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Phosphine Induced Changes in Various Esterase levels in 4th Instar Larvae of *Trogoderma granarium*

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Abstract.- Fourth instar larvae of two populations *viz.* Khanewal (KH) and Haroonabad 107 (107HR) of *Trogoderma granarium* were exposed to Phosphine at LC_{20} for 10, 20, 40 and 80 hours duration. In both populations, cholinesterase activity increased after prolonged administration, where as acetylcholinesterase, total esterase (TE) and arylesterase (AE) activities were decreased. AE activity showed a marked increase at 80 hours, whereas TE activity increased during 10 hours of exposure in 107 HR population.

Key words: Stored grain pest, insecticide resistance, acetylcholinesterase, cholinesterase, arylesterase.

INTRODUCTION

The stored grain losses produce severe drop in the world economy due to the damage caused by certain noxious pests (ICI, 1993). *Trogoderma granarium* commonly known as Khapra beetle is a cosmopolitan and serious insect pest of stored grains and their products (Cohen, 1982). There is need to control this serious pest as it causes enormous loss during its larval and adult forms. Several successful attempts have been made in the past to control this pest by the use of chemicals with effective killing potency. This method as usual has been wrought with the danger of development of insecticide resistance (Zettler *et al.,* 1989; Saleem *et al*., 2000; Guillemaud, 1998; Scott, 1999). This pest has developed resistance against phosphine (Udeaan, 1990; Irshad and Iqabal, 1994).

Insecticide exposure either occupational, accidental or any other type has been reported to induce esterase activity in insects and other organisms. The esterase level in resistant species has been reported to be very high (Terriere, 1984; Oi *et al*., 1990; West and McCaffery, 1992), which are responsible for breakdown or inactivation of various ester linkages of insecticides. These enzymes protect the target sites by catalyzing the hydrolysis of insecticides. Esterases have a wide range of specificity. They are able to cleave tri-ester-phosphates, halides, esters, thioesters, amides and peptides. The role of esterases in the defense mechanism against intoxication by insecticides is well documented (Guindy *et al*., 1982; Saleem and Shakoori, 1996). These enzymes are thought to be synthesized during various stages of larval development of the larvae. The level of esterase activity is not constant through the life cycle as larvae of some species exhibit higher tolerance to contact insecticides than adults (Nakakita and Winks, 1981)

There are reports about the resurgence of stored grain pests even after fumigation in most of the stored grain godowns in Pakistan (Irshad and Iqbal, 1994; Saleem and Shakoori, 1996; Taylor, 1986). The efforts have not been made to study the level of resistance in stored grain pests (especially *Trogoderma granarium*) against phosphine in Pakistan for effective management of the problem. There is no data available in Pakistan on the esterase level and resistance patterns of different populations of insects for comparing the enzyme induction and pesticide management for successful control measures.

The primary aim of this study is to investigate the level of resistance in various populations of *Trogoderma granarium* by using various esterases, as indicators. These studies may help in effective management of insect pests.

MATERIALS AND METHODS

Insects

Fourth instar larvae of *Trogoderma granarium*, collected from Khanewal (KH) and Haroonabad 107

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(107HR), were introduced in two desiccators, when they were kept without food and exposed to phosphine @ .0.8 ppm according to the standardized method mentioned in the FAO Plant Protection Bulletin (1975). Control group of each population (KH and 107HR) was also proceeded similarly except that only air instead of phosphine was administered in these desiccators. The larvae were exposed to phosphine fumes for 10, 20, 40, and 80 hours. Live larvae from each desiccator along with control groups were then weighed and used for estimation of total esterase, cholinesterase, acetylcholinesterase and arylesterase activities.

Cholinesterase activity was determined according to Rappaport *et al*. (1959). Acetylcholinesterase activity was determined according to Devonshire (1975a). Estimation of aryl esterase activity was done according to Junge and Klees (1981). For determination of TE activity Devonshire (1975a) was followed.

RESULTS AND DISCUSSION

Table I shows the analysis of various esterase activities of both the populations of *T. granarium* (KH and 107 HR) after 10, 20, 40 and 80 hours of phosphine exposure and control groups. In both populations AchE activity decreased during the entire exposure period. Almost similar decrease was observed in AE and TE activities in both populations but 107HR population showed significant increase in AE activity after 80 hours of exposure, whereas the TE activity increased in this population within 10 hours of exposure.

CE activity decreased in KH population after 10 hours and in 107 HR population after 80 hours of exposure. After longer administration the CE activity increased in both the populations (Table I).

AChE enzyme play very important role in the function of nerve impulse transmission. They are polymorphic enzymes and are known as inhibitors of organophosphate and carbamate insecticides (Toutant, 1989). In this study the AChE activity showed significant inhibition in almost both populations (KH and 107 HR) of *Trogoderma granarium*.

CE is another enzyme, which hydrolyzes cholinester to form choline and the corresponding fatty acid. CE activity showed significant rise in KH population as well as 107HR population after 20, 40 and 80 hours treatment, while after 10 hours treatment it also showed decreased activity in the KH population. This suggests that CE activity was induced after treatment with insecticide. The raised activity of this enzyme shows development of resistance in the populations.

AE are inhibited by organophosphate and eserine (Augustinsson, 1961). They preferentially hydrolyze aromatic esters and are also inhibited by parachloromercuribenzoate or parahydroxy mercuribenzoate. The AE activity decreased in KH population during the whole treatment period. In 107 HR population the activity of this enzyme was inhibited after 10,20 and 40 hours of exposure but there was increase in activity after 80 hours exposure. This shows induction of the enzyme after longer exposure of phosphine. The TE activity in KH population was inhibited throughout the treatment period, whereas in 107HR population, the activity increased during 10 hours treatment but remained inhibited during other treatment periods (Table I).

It is evident from the present studies that although some enzymes show evidence of induction after phosphine treatment, the overall results showed inhibition of esterases with phosphine under the present conditions of experiments. Results also show a relationship between the resistance and duration of treatment. AE activity is inducted with increasing exposure period, whereas the TE activity increased during first 10 hours, but on prolongation of exposure period the activity was inhibited (Table I). Irshad and Iqbal (1994) reported that each strain of *Trogoderma granarium* required different doses and exposure period for effective control. Borah and Chahl (1979) tested 2 strains of *T. granarium* from India and reported that strain P required higher dose of phosphine than strain N for the same level of kill.

Due to inhibition of detoxifying enzymes, the larval system could not cope with the phosphine and hence as a result mortality occurred. Increasing duration and concentration of toxicant can eliminate these insects (Rajendran, 1994; Pree *et al.,* 1989).

It is concluded from the present findings that the populations used in the experiments are susceptible to phosphine and can be controlled by

Table I.- Various esterase activities of Khanewal and Haroon Abad 107 populations of *Trogoderma granarium* against different doses of phosphine

Insect population	Exposure time (Hours)	AchE (m IU/mg)		CE (m IU/mg)		AE (m IU/mg)		TE (m IU/mg)	
		Control (n =3)	Treated (n =3)	Control (n =3)	Treated (n =3)	Control (n =3)	Treated (n=3)	Control (n =3)	Treated (n =3)
Khanewal	10	1.61 ± 0.01	1.30 ± 0.05	0.38 ± 0.03	0.25 ± 0.02	25.30 ± 2.2	21.72 ± 0.32	152.7 ± 0.22	149.9 ± 0.0525
	20	2.00 ± 0.12	1.9 ± 0.2**	0.55 ± 0.05*	0.59 ± 0.04	30.08 ± 1.95	25.5 ± 2.46	119.39 ± 6.2**	95.82 ± 0.25*
	40	1.72 ± 0.06	1.62 ± 0.06	0.32 ± 0.1	0.36 ± 0.1	16.79 ± 1.48	12.79 ± 1.22*	140.89 ± 0.59	108.44 ± 0.56*
	80	1.55 ± 0.6	1.4 ± 0.2*	0.38 ± 0.2	0.52 ± 0.2*	18.79 ± 0.71	15.41 ± 0.64*	163.92 ± 0.10	149.77 ± 0.10
Haroonabad 107	10	1.7 ± 0.7	1.6± 0.03**	0.67 ± 0.2	0.71 ± 0.02	23.1 ± 0.76	19.9 ± 0.36*	118.25 ± 0.36	125.5 ± 0.40**
	20	1.39 ± 0.07	0.95 ± 0.05	0.59 ± 0.05	0.62 ± 0.71	20.21 ± 0.84	17.31 ± 0.59	86.47 ± 0.23	78.26 ± 0.45***
	40	1.67 ± 0.07	0.9 ± 0.11	0.71 ± 0.06	0.74 ± 0.33	18.12 ± 0.14	9.56 ±0.12**	138.06 ± 0.45	118.61 ± 0.4**
	80	1.65 ± 0.09	0.95 ± 0.11	0.25 ± 0.03	0.21 ± 0.001	15.9 ± 0.34	20.00 ±0.45**	115.38 ± 0.24	109.80 ± 0.48**

Mean ± SEM
*P<0.05; **P<0.01; ***P<0.001 (student's t test)

proper management of dose and duration of treatment.

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