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Toxicity of Synthetic Pyrethroid, Fury, Against Different Developmental Stages of Three Strains of *Tribolium castaneum* (Herbst.)

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Abstract.- The LC_{50} of Fury, a synthetic pyrethroid, against Pak strain of red flour beetle *Tribolium castaneum* increased with the age during development up to newly emerged adult stage but the 15 days old adults were the most sensitive developmental stage and have much less LC_{50} as compared with the other developmental stages. The LC_{50} of Pak strain was 405, 648, 560 and 121 ppm, respectively, whereas it was 856, 1088, 555 and 58 ppm, respectively, for the insecticide susceptible FSS-II strain. In insecticide resistant CTC-12 strain the 4th and 6th instar larvae, and newly emerged and 15 days old adults had LC_{50} of 1442, 1258, 1420 and 671 ppm.

Key words: Lethal concentration, *Tribolium castaneum,* stored grain pest, red flour beetles, Fury, synthetic pyrethryoids.

INTRODUCTION

Large-scale production of synthetic pyrethroids at a reasonable cost has heralded a new era in pest control (MacCuaig, 1980; Ruscoe, 1977; Brees, 1977). They are highly potent neurotoxic insecticides which greatly affect the behavioural and neuro-endocrine functions of mammals. They alter the normal function of sodium (Na^+) channel. DDT and many of its structural analogues have similar mode of neuro-toxic action (Beeman and Schmidt, 1982). The introduction of ∝-cyano group and halogenation of esters further enhanced the activity and stability of pyrethroid. Pyrethroid encompass an increasingly diverse range of lipophilic and insecticidal compounds capable of assuming similar configuration on binding at the relevant neuroreceptor (Elliott, 1977). The development of a pyrethroid involved successive isosteric modifications of pyrethrin 1 with the goals of structural simplicity and enhanced potency and stability. The most rapidly metabolised and photolabile substituents of early pyrethroids were replaced with the newer compounds with groups of greater chemical stability. Metabolic studies in insects, mammals, plants and environmental system are needed to evaluate pyrethroid mode of action, resistance mechanism, interaction with synergists, selective toxicity relationship, residual properties and safety in use (Casida and Ruzo, 1980).

Pyrethroid insecticides are characterised by high knockdown and lethal activity, a wide spectrum, good residual activity together with repellent and antifeeding activity (Hirano, 1989). Pyrethroids possess high toxicity to insects, low toxicity to mammals, photostability, high degradability and effective application at minimum dose (Barlow *et al.*, 1971; Hadaway, 1972). With these characteristics, pyrethroid insecticides have become widely used for plant protection. Their major use has been for the control of bollworm and leafworms in cotton but they have also achieved wide spread use for controlling various species of lepidopterous pests of fruits and vegetables, aphids in cereals, and many other minor outlet. Although the early synthetic pyrethroids were ineffective against mites and soil pests, later additions such as fenpropathrin, have combined high acaricidal activity with insecticidal activity, and further

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pyrethroid are being introduced for use in soil. The extent of pyrethroid use has increased progressively since the first photostable pyrethroid was registered.

Red flour beetle, *Tribolium castaneum* is a serious grain pest which causes enormous losses in Pakistan. These insects have been reported from grain flour and other cereal products, beans, cottons seeds, shelled nuts, dried milk, dried vegetables, dried fruits, drugs and chocolates. They infest both in larval as well as adult conditions. The average loss of stored grain in 2005-2006 was 654,000 tonnes, which is 11% of the total stored grain in godowns.

T. castaneum can be controlled either through management practices or with the help of toxic chemicals *i.e.* residual insecticides and fumigants. Insecticides (organophosphates and synthetic pyrethroids) remain the most popular and effective chemicals for pest control. *T. castanum* has been reported to develop great resistance against different chlorinated hydrocarbon and organophosphates insecticides (Speirs and Zetler, 1969; Champ and Campbell-Brown, 1970; Dyte and Blackman, 1970; Dyte *et al.*, 1973). The increasing prevalence of resistance to insecticide suggests that other new insecticides, such as pyrethroids may also be used for chemical control. The synthetic pyrethroid are known to possess many desirable properties including high toxicity to insects, high biodegradability and effectiveness at low doses (Barlow *et al.,* 1971; Hadaway, 1972).

The present study is aimed at evaluating of toxicity of new synthetic pyrethroids (Fury) against three strains of *T. castanum* for successful control of this noxious stored grain pest in Pakistan.

MATERIALS AND METHODS

Insect

Three strains of *Tribolium castaneum* (Herbst.) *viz*. Pak, FSS-II and CTC-12 were used in this study. The master culture of Pak strain was obtained from Food Storage Division of Pakistan Agricultural Research Council, Malir Halt, Karachi. The susceptible (FSS-II) and resistant (CTC-12) strains were obtained from University of New Castle upon Tyne, England. FSS-II is susceptible to malathion (Lloyd and Ruczkowski, 1980), whereas CTC-12 is a multi-OP resistant strain of the above pest, reported first by Champ and Campbell-Brown (1970).

New cultures were set up in the sterilized jam jars covered with muslin cloth tied with rubber bands. Whole meal flour was used as culture medium. The culture was maintained in the laboratory at 30±1°C and 65±5 relative humidity. Insects were left in the culture media for three days for egg laying and then were removed with the help of separate sieves and fine camel hair brushes. Flour with eggs was placed in the same jar, from where beetles and larvae were obtained for toxicological studies.

Insecticides used

Fury is designed to provide effective control of a wide range of insects on cotton and other crops. In addition to outstanding insect control, Fury provides excellent plant safety and minimal environmental impact. This pesticide is extremely toxic to fish and aquatic invertebrates, and it is highly toxic to bees exposed to direct treatment or residues on blooming crops or weeds. It has a broad-spectrum of activity, including tobacco bud worm, cotton boll worm, cabbage looper, corn earworm, codling moth, soya bean looper, tomato fruit worm and peach borer. The commercial formulation of the synthetic pyrethroid Fury 18.1 EC (α-Cyano (2-phenoxyphenyl (±) cis/trans 3-(2,2-dichloroethenyl)-2,2 dimethylcyclopropane carboxylate) was obtained from the FMC (United), 7-Canal Bank Road, Gulberg V, Lahore.

Estimation of LC_{50}

Different concentrations of insecticides were prepared as serial dilutions using acetone as solvent. The range of doses used for determination of LC_{50} of Pak strain larvae and beetles after treatment for 48 hours were:125 to 1000 ppm for 4^{th} instar larvae, 250 to 2000 ppm for 6^{th} instar larvae, 375 to 3000 for newly emerged and 125 to 1000 ppm for 15 day old adult beetles. Similarly for FSS-II strain the range of doses used for 4^{th} instar larvae were: 187 to 1500 ppm, 250 to 2000 for 6^{th} instar larvae, 187 to 1500 ppm for newly emerged and 18.75 to 150 ppm for15 day old adult beetles, respectively. The CTC-12 strain treated comparatively high dose like 500 to 4000 for 4^{th} instar larvae, 625 to 5000 for 6^{th} instar larvae, 375 to 3000 for newly emerged adult and 250 to 2000 for 15 day old adult beetles.

Different doses were spread uniformly (1.3 ml/plates) on glass Petri plates. The control plates had no insecticide, but only the acetone. Three replicates of each dose were run simultaneously. After evaporation of acetone and drying of Petri dishes about 10 beetles of similar size and weight were introduced to each Petri dish. The mortality counts were done after 48 and 72 hours. The percentage kill was corrected by Abbott's formula (Abbott, 1925) for control mortality.

The criterion of mortality used in this study was the one described by Lloyd (1969). The mortality data were, thereafter, subjected to probit analysis by Finney (1971). LC_{50} values were derived from these analyses and expressed in ppm as well as $\mu g/cm^2$ of insecticide. The mortality data was subjected to logit analysis using POLO-PC (LeOra Software, 1987) to estimate the LC_{50} and confidence limits.

RESULTS

Table I shows the LC_{50} of a synthetic pyrethroid, Fury, against 4th instar larvae, 6th instar larvae, newly emerged adult beetles and 15 days old beetles of Pak, FSS-II and CTC-12 strains of *T. castaneum*.

The LC_{50} for the 4th instar larvae of Pak strain was 405 ppm, whereas the 6th instar larvae and newly emerged adults required, respectively, 60 and 38% (1.6 and 1.4-fold), more insecticide for LC_{50}, when compared with the 4th instar larvae. The 15 days old adult beetles, however, required 70% less concentration as compared with the 4th instar larvae. In susceptible strain (FSS-II), the LC_{50} of Fury against 4th instar larvae was 856 ppm. The 6th instar larvae, however required 27% (1.3-fold) more insecticide for LC_{50} than the 4th instar larvae. The newly emerged and 15 days old beetles required 35 and 93% less dose than the 4th instar larvae. The 4th instar larvae of resistant strain (CTC-12) required 1442 ppm Fury for $LC_{50.}$ The 6th instar larvae, newly emerged adult beetles and 15 days old beetles required 11, 53 and 1.5% less dose than the 4th instar larvae.

The 4th instar larvae of Pak strain required 53% (2.11-fold) less, while the 4th instar larvae of CTC-12 required 68% (1.7-fold) more insecticide concentration as compared with the 4th instar larvae of FSS-II. The Pak 6th instar larvae required 38% (1.67-fold) less and CTC-12 6th instar larvae 31% (1.18-fold) higher concentration of insecticide than the 6th instar larvae of FSS-II strain. The newly emerged adults of Pak and FSS-II strain had the same $LC_{50,}$ whereas the newly emerged adults of CTC-12 strain required 156% (2.55-fold) more insecticide than those of FSS-II. The 15 days old beetles of Pak and CTC-12 strain required 109 and 1055% (2.08 and 11.56-fold) more insecticide than those of FSS-II. The 15 day old adults are however, still the most sensitive developmental stage, as compared to newly emerged adults and larvae.

DISCUSSION

Insecticides are likely to cause extensive damage to insect blood, beside other system at lethal dose level. Hence the present study was undertaken to determine the sub lethal effect of Fury (pyrethroid) against three different strains at four different developmental stages.

When LC_{50} values against Fury for the Pak and CTC-12 strains *T. castaneum* 4th and 6th instar larvae were compared with FSS-II strain larvae, it was found that Pak strain larvae had low LC_{50}, whereas CTC-12 strain larvae had high $LC_{50.}$ Anspaugh *et al*. (1994) reported that on the topical application of different concentrations of permethrin, the KD for resistant German cock roaches was 20 times greater than that for susceptible insects, which was because of reduced penetration of permethrin in resistant insects along with increased *in vivo* metabolism as compared with susceptible controls. Yu and Nguyen (1996) reported that pyrethroid resistance in diamondback moths *Plutlla xylostella* (L) was most likely attributed to decreased target site sensitivity.

The above mentioned results also suggest that Fury may be of value of combating the growing threat of insecticide resistance. Lloyd (1973) and Carter *et at*. (1975) have reported almost similar results. No cross resistance was detected in the present study. Lloyd and Ruczkowski (1980) concluded that strain of *T. castaneum* with specific resistance to malathion and analogs showed no cross resistance to natural pyrethrins and synthetic pyrethroids. Saleem and Shakoori (1989) have reported that no cross-resistance was recorded against cypermethrin and permethrin in the 6th instar larvae of *T. castaneum.*

Table I.- LC_{50} of Fury (18.1 EC), a synthetic pyrethroid, against four different developmental stages of three strains (Pak, FSS-II, CTC-12) of stored grain pest, *T. castaneum.*

Strains	Developmental stages	Slope	LC_{50}	95% CL	Regression equation	Resistance factor*
Pak	4th instar larvae	1.127	405	236.78-804.53	4.94048+1.12741 (X-12.5544)	0.47
	6th instar larvae	2.387	648	497.03-833.33	5.05288+2.38702 (X-12.8336)	0.59
	Newly emerged adults	2.052	560	353.51-749.63	5.38904+2.05254 (X-12.9377)	1.00
	15 days old beetles	1.159	121	18.95-209.36	5.48039+1.15908 (X-12.4978)	2.08
FSS-II	4th instar larvae	1.630	856	608.45-1471.5	4.73178+1.63033 (X-12.7677)	
	6th instar larvae	2.347	1088	846.71-1508.7	4.73705+2.34714 (X-12.9247)	
	Newly emerged adults	2.264	555	425.70-730.40	4.9718+2.26458 (X-12.73217)	
	15 days old beetles	2.217	58.0	44.08-76.54	4.94915+2.21779 (X-11.7378)	
CTC-12	4th instar larvae	2.76	1442	1149.43-1812.7	4.98904+2.76590 (X-13.1550)	1.68
	6th instar larvae	3.170	1285	1026.37-1571.7	5.15671+3.17032 (X-13.1584)	1.18
	Newly emerged adults	2.90	1420	1147.76-1807.2	4.84114+2.90426 (X-13.0977)	2.55
	15 days old beetles	2.632	671	527.45-847.71	5.03069+2.63276 (X-12.8382)	1158

*Resistance factor: LC_{50} of Pak/LC_{50} of FSS-II; LC_{50} of CTC-12/LC_{50} of FSS-II.

Newly emerged adults of Pak and FSS-II strains have approximately the same LC_{50}, whereas the CTC-12 strain's newly emerged beetles required more than double the amount of Fury than in the other two strains. The organisms are becoming resistant to pyrethroids with the passage of time (Pree *et al*; 1989; Muller, 1989). The LD_{50} and knockdown effect of deltamethrin, permethrin and DDT to resistant and susceptible *Musca domestica* showed that susceptible strain had already developed very high level of resistance to knockdown by pyrethroid as well as DDT. This pyrethroid resistance is probably due to knockdown resistant gene mechanism. Stuart *et al.* (1998) reported that the *T. castaneum* (Herbst.) strain QTC 279 is highly resistant to deltamethrin and other synthetic pyrethroids.

The 15 days old beetles in this study were found to be the most susceptible stages of life cycle. Saleem and Shakoori (1990) have also reported that the adult beetles were more susceptible to insecticide treatment as compared with the 6th instar larva.

Gunning *et al*. (1998) used relatively small quantities of OP principally methyl parathion and profenofos to control *Helicoverpa armigera* on the late season cotton, while resistance to porofenofos was detectable from time to time by bioassay, OP resistance did not give control problem in the field. More recently, however, increasing resistance of *Helicoverpa armigera* to alternative control chemical such as endosulfan, pyrethroid, and carbamate resulted in an expanded use of the OPs (methyl parathion, profenofos, and chlorpyrifos) on cotton and other crops leading to serious field-resistance problem in Australia. Gunning *et al.* (1999) also reported that one hundred percent mortality could he achieved when *Helicoverpa armigera* were pretreated with some OP and then dosed with pyrethroid.

Biochemical studies showed that pyrethroid resistance associated resistance esterases in *Helicoverpa armigera* were inhibited by OP compound, such as ethion, chloropyrifos and its oxon, profenofos and acephate. The OP binds to the active site of enzyme thus preventing pyrethroid detoxication. The two most important detoxification pathways for pyrethroids involve esterase cleavage and hydroxylation by MFO enzymes which involve cytochrome P-450s. This enzyme shows large number of forms, each of which exhibits a broad substrate specificity (Shono *et al.*, 1979; Bigley and

Plapp, 1978; Lee *et at*., 1989). Similarly, a range of different forms of the enzyme is also found in insect (Terriere and Yu, 1979). These enzymes are thought to have evolved in animals as a general detoxification mechanism (Hodgson, 1984). In many strains of insects, more than one of these mechanisms may be present and making it difficult to determine the relative importance of each in specific case of resistance (Brattsten *et at*., 1986). The detoxifing enzymes *e.g*. the glutathione transferases and CE cetalyse reactions that render the insecticide molecules less toxic or non toxic (Terriere and Yu, 1974).

On the basis of the above result it is concluded that 15 days adult beetles of Pak, FSS-II and CTC-12 strains are susceptible against Fury, while the larval stages and newly emerged adult beetles are resistant against this insecticide. This is probably because larva is metabolically a very active stage of life, during which reorganization of differentiating tissue is taking place and hence are able to metabolize and detoxify the toxic compounds quickly and more efficiently as compare with the adult beetles. After emergence from pupa, it will not be possible to activate all its system at once. The new adult move slowly and develop new cuticle so intake of insecticide is very low. In this way newly emerged adult require high dose for mortality as compare to the larvae and adult beetles. So *T. castaneum* could best be control with Fury at 15 days old adult stage.

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