectively. After phosphine treatment the susceptible Khw strain showed significant increase of glucose, whereas the resistant Chi strain depicted significant decrease. The average lipid content of Chi and Khw strains were 62.96 ± 1.56, and 60.79 ± 0.80g/l, respectively. In both the strains moderate but significant increase was observed at LC_{10} after 24 hours treatment of fumigation. The average FAA contents of both Chi and Khw strains were 26.0± 0.4 and 17.11± 0.50, respectively. After treatment significant increase was observed in Chi strain, whereas Khw strain showed non-

significant decrease at LC_{10}. The average control protein contents were 16.26±0.40 and 9.89± 0.302, respectively in Chi and Khw strain, after Khw strains depicted highly significant decrease (Table-III). The average glycogen content of Chi and Khw strains were 6.26 ± 0.06 and 2.80 ±0.03, respectively. Khw strain showed highly significant decrease after treatment of phosphine at LC_{10} (Table-III). The average trehalose content of Chi and Khw strains were 1.62 ± and 1.41 ±0.03, respectively (Table- II-III). Khw strain showed highly significant decrease after treatment of phosphine (Table-III). Table II-III showed biochemical analysis of Chi and Khw strains of *T. granarium* after 24 hours, Parameter glucose, glycogen, Lipid, trehalose, protein and FAA at LC_{10}, LC_{20} and $LC_{30.}$

4. Discussion

The development of resistance to chemical insecticides in arthropod pests constitutes a worldwide economic problem [16,17,18]. The khapra beetle, *T. granarium*, is one of the most important pests of household, commercial food processing establishments and flour mills in Pakistan and many countries around the world. It is also a significant pest of trade for many grain exporting countries. This study revealed that glucose contents increased throughout experimental study in the Phosphine susceptible Khw strain (Table-I) after 24 hours and glycogen contents decreases against all doses of *T. granarium* same results were reported that 48 hours treatment of Talcord when compared with their respective control. Result showed glycogen content was utilized drastically whereas glucose content showed elevation possibly due to inter conversion of polysaccharides to monosaccharide [19]. Ripcord treatment also increased glucose, fructose, total lipids and cholesterol contents, while glycogen content was decreased tremendously, when *Tribolium castaneum* treated larvae were compared with their respective controls [20]. At all doses $LC_{10,}$ LC_{20} and LC_{30} (Table-I) lipid and soluble protein contents were increased in Chi strain, so elevation of lipid and soluble protein contents could be attributed to their possible conversion under phosphine stress conditions in resistant strain. Raised level of protein may be related increased activities of various enzymatic activities [19,20]. Various insecticide induced biochemical abnormalities have also been reported in susceptible and resistant strain of *T. castaneum* [21,22]. Changes in metabolism and adverse effects on the behaviour and reproductive performance in insects also reported [23]. In this study *T. granarium* larvae showed significant increase in total protein contents throughout experiments in resistant strain. Similar results also depicted that the increase in glycogen and total lipid in T. *castaneum* provide primary source of energy after 4 days treatment of Ripcord while total protein provided secondary source of energy as it showed increase in the first two days of treatment [20]. Figure 1 showed five (glycogen, lipids, trehalose, protein and FAA) macromolecular contents in this study increase throughout the experiment with the treatment of phosphine at all sublethal doses but prolonged use of this fumigant might be develop molecular abnormalities which could be sufficient to play an important role in the pest control programme.

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