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Original Article

Toxicity of phosphine against tolerant and susceptible populations of *Trogoderma granarium* collected from Punjab, Pakistan

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Abstract

Khapra beetle, *Trogoderma granarium* is one of the most notorious pests of stored grains. This study was aimed to evaluate the toxicity of phosphine to 4th, 6th instar larvae and adult beetle in phosphine resistant populations *viz.,* Gujranwala, Mandi Bahauddin-I (M.B.Din-I), Mandi Bahauddin-II (M.B.Din-II),Gujrat and Sargodha and a susceptible population of *T. granarium.* The 4th, 6th instar larvae and adult beetles of *T. granarium*were exposed to different sub lethal concentrations of phosphine for 20 hours. Based on LC_{50} data, Gujranwala population was found to be most resistant to phosphine whereas Sargodha population was found the least resistant population. Gujranwala population required 113.5, 128.8 and 168.1% more phosphine concentration in 4th, 6th instar larvae and adult beetles, respectively with reference to susceptible population of *T. granarium.*The various insect populations based on LC_{50} of phosphine is graded as Gujranwala > MBDin-I > Gujrat > MBDin-II > Sargodha > control.The LC_{50} values of phosphine against adult were always less than of 6th instar larvae and likewise LC_{50} of phosphine against 6th instar larvae is always less compared to 4th instar larvae in all populations. The 4th instar of Gujranwala required 17.03 and 25.39% more phosphine than 6th instar larvae and adult beetle, respectively. Based on LC_{50} data, gradation followed by developmental stages is 4th instar > 6th instar > adult. Adult insects seemed to be more sensitive to phosphine compared to 6th and 4th instar larvae.

Key words: Phosphine, *Trogoderma granarium*, Toxicity

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INTRODUCTION

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 used to eliminate the insects of storage commodities by killing the insects without affecting the viability of grains. It is relatively cheap, versatile and easy to use. 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). *Trogoderma granarium* commonly called Khapra beetle is a notorious pest of stored grains.

It is associated with man and human settlements. They are found in grain stores, malt houses, food stores, dried milk factories, seed and fodder processing plants. Larvae feed on a variety of food products especially whole grains and cereal products but they also feed on dried foods with proteinaceous contents such as fruits, grains, spices, dried seeds and gums. Larvae invade through the pericarp of grain or seed, feed on the damaged seed and cause considerable loss of weight and seed quality. Serious infestations of cereal grain by *T. granarium* can make it indigestible, unpleasant and reduced market value. Total protein and carbohydrates contents of the grain are also affected by infestation (Jood and Kapoor, 1993; Jood *et al*., 1993). During infestation, the beetle contaminates the grains with skin and setae which cause dermatitis, gastrointestinal irritation and allergic reactions (Jood *et al*., 1996).

Phosphine is a nucleophile reducing agent, which acts by interacting with and inhibiting critical cellular enzymes involved in metabolic processes. It has specific inhibitory effects on mitochondrial cytochrome c oxidase (Chefurka *et al.,* 1976). The inhibition of cytochrome c oxidase and other enzymes

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causes the synthesis of superoxide radicals and cellular peroxides. These all lead to subsequent cellular injury through lipid peroxidation and other oxidant mechanisms (Bolter and Chefurka, 1990; Chugh *et al*., 1996; Sudakin, 2005).

MATERIALS AND METHODS

Collection 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 resistant to phosphine were collected from different go downs of Punjab province *viz*., Mandi Bahauddin-I (MBDin-I), Mandi Bahauddin-II (MBDin-II), Gujrat, Gujranwala and Sargodha. These go downs have more than ten 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.

Six untreated populations of Lahore, Muzaffargarh, Khanewal, Chistian, Haroonabad and Faqeer wali were taken from ten years old culture maintained in the culture room of department of Zoology, University of the Punjab, Lahore. These populations were not exposed to any insecticide or fumigant for the last ten years. The LC_{50} values of Lahore, Khanewal, Chishtian, Muzaffergarh, Haroonabad and Faqeer wali were not significantly different from each other so these all were pooled as one susceptible population (control).

Maintenance of culture

The master cultures of *T. granarium* (six populations) were maintained in temperature and humidity controlled room at 35±1°C and 65±5% RH (Riaz *et al*., 2014; Shakoori *et al*., 2016). A pure homogeneous stock of each population was developed in the culture room. Crushed wheat grains and flour was used to feed the larvae because adult beetle do not feed. Wheat (5kg) was treated with phosphine tablet to eliminate other insect pests.

One phosphine tablet was wrapped in muslin cloth and then it was kept in an empty match box and was placed in air tight jar containing wheat for 48 hours. Phosphine treated sterile wheat was spread on clean sheet for 7 hours in air so effect of phosphine was last and then sterilized wheat was stored in air tight container for use. The beetles were reared in 300 ml sterilized glass jars covered with muslin cloth to prevent the escape of larvae and beetles and entry of mite and ticks. Glass jars were washed with detergent and sterilized overnight at 180°C in an oven (Cuperus *et al.,* 1986). Sterilized jars were then labeled with date and names of six populations of *T. granarium.* Each jar was filled 1/3rd with crushed wheat and flour and 100 adult khapra beetles of each population were introduced in their respective jar. Although adult khapra beetle do not feed but as beetle laid eggs and eggs hatched into 1st instar larvae so feed was provided for eggs. These 1st instar larvae could not break the whole grain so flour is provided for their feed. Adult beetle were left in the culture medium for 5-6 days to ensure egg laying. By using separating sieve and camel hair brush dead beetles were discarded and flour containing eggs were separated. The eggs developed into adult beetle via larval and pupal period. These adult beetles were again transferred to next jars for continuity of the culture and homogeneous stock was maintained for further studies. From 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} of phosphine against T. granarium

For determination of LC_{50} against *T. granarium*, phosphine was generated from aluminium phosphide in the laboratory. Commercially available aluminium phosphide (AlP) pellets containing (approximately 0.2g) are recommended as the most suitable source of phosphine (PH_3).

$$\mathrm{AlP} + 3\mathrm{H_2O} \rightarrow \mathrm{Al(OH)_3} + \mathrm{PH_3}$$

Generation of phosphine gas

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.

Administration of phosphine

All 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 of suitable thickness so that syringe needle may pass through it. A thin layer of grease was applied on the edge of the lid to make it air tight. Saturated solution of sodium nitrite in Petri dish was placed to maintain the RH 65±5%. A ceramic plate with holes was

placed over the bottom narrow compartment of desiccators to place the insect vials with holed lids. Twenty insects of each 4th, 6th instar larvae and adult beetles (24 hours old) were introduced to each vial with three replicates for each dose. Phosphine doses 0, 2, 4, 6, 8, 10, 12, 14 and 16ppm were used for evaluation of LC_{50} of each population.

Determination of dose volume

Different doses of phosphine were calculated according to the formula mentioned in FAO Bulletin (1975). Phosphine source (86%) is equivalent to a concentration of 1.195µg/µl.

Fumigation procedure

Insect vials containing desiccators were transferred to the fume hood for fumigation purpose. Sterilized Hamilton microsyringe was used for dispensing phosphine doses, before taking the first dose with Hamilton microsyringe; it was flushed with small quantity of phosphine by withdrawing small volumes of phosphine and then expelled it into fume hood. While drawing the phosphine, plunger of syringe was moved very slowly. Dose volumes for each concentration of 0, 2, 4, 6, 8, 10, 12, 14 and 16 ppm were withdrawn and injected into the appropriate desiccators. Following dosing, the desiccators were placed in temperature controlled room throughout the exposure period for 20 hours and exposure period (20 hours) remained the same for all concentrations used in the study. Control desiccators were also prepared in the same way but they were not fumigated.

Mortality assessment

After 20 hours, desiccators were opened and insect vials were taken out. The 4th and 6th instar larvae were transferred to separate crushed wheat medium and maintained at 30±1°C and 60±5% RH. The adult beetles were kept in empty glass jars.

Mortality of 4th, 6th instar larvae and adult beetles was assessed after 48 hours from the end of the exposure period. Lloyd (1969) criterion was followed "insect judged to be dead when the pressure from a brush failed to reproduce a response". The % mortality was corrected by Abbot's formula (Abbot, 1925).

$$\text{Corrected \% mortality} = \frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}} \text{ X } 100$$

Data was analyzed by the method described by Finney (1971). Each analysis produced the LC_{50} value in ppm for 4th, 6th instar larvae and adult beetles of *T. granarium*.

RESULTS

The LC_{50} of phosphine against 4th, 6th instar larvae and adult beetles of five tolerant populations *viz.,* Gujranwala, MBDIN-I, Gujrat, MBDIN-II, Sargodha and a susceptible population of *Trogoderma granarium* are shown in Table I.

In MBDIN-I population, the LC_{50} of 4th, 6th instar larvae and adult beetles was 13.4, 11.9 and 9.8ppm, respectively while in MBDIN-II population, the LC_{50} of 4th, 6th instar larvae and adult beetles was 12.2, 11.1 and 9.2ppm, respectively. The LC_{50} of 4th, 6th instar larvae and adult beetles of Gujrat population was 13.1, 12.3 and 11.4, respectively. In Gujranwala population, the LC_{50} of 4th, 6th instar larvae and adult beetles was 15.8, 13.5 and 12.6ppm respectively while in Sargodha population; the LC_{50} of 4th, 6th instar larvae and adult beetles was 11.6, 10.4 and 8.2ppm, respectively. On the basis of LC_{50} of phosphine, population having highest LC_{50} was termed as phosphine-tolerant and the one having lowest LC_{50} was termed as susceptible (control) population. Gujranwala population was found to be the most tolerant population. The various insect populations based on LC_{50} of phosphine is graded as Gujranwala > MBDIN-I > Gujrat > MBDIN-II > Sargodha > susceptible. It was observed that phosphine toxicity did not vary only in different populations but it also varied among different developmental stages. In susceptible and other phosphine-tolerant populations, the LC_{50} of phosphine against adult is less than that of 6th instar larvae and likewise LC_{50} of phosphine against 6th instar larvae is less compared to 4th instar larvae. Based on LC_{50} data, various developmental stages can be graded as: 4th instar larvae > 6th instar larvae > adult. Adult insects seem to be more sensitive to phosphine compared to 6th and 4th instar larvae.

DISCUSSION

Five phosphine-tolerant populations (MBDin-I, MBDin-II, Gujrat, Gujranwala and Sargodha) and a susceptible population of *T. granarium* investigated in present study behaved differently after exposure to phosphine in

laboratory. Exposure to phosphine revealed that MBDin-I, MBDin-II, Gujrat, Gujranwala and Sargodha populations were differently tolerant to phosphine. On the basis of LC_{50} of phosphine, Gujranwala population was found to be the most tolerant population with 2.135 fold more tolerance in 4th instar larvae than susceptible population. Other population *viz*., MBDIN-I, MBDIN-II, Gujrat and Sargodha populations of *T. granarium*showed 1.81, 1.77, 1.64 and 1.54 fold more tolerance in 4th instar larvae with reference to susceptible population. Price (1984) and Chaudhry and Price (1990) proposed that phosphine resistant populations uptake less phosphine than susceptible population, so phosphine concentrations that causes mortality in susceptible population cannot cause significant mortality in resistant population.

Table I: LC_{50} of phosphine against three different developmental stages of susceptible and five tolerant populations of *T. granarium* collected from Punjab and percent increase and fold increase in LC_{50} of tolerant populations compared with susceptible population

Sr. No.	Populations	Developmental stages	LC_{50}(ppm)	% increase in LC_{50} (ppm)*	Fold increase in LC_{50}*
1	Gujranwala	4th instar larvae	15.8	113.51	2.135
		6th instar larvae	13.5	128.81	2.288
		Adult	12.6	168.1	2.68
2	MBDIN-I	4th instar larvae	13.4	81.08	1.81
		6th instar larvae	11.9	101.7	2.01
		Adult	9.8	108.51	2.08
3	Gujrat	4th instar larvae	13.1	77.02	1.77
		6th instar larvae	12.3	108.47	2.08
		Adult	11.4	142.55	2.42
4	MBDIN-II	4th instar larvae	12.2	64.86	1.64
		6th instar larvae	11.1	88.13	1.88
		Adult	9.2	95.74	1.95
5	Sargodha	4th instar larvae	11.6	56.75	1.54
		6th instar larvae	10.4	76.27	1.76
		Adult	8.2	74.46	1.74
6	Susceptible	4th instar larvae	7.4	0.00	0.00
		6th instar larvae	5.9	0.00	0.00
		Adult	4.7	0.00	0.00

*With reference to the susceptible population

Among three developmental stages (4th, 6th instar larvae and adult beetles), the 4th instar larvae were found to be the most tolerant developmental stage than 6th instar larvae and adult beetles. These results showed that phosphine toxicity not only vary in different populations but also in different developmental stages. Adults seemed to be more susceptible than 4th and 6th instar larvae. Saleem and Shakoori (1990) also reported 4th instar larvae, the most resistant stage of *T. castaneum.* Bell and Wilson (1995) investigated that individual tolerance to phosphine was higher in larval stages.This may be because larvae are metabolically more active than adult beetle. In larval stages reorganization of differentiating tissues are accompanying and they have potential to quickly metabolize and detoxify the toxic compounds effectively as compared to adult beetle. The adult beetle is emerged from inactive pupae so it might not be possible for adult to activate all the metabolic system at once

when compared with larval stages. Eggs and pupae were found more tolerant to phosphine (Hole *et al.,* 1976; Bell and Wilson, 1995). Adu and Muthu (1985) reported on the basis of LC_{95} of *Callosobruchuschinensis* that susceptibility of developmental stages decreased from larvae > adults > pupae > eggs.

CONCLUSION

Phosphine toxicity varied among different developmental stages representing that adult beetles are most susceptible than developmental stages (4th and 6th instar larvae).

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