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Effect of Phosphine on Esterases of Larvae and Adult Beetles of Phosphine-Exposed Populations of Stored Grain Pest, *Trogoderma granarium* Collected from Different Godowns of Punjab

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Authors' Contributions
SSA, FRS and TR designed the research project, analysed the data and wrote the article. TR conducted the experimental work. FRS supervised the work.

Key words
***Trogoderma granarium*, Phosphine, Esterases, Insecticide tolerance, Fumigation.**

ABSTRACT

The present study was aimed at determining the possible role of esterases in development of tolerance/ resistance in phosphine-tolerant populations. The level of total esterases, carboxyl esterases, choline esterases, acetylcholine esterases and aryl esterases were determined in 4th & 6th instar larvae and adult beetles of phosphine-tolerant populations (previously exposed to phosphine for 15 years) of wheat grain pest, *Trogoderma granarium* collected from various storage facilities of Punjab, Pakistan *viz*., Mandi Bahauddin-I, Mandi Bahauddin-II, Gujrat, Gujranwala and Sargodha. The activities of all esterases tested were significantly increased in all field collected phosphine-tolerant populations when compared with phosphine-susceptible population. Among developmental stages, the 4th instar larvae possessed higher esterase activities than 6th instar larvae and adult beetles in all populations of *T. granarium*. The increased level of esterases in phosphine tolerant populations as compared to susceptible population has pointed some correlation between esterase activities and phosphine tolerance.

INTRODUCTION

Khapra beetle, *Trogoderma granarium* is one of the most notorious pests of stored grains in many tropical and subtropical regions of the world (Ahmedani *et al*., 2011). To overcome the problem of pest development, control measures of different nature are being adapted at farm, market and public sector storages (Haq *et al*., 2005), that consist of the use of native or natural method of control by plant material (Prakash and Rao, 2006; Kestenholz *et al*., 2007; Neoliya *et al*., 2007; Gandhi *et al*., 2010) and also by use of contact insecticides and fumigants. Insecticides and fumigants are used to restrict insect damage. Due to impaired ability of pesticides to control these stored grain pests, the fumigation technique with methyl bromide and phosphine as fumigant is being widely used in godowns (Walter, 2006). Fumigation plays a major role in insect pest elimination in stored products. The use of phosphine is preferred now a-days due to its minimum residual effects (Atkinson *et al*., 2004; Walter, 2006; Wang *et al*., 2006; Pimentel *et al*., 2007).

Indiscriminate and unplanned use of pesticides and fumigants has resulted in wide spread resistance in pests against these chemical (Zettler and Arthur, 2000; Bughio and Wilkins, 2004; Assie *et al*., 2007; Daglish, 2008). The development of resistance has become a global issue and control of insect pest is becoming difficult due to this phenomenon (Collins *et al*., 2005; Pimentel *et al*., 2009).

The increased tolerance and development of resistance against sub lethal doses of insecticides may be due to increased levels of several insecticide degrading enzymes and other metabolites. The up-regulation is usually attributed to a genetic change in the insecticides degrading enzymes. Insecticide/fumigant exposure has been reported to induce esterase activity in insects and these esterase activities have been found very high in resistant species, which are responsible for hydrolysing or inactivation of various ester linkages of insecticides. They are able to cleave esters, tri-ester phosphates, halides, amides and thio-esters. No efforts have been made to study the level of resistance in *T. granarium* against phosphine in Pakistan for effective management of the problem. Although in literature, the mode of action of phosphine has been mentioned as uncoupler of oxidative phosphorylation

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in electron transport chain but this study was specifically planned to evaluate the direct/indirect effect of phosphine on various esterase levels to correlate its activities with development of tolerance in *T. granarium*.

MATERIALS AND METHODS

Rearing and maintenance of insect culture

Six populations of stored grain pest, *Trogoderma granarium* (Everts) with common name khapra beetle were used in this study. Master cultures of five populations of khapra beetle tolerant to phosphine (previously exposed to phosphine for 15 years) were collected from different godowns *viz*., Mandi Bahauddin-I (MBDIN-I), Mandi Bahauddin-II (MBDIN-II), Gujrat, Gujranwala and Sargodha of Punjab province. These godowns have more than 15 years history of phosphine fumigation to wheat. The wheat samples containing *T. granarium* (Everts) were collected in sterilized plastic bags and brought to laboratory for study. A phosphine-susceptible (population never exposed to phosphine previously) were taken from 10 years old culture maintained in the culture room of department of Zoology, University of the Punjab, Lahore. The master cultures of *T. granarium* (six populations) were maintained in temperature and humidity controlled room at 30±1°C and 65±5% RH. A pure homogeneous stock of each population was developed in the culture room according to Riaz *et al*. (2014) and Shakoori *et al*. (2016). From homogeneous stock of each population 4th, 6th instar larvae and adult beetles were used for further experiments.

Generation and administration of phosphine

To evaluate the LC_{50} value of phosphine for 4th and 6th instar larvae and adult beetles (24 h old), gaseous phosphine was generated from aluminium phosphide in the laboratory and different doses were calculated according to the technique given in FAO plant protection bulletin (1975). Commercially available aluminium phosphide (AlP) pellets containing (approximately 0.2g) are recommended as the most suitable source of phosphine. The LC_{50} value of each population was recorded according to procedure given by Riaz *et al*. (2016).

Biochemical analysis for esterase activities

Biochemical analysis of various esterase activities including total esterases (TE), carboxylesterase (CE), cholniesterase (ChE), acetylcholineesterase (AChE) and aryleesterase (AE) were performed. Absorbance of some enzyme activities like TE, CE and ChE were converted into activity/quantity from their standard curve.

Twenty adults and twenty larvae of 4th and 6th instar larvae of khapra beetle from each population were taken in three replicates. They were weighed and homogenized in their respective extraction buffer with the help of motor driven Teflon glass homogenizer with constant cooling in crushed ice. Total esterase and carboxyl esterase activities were estimated according to Devonshire (1975b). Acetylcholine esterase activity was estimated according to Devonshire (1975a). Cholinesterase activity was determined according to Rappaport *et al*. (1959). Estimation of aryle esterase activity was done according to Junge and Klees (1981).

Analysis of variance (ANOVA) followed by tukey's Post Hoc test was applied to all the data of biochemical parameters to compare pair wise means of various populations to determine the significant difference at $P<0.05$ using SPSS software.

RESULTS

Various esterases (TE, CE, ChE, AChE and AE) activity in three different developmental stages (4th and 6th instar larvae and adult beetles) of phosphine-susceptible population and five phosphine-tolerant populations (Gujranwala, MBDIN-I, Gujrat, MBDIN-II and Sargodha) of *T. granarium* are shown in Table I. Percent increase in TE, CE, ChE, AChE and AE activity of tolerant populations in comparison with susceptible is shown in Figure 1. In phosphine-tolerant populations, TE, CE, ChE, AChE and AE activity was raised significantly when compared with phosphine-susceptible population at $P<0.05$. Among tolerant populations, TE, CE and AE activity was significantly different from each other while ChE and AChE activity was not significantly different in various populations at $P<0.05$. In 4th instar larvae, the ChE activity was not significantly different in (Sargodha and MBDIN-I population) and (MBDIN-II and Gujrat population), likewise in 6th instar larvae the ChE activity in Gujrat population was not significantly different from MBDIN-II and Sargodha populations at $P<0.05$. In adult beetles MBDIN-I was not significantly different from Gujrat and Sargodha populations in ChE activity at $P<0.05$. In 4th instar larvae the AChE activity was not significantly different in Sargodha and MBDIN-I populations, likewise in 6th instar larvae, AChE activity was not significantly different in Sargodha and Gujranwal populations at $P<0.05$. In adult beetles non-significant difference was observed in AChE activity of (MBDIN-II and Gujrat populations) and (Sargodha, Gujrat and Gujranwala populations) at $P<0.05$. In phosphine-tolerant populations, AE activity was raised significantly when compared with susceptible population at $P<0.05$. The AE activity was not significantly different in adult beetles of MBDIN-II and MBDIN-I population at $P<0.05$.

Table I.- Activities of various esterases (IU/mg body weight) of 4th instar larvae, 6th instar larvae and adult beetles of susceptible and five tolerant populations of *T. granarium*.

Populations	TE	CE	ChE	AChE	AE
4th instar larvae					
Susceptible	*116.86 ± 0.149[a]	*23.31 ± 0.201[a]	*24.31 ± 0.658[abc]	*36.32 ± 0.701[ab]	*52.71 ± 0.277[a]
MBDIN-I	133.87 ± 0.249[a]	45.49 ± 0.244[a]	30.98 ± 0.829[ac]	53.94 ± 0.702[a]	98.78 ± 0.415[a]
MBDIN-II	131.34 ± 0.503[a]	42.95 ± 0.127[a]	49.24 ± 0.742[ab]	59.26 ± 0.671[ab]	102.37 ± 0.694[a]
Gujrat	136.34 ± 0.213[a]	39.41 ± 0.129[a]	46.67 ± 0.758[ab]	66.32 ± 0.706[ab]	90.83 ± 0.658[a]
Gujranwala	138.98 ± 0.329[a]	37.54 ± 0.216[a]	53.1 ± 0.695[abc]	71.68 ± 0.721[ab]	114.98 ± 0.741[a]
Sargodha	122.66 ± 0.229[a]	29.72 ± 0.205[a]	31.45 ± 1.256[ac]	56.28 ± 0.538[a]	61.79 ± 0.669[a]
6th instar larvae					
Susceptible	108.36 ± 0.224[a]	23.43 ± 0.245[a]	21.95 ± 0.843[abc]	35.11 ± 0.70[ab]	49.21 ± 0.322[a]
MBDIN-I	130.81 ± 0.205[a]	42.43 ± 0.222[a]	26.97 ± 0.727[abc]	50.92 ± 0.871[ab]	95.63 ± 0.688[a]
MBDIN-II	129.33 ± 0.110[a]	37.29 ± 0.311[a]	46.32 ± 0.979[ac]	56.43 ± 0.666[ab]	100.12 ± 0.671[a]
Gujrat	132.48 ± 0.219[a]	45.42 ± 0.163[a]	47.19 ± 0.657[a]	61.05 ± 0.876[ab]	104.01 ± 0.642[a]
Gujranwala	137.62 ± 0.117[a]	48.21 ± 0.159[a]	50.45 ± 0.948[ab]	69.11 ± 0.717[a]	112.81 ± 0.768[a]
Sargodha	126.31 ± 0.119[a]	33.98 ± 0.191[a]	36.15 ± 0.663[abc]	68.11 ± 0.498[a]	90.49 ± 0.628[a]
Adult beetles					
Susceptible	87.14 ± 0.879[a]	19.89 ± 0.161[a]	17.56 ± 0.756[ade]	33.27 ± 0.721[ade]	47.38 ± 0.255[cd]
MBDIN-I	125.31 ± 0.160[a]	41.84 ± 0.356[a]	22.76 ± 0.591[ae]	47.48 ± 0.81[ade]	93.95 ± 0.743[c]
MBDIN-II	122.56 ± 0.354[a]	35.83 ± 0.126[a]	33.98 ± 0.603[ade]	54.19 ± 0.683[ae]	96.06 ± 0.732[c]
Gujrat	126.62 ± 0.266[a]	44.69 ± 0.193[a]	39.21 ± 0.786[ad]	57.41 ± 0.598[a]	101.14 ± 0.650[cd]
Gujranwala	129.67 ± 0.196[a]	45.94 ± 0.164[a]	41.10 ± 0.482[ad]	63.18 ± 0.801[ade]	106.90 ±0.731[cd]
Sargodha	119.93 ± 0.171[a]	30.09 ± 0.137[a]	21.87 ± 0.658[ae]	60.05 ± 0.655[ad]	86.39 ± 0.714[cd]

TE, total esterases; CE, carboxyl esterase; ChE, cholin esterase; AChE, acetylcholin esterase; AE, aryle esterase.

DISCUSSION

Khapra beetle, *T. granarium* is a serious pest of grains and stored products all over the world especially in tropics and subtropics including Pakistan. In current investigation, populations of Khapra beetle were collected from godowns where insects are consecutively exposed to sub-lethal doses of phosphine because storage facilities are insufficiently gas tight. So, as a result of repeated exposure to low doses of phosphine, these insects develop tolerance against phosphine. It was investigated that continuous exposure of phosphine to *T. granarium* in the stored grain facilities resulted in elevated level of TE, CE, ChE, AChE and AE activities in all phosphine-tolerant populations. Sher *et al*. (2004) reported that in 4th instar larvae of *T. granarium,* the activity of TE was increased in Haroonabad (107HR) population after 10 h exposure of phosphine concentration. CE are enzymes that catalyze the hydrolysis of carboxyl esters with addition of water and they belong to α/β- hydrolase family (Junge *et al*., 1975; Ollis *et al*., 1992; Cygler *et al*., 1993; Oakeshott *et al*., 1999; Satoh and Hosokawa, 2006; Hosokawa *et al*., 2007). The development of resistance to agrochemicals, pesticides and fumigants involves high level of CE reported by Newcomb *et al*. (1997), Byrne *et al*. (2000), Oakeshott *et al*. (2005) and Cui *et al*. (2007). Tang *et al*. (1988) reported 23 resistant species of pests in China and it was evaluated that resistance was caused by increased level of CE activity which play major role in development of resistance than MFOs.

ChE and AChE are enzymes that belong to a/b family of hydrolyzing enzymes to terminate nerve impulses by breaking the neurotransmitter at cholinergic synapses (Ollis *et al*., 1992). Sher *et al*. (2004) reported that in 4th instar larvae of *T. granarium* the activity of ChE was increased as a result of exposure to phosphine. In literature, there is couple of studies who have reported the inhibition of AChE activity after exposure to phosphine. Sher *et al*. (2004) reported the inhibition of AChE activity in 4th instar larvae of *T. granarium* after exposure to phosphine. These all reports on inhibition of AChE activity were documented in various test organisms after a single exposure to phosphine but in godown as discussed earlier beetles are exposed to under dosage of phosphine. So, with increased AChE activity, the normal breakdown process of acetylcholine into choline will occur normally at the time of need and different systems of insect may co-ordinate efficiently, providing it protection and tolerance against fumigant.

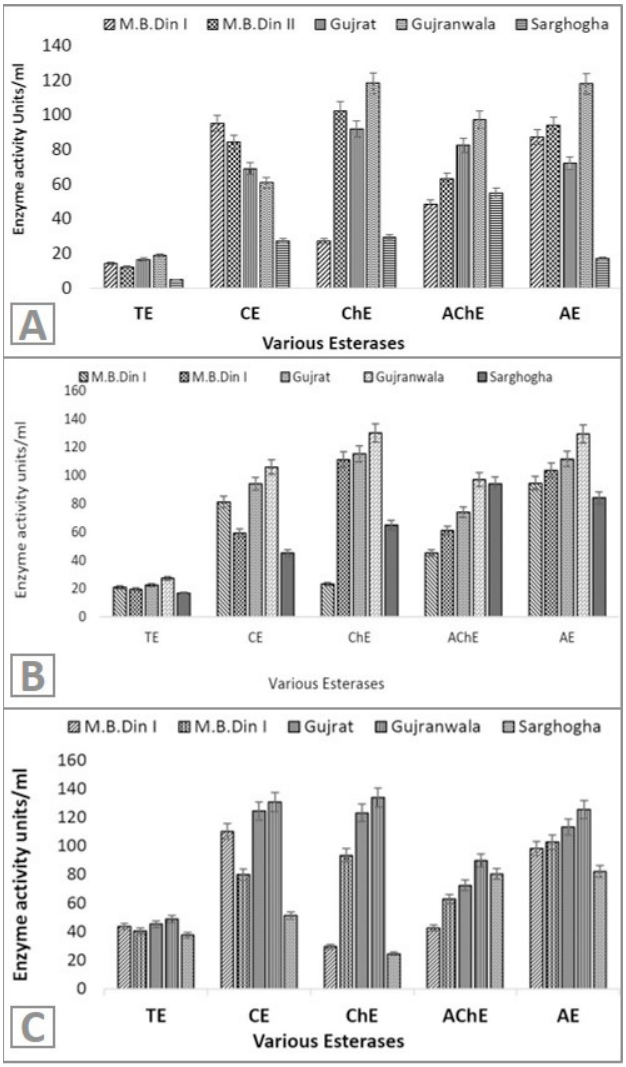

Fig. 1. Increase (%) in activities of various esterases of 4th instar larvae (A), 6th instar larvae (B) and adult beetles (C) of tolerant populations of *T. granarium* with reference to susceptible population.

The involvement of AChE in insecticide resistance is primarily related to the presence of altered AChE binding site in resistant insects which makes the enzyme insensitive to inhibition by the insecticides. Modified AChE (MACE), with alterations in the primary structure of the enzyme, results in a reduced sensitivity of AChE to OPs and carbamates and provides to the insect some levels of resistance (Fournier and Mutero, 1994). Charpentier and Founeir (2001) reported that the amount of AChE is positively correlated with resistance to OP insecticides. Alon *et al*. (2008) and Cao *et al*. (2008) reported that increased expression of esterases results from increased transcription levels, due to upregulation of the corresponding gene.

Zhu and He (2000) reported that elevated level of AE in *Schizaphis graminum* and Sher *et al*. (2004) in *T. granarium* reported that AE was responsible for development of resistance against organophosphates/ fumigants.

Although, the known literature does not indicate any increase in esterase activity with exposure to phosphine as it is well known that phosphine is a gas without any ester bond so, it is assumed that rise in esterase level is not possible with this toxicant. But during this study, the experiments were formed five times to make assure the change in enzyme activity and every time an increase in esterase activity has been observed. This increased esterase level is indicator of some indirect effects of phosphine on esterase activities. In literature several studies have shown that phosphine undergo metabolism in living system with the formation of organophosphine compounds, phosphates, hypophosphite and phosphite derivatives by replacing hydrogen atom (R_3P) with other organic molecules like alcoholic group of proteins, amino acids and sugars with the formation of esters. In living system there is possibility that rise in these organic phosphate esters might be responsible for rise in various esterase activities.

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Conflict of interest statement

The authors have declared no conflict of interest.

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