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**TOXICITIES OF AZADIRACHTIN AND POLYCHLORINATED PETROLEUM HYDROCARBON AGAINST RESISTANT AND SUSCEPTIBLE STRAINS OF *TRIBOLIUM CASTANEUM* (COLEOPTERA: TENEBRIONIDAE) ADULTS**

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**ABSTRACT:-** The  $LC_{50}$  values for malathion-resistant (PAK) and organo-phosphate-susceptible (FSS-II) strains of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) adult beetles were determined through filter paper impregnated method against azadirachtin (Nimbokil 60 EC) and polychlorinated petroleum hydrocarbon (Tenekil 100 EC). The  $LC_{50}$  values of these insecticides were worked out as 12830 and 9331 ppm for azadirachtin and 5148 and 4047 ppm for Tenekil 100 EC against PAK and FSS-II strains, respectively. The results revealed that polychlorinated petroleum hydrocarbon was more toxic than the azadirachtin. Furthermore, both the insecticides were equally toxic to the adult beetles of *T. castaneum* as the difference was non-significant because of overlapping 95% FLs to  $LC_{50}$ .

*Key Words:* *Tribolium castaneum*; Insecticides; Polychlorinated Petroleum Hydrocarbon; Azadirachtin; Toxicity; Pakistan.

## INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst), is an important pest of stored grains and stored products specially pulses, millets and cereals in Pakistan (Hamed and Khattak, 1985) and other parts of the world (Obeng-Ofori, 2008). Insecticide resistance in the populations of *T. castaneum* is widespread throughout the world. This pest has developed resistance against lindane (Kumar and Bhatia, 1982); DDT (Spiers et al., 1971); malathion (Saleem and Shakoori, 1989; Hussain et al., 1996a, b; Hasan et al., 1996) cypermethrin, deltamethrin, cyfluthrin, fenvalerate and permethrin (Collins, 1990; Hasan et al.,

1996; Hussain et al., 1996a; b) as well as against some juvenile hormone analogues (Dyte, 1972).

Phytochemical azadirachtin found in neem (*Azadirachta indica* A. Juss), is an ecdysone antagonist that disrupts insect molting (Tomlin, 2000). It is widely used against Coleoptera, Diptera, Hemiptera, Homoptera, Lepidoptera, Thysanoptera, Tetranychidae, and some Nematoda species (Ishaaya and Degheele, 1998). Some scientists also reported the effectiveness of azadirachtin against larvae and adult beetles of *Cryolestes pusillus* (Schon.) (Rahman et al., 2005); *T. castaneum* (Hameed et al., 2012; Mostafa et al., 2012) as well as *Papilio demoleus* L (Khan and Ahmed, 2003);

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*Myzus persicae* (Sulzer) (Dela et al., 2014); *Dermestes maculatus* DeGeer larvae and adults (Baba et al., 2014); *Callosobruchus chinensis* (L) (Devi, 2014) and sugarcane borer (Da Silva et al., 2013). Hussain et al. (2005) found azadirachtin comparatively least toxic to larvae of *T. castaneum*.

Polychlorinated petroleum hydrocarbon belongs to chlorinated hydrocarbon group and was formulated by the scientists of the Pakistan Council for Scientific and Industrial Research (PCSIR), Karachi. It is being marketed with a brand name of Tenekil 100 EC by the Scientific and Technological Development Corporation (STEDEC), Ministry of Science and Technology, Government of Pakistan, against termites, borers, nematodes and sucking pests of various crops (STEDEC, 2004). Hussain et al. (2005) studied that polychlorinated petroleum hydrocarbon on the basis of  $LC_{50}$  proved more effective against larvae of organophosphate-susceptible (FSS-II) strain as compared to malathion-resistant (PAK) strain of *T. castaneum*.

For combating the problem of resistance to synthetic insecticides, the present studies were arranged to find out alternative of insecticides being used against the pest.

## MATERIALS AND METHOD

### Insect Strains

The malathion-resistant (PAK) and the organophosphate-susceptible (FSS-II) strains of *T. castaneum* were obtained from the Department of Zoology, University of the Punjab, Lahore, Pakistan. The adults of PAK strain had developed 56-fold resistance against Malathion while comparing  $LC_{50}$  of FSS-II strain with that of PAK strain (Saleem and

Shakoori, 1989). The beetles of these two strains were reared in empty glass jars of 300 ml capacity in the Ecotoxicology Laboratory, Department of Entomology, University of Agriculture, Faisalabad, Pakistan. Wheat flour was used as the culture medium for insect. Prior to use, the culture medium was placed in an oven at 60°C for 3 h which reduce contamination maximally. Both the strains were bred at 30±1°C and 60±5% relative humidity (Saleem and Shakoori, 1985). The new cultures were prepared every third day to obtain sufficient beetles for the experiments. Each jar was 1/4<sup>th</sup> filled with sterilized wheat flour and about 50 adult beetles were introduced into each. Ten-day old adult beetles were collected and used in insecticidal bioassays.

### Insecticides

Commercially available formulations of insecticides (Table 1) were used in the present study.

### Determination of Toxicities

Initial dose response tests of azadirachtin and polychlorinated petroleum hydrocarbon were conducted using filter paper impregnated method to find out dose ranges, which would be expected to yield  $LC_{50}$  values of the insecticides. For all experiments, calculated quantities of insecticides were dissolved in acetone. The insecticide solutions were then diluted to give a geometric series of at least 3-5 different concentrations. The insecticide dilutions (1 ml/ dish) of azadirachtin and polychlorinated petroleum hydrocarbon were applied on Whatman grade 41 filter paper akin to glass Petri dishes (130 cm<sup>2</sup>). Acetone was

**TOXICITIES OF AZADIRACTIN AND POLYCHLORINATED**

**Table 1. Details of toxicants used**

Common Name	Brand Name	Group	Name and address of manufacturer/distributor
Azadirachtin	Nimbokil 60EC	Insecticides with natural origin	STEDEC Pakistan, STEDEC House, Lahore, Pakistan
Polychlorinated petroleum hydrocarbon	Tenekil 100EC	Chlorinated hydrocarbon	STEDEC, Pakistan, STEDEC House, Lahore, Pakistan

used as control treatment. Three replicates of each dose were used simultaneously for both insecticides. After evaporation of acetone at room temperature, 20 healthy and 10-day old adult beetles were released in each dish. They were kept without food and mortality counts were made after 48 h exposure to insecticides and the percent mortality was corrected by following Abbott's formula for any control mortality (Abbott, 1925).

$$\text{Mortality(\%)} = \frac{\text{Test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

Larvae giving no response after squeezing with a brush were considered as dead (Lloyd, 1969). The mortality data so obtained were subjected to Probit Analysis. The LC<sub>50</sub> values were calculated as outlined by Busvine (1971) and the Chi-squares (X<sup>2</sup>)

values were found significant, the variances were adjusted for heterogeneity to have the good fit of regression lines.

**RESULTS AND DISCUSSION**

The LC<sub>50</sub> values for adult beetles and sixth instar larvae of PAK and FSS-II strains of *T. castaneum*, were determined as follows:

**Toxicity of Azadirachtin**

The LC<sub>50</sub> values for azadirachtin against the adult beetles of PAK and FSS-II strains of *T. castaneum* were 12863 and 9331 mg l<sup>-1</sup>, respectively (Table 2). However, the results showed clearly that the LC<sub>50</sub> of both strains were not different significantly at 95% confidence limits. This indicated that azadirachtin was equally toxic to the adult beetles of the malathion-resistant (PAK) and organophosphate-susceptible (FSS-II)

**Table 2. Relative toxicities of azadirachtin and polychlorinated petroleum hydrocarbon against PAK and FSS-II strains of *T. castaneum* adult beetles**

Insecticide	Strain	LC <sub>50</sub> (ppm)	95 % Fiducial limits	Slope ± S.E.	Chi-square(X <sup>2</sup> ) at 2df
Azadirachtin (Nimbokil 60EC)	PAK	12863	4198-15776	1.92 ± 0.065	5.96
	FSS-II	9331	7906-11015	2.33 ± 0.104	4.82
Polychlorinated petroleum hydrocarbon (Tenekil 100EC)	PAK	5148	4027-6531	1.63 ± 0.039	0.95
	FSS-II	4047	3221-5081	1.68 ± 0.05	2.91

strains of *T. castaneum*.

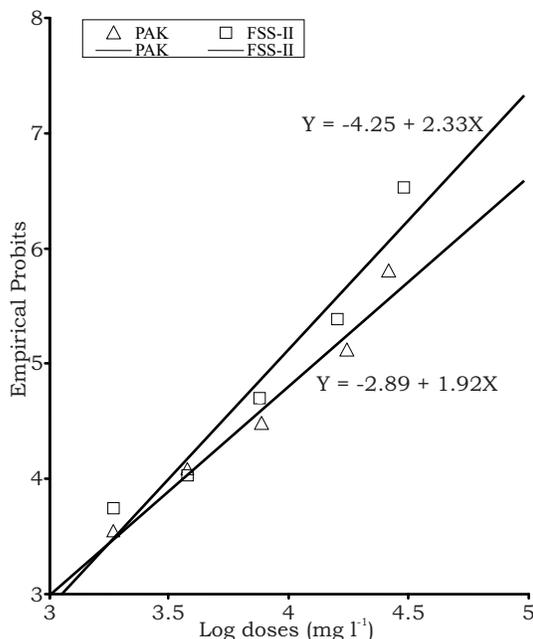
But the slope values gave the regression lines relatively flatter in PAK strain ( $b= 2.77$ ) and steeper in FSS-II strain ( $b= 3.93$ ) (Figure 1). It indicated the presence of some resistant individuals in the population of PAK strain at mortality above  $LC_{50}$ . Azadirachtin had been effective against adult beetles of PAK and FSS-II strains of *T. castaneum* in the present studies by filter paper impregnated method. Its effectiveness against stored grain pests has also been recorded by several previous workers (Paul et al., 1963; Jotwani and Sircar, 1965, 1967; Jairaj, 1976; Savitri and Rao, 1976; Tabassum et al., 1994; Mathur and Nigam, 1996; Muda and Cribb, 1999; Zahid et al., 2000; Qureshi et al., 2002; Hussain et al., 2005; Hameed

et al., 2012; Mostafa et al., 2012). Some scientists have reported the effectiveness of neem insecticide against larvae and adult beetles of *Crypolestes pusillus* (Schon.) (Rahman et al., 2005); *T. castaneum* and *Papilio demoleus* L (Khan and Ahmed, 2003); *Myzus persicae* (Dela et al., 2014); *Dermestes maculatus* larvae and adults (Baba et al., 2014); *Callosobruchus chinensis* (Devi, 2014) and sugarcane borer (Da Silva et al., 2013). Hussain et al. (2005) in a research found azadirachtin comparatively least toxic to larvae of *T. castaneum*.

Azadirachtin an environment friendly insecticide had been exempted from residue tolerance requirements, by the US Environmental Protection Agency, for food crop applications (Immaraju, 1998). The results of the present investigations, together with the results of the previous workers suggested that azadirachtin was a potential insecticide to be used against stored grain pests.

#### Toxicity of Polychlorinated Petroleum Hydrocarbon (Tenekil 100EC)

The  $LC_{50}$  values against PAK and FSS-II strains were 5148 and 4047  $mg\ l^{-1}$ , respectively and these differences were non-significant because of overlapping 95 % FLs to  $LC_{50}$  (Table 2). It is also evident from the slopes of regression line for PAK ( $b= 1.63$ ) and FSS-II ( $b= 1.68$ ) strains (Figure 2). There are numerous reports on development of resistance in *T. castaneum* against organochlorine insecticides (Dyte and Blackman, 1970; Kumar and Bhatia, 1982; Brun and Attia, 1983). In the present studies, polychlorinated petroleum hydrocarbon showed efficacy against adults of malathion-resistant (PAK) and organophosphate-susceptible



**Figure 1. Regression lines of toxicity of azadirachtin against PAK and FSS-II strains of *T. castaneum* adult beetles**

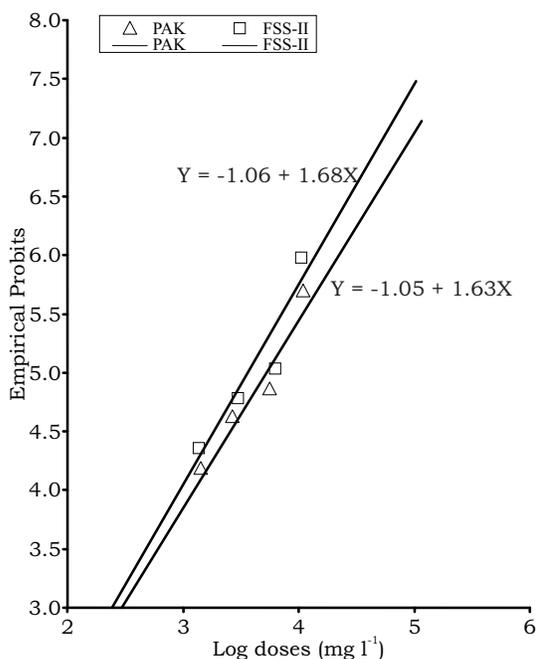
(FSS-II) strains of *T. castaneum*, but higher  $LC_{50}$  indicated that the pesticide was least toxic to adults of PAK strain. Similar studies on larvae of *T. castaneum* done by Hussain et al. (2005) not only revealed that the insecticide was least toxic to larvae of PAK strain ( $LC_{50}$  = 9933 ppm) as compared to FFS-II strain ( $LC_{50}$  = 6286 ppm) rather it proved least toxic to larvae as compared to adult beetles in the light of present studies. In a study conducted by Barwal and Kalra (1982) lindane resistance in *T. castaneum* did not extend to malathion. In another study, a lindane-resistant strain was more susceptible as compared to DDT-resistant strain of *T. castaneum* to an inactive isomer of hexachlorocyclohexane (Saxena, 1983). Poly-

chlorinated petroleum hydrocarbon had  $LD_{50}$  value  $>5000$   $mg\ kg^{-1}$  for rats. These characteristics together with the present results, suggested that the insecticide may be of value in stored grains pest management.

The results of the present investigations suggest that azadirachtin and polychlorinated petroleum hydrocarbon may be potential insecticides to be used against stored grain pests *T. castaneum*, but the latter requires further investigations regarding its persistence and degradation in the environment.

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**Figure 2. Regression lines of toxicity of polychlorinated petroleum hydrocarbon against PAK and FSS-II strains of *T. castaneum* adult beetles.**

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#### AUTHORSHIP AND CONTRIBUTION DECLARATION

S. No	Author Name	Contribution to the paper
1.	Dr. Riaz Hussain	Conceived the idea and Conducted research
2.	Dr. Muhammad Riaz	Conceived the idea, Technical input at every step
3.	Dr. Mushtaq A. Saleem	Supervised the research

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