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Effect of Sub-lethal Doses of Phosphine on Macromolecular Concentrations and Metabolites of Adult Beetles of Stored Grain Pest, *Trogoderma granarium*, Previously Exposed to Phosphine

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ABSTRACT

Effect of different concentrations of phosphine for various exposure periods (24-120h) has been studied on macromolecular concentrations and metabolites of adult beetles of a stored grain pest *Trogoderma granarium* (Everts) previously exposed to phosphine (= resistant) and those not previously exposed (= susceptible) strain. The LC_{50} values for adult beetles of phosphine-susceptible and resistant populations were 4.5 and 4.87 ppm, respectively. Sub lethal dose of phosphine (LC_{20}) significantly decreased the glycogen, lipids, DNA and RNA contents of all adult beetles, while free amino acids and glucose contents were increased in resistant population throughout the exposure period. Soluble proteins in resistant population, free amino acids and glucose contents in susceptible population was first increased after exposure to sub lethal dose of phosphine but then started to decrease after 72 h exposure period. Soluble protein contents of susceptible population was first raised after exposure to sub lethal dose but then started to decrease after 48 h exposure. Resistant population showed a significant increase in soluble proteins, amino acids and glucose contents from 24-96 h with reference to susceptible population while resistant population, showed a significant decrease in glycogen, lipids, DNA and RNA contents with reference to susceptible population. These metabolic derangements induced by phosphine over various exposure periods provide a raw biochemical data to adopt better control strategy by regulating exposure period for this stored grain pest.

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FRS designed and supervised the study. FRS, AF and TR executed the experimental work and wrote the article.

Key words
***Trogoderma granarium*, stored grain pest, phosphine, metabolites**

INTRODUCTION

Stored cereal grains are infested by a number of stored grain pests but Khapra beetle, *Trogoderma granarium* (Everts) is the most notorious pest of wheat causes a huge loss (Khare *et al*., 1974; Arbogast, 2004; Ahmedani *et al.*, 2009). Control measures of different nature *i.e*., control by plant extracts (Dwivedi and Garg, 2003; Kestenholz *et al*., 2007), chemical control by pesticides, fumigation with methyl bromide and phosphine, (Atkinson *et al*., 2004; Wang *et al*., 2006) are being adapted. Phosphine was discovered in 17th century and has been used as important fumigant to control the stored grain pests all over the world since 1930. It is a highly effective fumigant for disinfestations of bulk grain without affecting the viability of grains. It is very toxic to aerobically respiring organisms but non toxic to anaerobic and metabolically dormant organisms (Fluck, 1973; Berners and Sadler, 1988; Chaudhry, 1997). Mechanism of phosphine toxicity is not well understood but biochemical and physiological changes which occur as result of phosphine exposure can be classified as neural, metabolic and redox related response (Nath *et al*., 2011).

The unplanned use of phosphine leads to development of resistance in *T. granarium* (Benhalima *et al*., 2004). Since then the situation has worsened with resistance being detected by different workers around the world with increasing frequency (Srivastava, 1980; Conway, 1981; Mills, 1983, 1986; Taylor, 1986; Zettler, 1991). Being a fumigant, the efficacy of phosphine depends on time and concentration. Generally, higher concentrations of fumigant are required for less exposure periods to achieve proper control of the pest, and the relationship between time and concentration can be linear on a log–log scale between certain concentrations. The equation $C^n=t$ has described this relationship (Winks and Waterford, 1986; Bell, 1992; Ho and Winks, 1995; Daglish *et al.*, 2002; Ahmedani, 2009).

Energy consumption could be measured using the electron transport activity (at the mitochondrial level), whereas reserve energy for metabolism could be measured by measuring total lipids, protein and sugar contents by spectrophotometric analysis. The differences between energy consumption and the energy reserves suggest the energy available for growth and biomarker of fitness cost in resistant populations (Guedes *et al*., 2006;

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Araujo *et al*., 2008a,b; Lopes *et al*., 2010). The aim of the present study was to evaluate the effect of sub-lethal concentration (LC_{20}) on various metabolites over wide range of exposure periods. There is no report on the effect of sub lethal concentration of phosphine (LC_{20}) on the activities and level of metabolites over wide range of exposure periods.

MATERIALS AND METHODS

Rearing and maintenance of beetles

In this study a phosphine-susceptible (a population never exposed to phosphine previously) and phosphine-resistant populations (a population previously exposed to phosphine for at least 15 generations) of *T. granarium* were used. Master culture of resistant population was collected from PASCO godowns of Gujranwala which have more than fifteen years history of phosphine fumigation while susceptible population was obtained from the Department of Zoology, University of the Punjab, Lahore and this culture was never exposed with any type of insecticide or fumigant since thirteen years. The culture of *T. granarium* was reared in the culture room of Department of Zoology, University of the Punjab Lahore. The culture was maintained at 35±2°C and 60±5% relative humidity (Riaz *et al*., 2014). The beetles were fed on sterilized broken wheat and wheat flour in sterilized glass jars of 300ml and these glass jars were covered with muslin cloth which were tighten with rubber band to avoid the escape of beetles and also to protect the beetles from the pervasion of rodents, lizards and other insects. The culture was reared to obtain age wise homogeneous stock of adult beetles. The homogeneous stock was maintained for further experiments.

Phosphine generation and administration

Gaseous phosphine was generated in laboratory and different doses of phosphine were calculated according to the method described in FAO Plant Protection Bulletin (1969). Approximately 20 newly emerged adult beetles of both populations of *T. granarium* were introduced in their respective vials with five replicates for each dose. Insects containing vials were exposed to different concentrations of phosphine *i.e.*, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5, 5.5, 6, 6.5, 7.0, 7.5 and 8.0 ppm in their respective gas-tight glass desiccators for 20 h (FAO, 1969). Mortality data against each dose was recorded after ventilation for 48 h and data was subjected to Probit analysis (Finny, 1971) for the determination of LC_{50}. Corrected mortality was determined by using Abbot Formula (Abbott, 1925).

Effect of LC_{20} on various metabolites over wide range of exposure periods

Approximately 300 newly emerged adult beetles of both population of *T. granarium* were exposed separately to their respective LC_{20} for 24, 48, 72, 96 and 120 h, at 35±2°C and 60±5% RH. Control desiccators of both populations were prepared in the same way but they were not exposed to phosphine. After exposure to LC_{20} live adult beetles of both populations were used immediately for biochemical analysis.

Biochemical analysis

Twenty adult beetles (treated and control) from both populations of khapra beetle were weighed and then macerated separately in 1.5ml saline (0.89%) with the help of motor-driven Teflon glass homogenizer at 4°C. They were centrifuged at 3000×g for 30 min in refrigerated centrifuge at 4°C. Thus, clear supernatant was used for the estimation of soluble proteins and glucose contents. Soluble proteins contents of beetle extract were determined according to the method described by Lowry *et al*. (1951) and glucose contents of beetle extract were determined by the *o*-toluidine method described by Hartel *et al.* (1969). Total lipids, nucleic acids and free amino acid (FAA) contents were estimated from ethanol extract of treated and control beetles. For total lipids, nucleic acid and FAA estimation the method of Zöllner and Kirsch (1962), Schneider (1957) and Moore and Stein (1954) were adopted, respectively. Glycogen contents were extracted by crushing the whole beetles in KOH and estimated by the anthrone method of Consolazio and Lacono (1963).

Statistical analysis

Statistical analysis was carried out in Minitab 16. Mortality data was subjected to two way ANOVA and comparison of mean mortality with respect to exposure periods was done by Tukey's test at 95% confidence limit. While data pertaining to effects of sub-lethal dose of phosphine (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.

RESULTS

Determination of LC_{50}

Toxicity of phosphine for resistant and susceptible populations of *T. granarium* was determined in terms of LC_{50} by Probit analysis at 95% fudicial limit. The LC_{50} values for adult beetles of susceptible population was 4.50 and for resistant population was 4.87ppm.

Effect of LC_{20} on the metabolites of adult beetles

The effect of sub-lethal concentration of phosphine (LC_{20}) was evaluated on metabolites of adult beetles of resistant and susceptible populations of *T. granarium* on daily basis for five days.

Soluble protein contents

Soluble protein contents of susceptible population were first significantly raised (10 and 24%) after 24 and 48 h exposure to sub lethal dose but after 48, 72, 96 and 120 h a significant decreased was observed with reference to control beetles of susceptible population. Soluble proteins in resistant population were significantly increased till 72 h but then concentration started to decrease and at 120 it concentration was significantly decreased (11%) with reference to control (Fig. 1A).

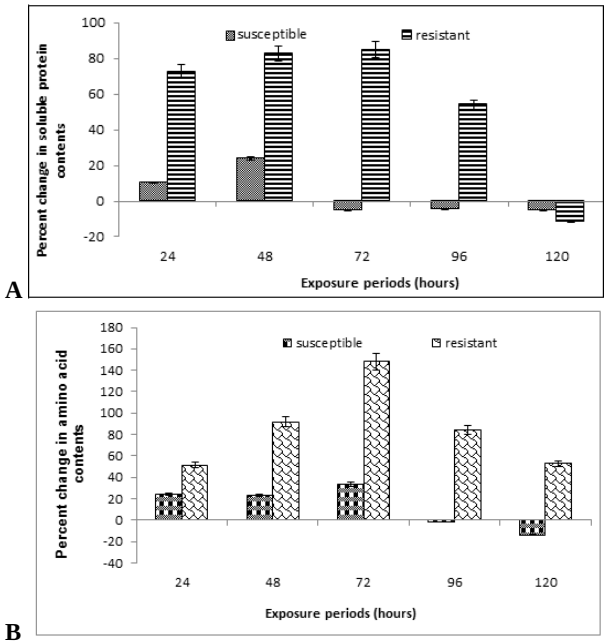

Fig. 1. Effect of sub-lethal dose of phosphine on soluble protein contents (A) and amino acid contents (B) of adult beetles of susceptible and resistant populations of *T. granarium*. The percentage change has been calculated with reference to the control which was susceptible strain, but not treated with subl-ethal dose of phosphine.

Free amino acids

Level of free amino acids in adult beetle of susceptible population was significantly increased (24, 23 and 34%) after the exposure of 24, 48 and 72 h, respectively. Whereas, after exposure of 96 and 120 h level of free amino acids was significantly depleted by 2 and 14%, respectively. The adult beetles of resistant population possessed higher levels of free amino acids after each exposure period with reference to control but a significantly decreasing trend (84 and 53%) was 96 and 120 h, respectively (Fig. 1B).

Glucose contents

Glucose contents in adult beetle of susceptible population were significantly increased (13, 23 and 13%) after exposure of 24, 48 and 72 h, with reference to control, respectively. Whereas, after exposure of 96 and 120 h, glucose level was significantly decreased by 5 and 18% with respect to control, respectively. Although in adult beetles of resistant population glucose contents were significantly increased by 50, 149 and 235 % after exposure of 24, 48 and 72 h, respectively but after 72 h glucose level was started to decrease and at 120 h glucose level was significantly decreased from control sample (Fig. 2A).

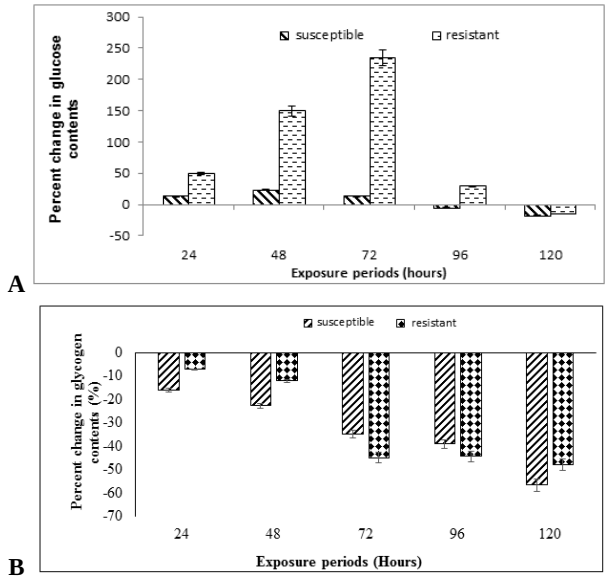

Fig. 2. Effect of sub lethal dose of phosphine on glucose contents (A), and glycogen contents (B) of adult beetles of susceptible and resistant populations of *T. granarium.* The percentage change has been calculated with reference to the control which was susceptible strain, but not treated with sub lethal dose of phosphine.

Glycogen, Lipids and Nucleic acids contents

In adult beetles of susceptible and resistant population glycogen (Fig. 2B), lipids (Fig. 3) and nucleic

acid contents (Fig. 4) were significantly decreased after exposure periods of 24, 48, 72, 96 and 120h.

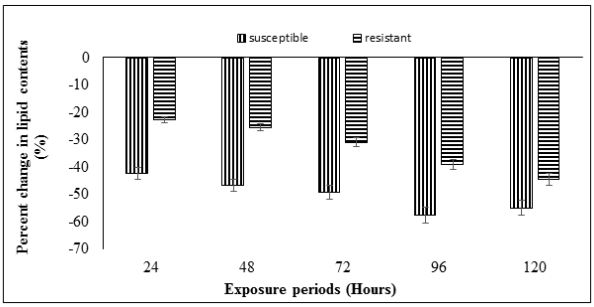

Fig. 3. Effect of sub lethal dose of phosphine on lipid contents of adult beetles of susceptible and resistant populations of *T. granarium*. The percentage change has been calculated with reference to the control which was susceptible strain, but not treated with sub lethal dose of phosphine.

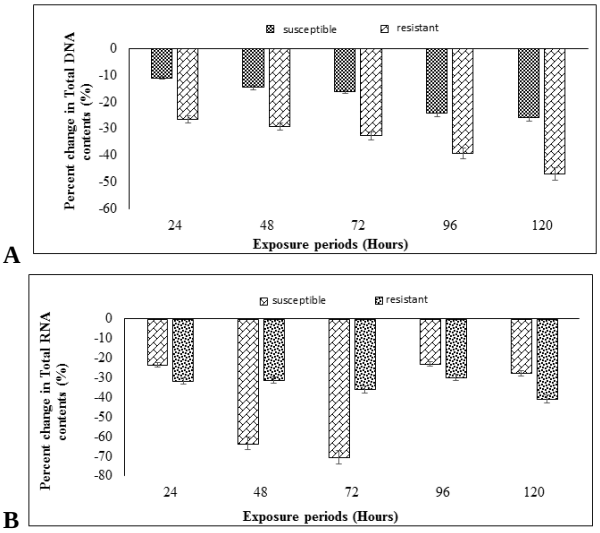

Fig. 4. Effect of sub lethal dose of phosphine on DNA (A) and RNA contents (B) of adult beetles of susceptible and resistant populations of *T. granarium.* The percentage change has been calculated with reference to the control which was susceptible strain, but not treated with sub lethal dose of phosphine.

DISCUSSION

The insecticide resistance problem cannot be resolved only by increasing the concentration of phosphine because it is not only uneconomical but may also lead to necrosis, which in turn increase survival of insects (Nakakita and Kuroda, 1986). It is evident from the results of present investigation that sub-lethal concentrations of phosphine in conjunction with exposure periods had significant effect on the mortality of adult beetles of resistant and susceptible populations of *T. granarium.* It was also revealed from the results that phosphine concentrations had positive correlation alone on adult beetle mortality; as the phosphine concentration increased; mortality was also increased irrespective of the exposure period. Likewise, exposure periods as well as had significant interactions with phosphine toxicity irrespective of phosphine concentrations. Overall findings regarding efficacy of phosphine of present research are in accordance with Haber's rule (1924). This is true for the fumigants or insecticides that their effect take some time to become effective to achieve end point (Sun, 1946). It was reported by Price and Mills (1988); Mills and Pacheco (1996) that exposure time come to be most effective factor rather than phosphine concentrations for proper management of target pests.

Exposure of adult beetles of susceptible and Resistant populations of *T. granarium* to their respective LC_{20} showed changes in activities of soluble protein, free amino acids, glucose, glycogen, lipids, RNA and DNA contents. It is evident from the present studies that soluble protein contents were significantly increased in adult beetles of resistant population after exposure of 24, 48, 72 and 96 h and decreased significantly after exposure of 120 h. But in adult beetles of susceptible population soluble protein contents were increased significantly after exposure of 24 and 48 h and decreased significantly after the exposure periods of 72, 96 and 120 h with respect to their controls. Soluble proteins consist of albuminous fractions, antibodies and enzymes, although as the duration of exposure period was increased there were depletion in soluble protein contents because of the inhibition of protein synthesis by phosphine. Landa *et al.* (1991) reported that the exogenous substances can adversely affect proteins and peptides by three ways like inhibition of protein synthesis, inhibition of enzymes, and induction of enzymes.

Level of free amino acids was increased in resistant population after each exposure period with respect to controls. These results are in accordance with Hussain *et al.* (2012) who reported elevated level of free amino acids after treatment with abamectin in resistant adult than susceptible adults of *T. castaneum* populations. Glucose contents after exposure periods of 24, 48, 72 and 96 were significantly increased in resistant population and significantly decreased after exposure of 120 h. Whereas in susceptible population, glucose contents were significantly elevated after exposure of 24, 48 and 72 h, while reduced significantly after 96 and 120 h with respect to control. Glycogen, lipids and nucleic acid level

was significantly reduced in both populations after each exposure period.

Although typically in the insect body free glycogen floats in the haemolymph but in order to maintain glucose level in blood glycogen is broken down and released glucose. Such change gives sufficient stimulus to initiate glycogenolysis in insect tissues and accelerate the development of glycogen units in response to stress inflicted by pesticide exposure to adapt the insecticide induced stress that cause the release of glucagons, corticosteroids and catecholamines stimulating glucose synthesis from the breakdown of glycogen to reduce energy demand (Dezwann and Zandee, 1972; Shoba *et al*., 2011). The reduction in the total lipid contents could be attributed to its conversion to proteins to compensate the reduction in protein contents or generate supplementary energy. These findings are in accordance with those results reported by many investigators (Abo-Ela *et al*., 1998; Omar *et al*., 2005).

The growth of insects is under control of molting hormone and juvenile hormone these hormones are regulated by enzymes. It was reported by Socha and Sehnal (1973) that molting hormone activates the synthesis of RNA and juvenile hormone induced duplication of DNA. So, it is suggested that as DNA and RNA contents were reduced and their reduction was enhanced as duration of exposure period increased thus phosphine may acts as insect growth regulators by inflicting its toxicity by targeting molting hormone and juvenile hormone.

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