Topical Application of Some Pyrethroids against the Housefly, *Musca Domestica* L.

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Abstract: In this study, three pyrethroid insecticides 'Deltamethrin, Cypermethrin and Cyfluthrin' were evaluated against field and laboratory strains of the adult females of the housefly, *Musca domestica* L. using topical application bioassay technique under laboratory conditions. Different concentrations were used, which were ranged between 0.001-0.5 ppm against laboratory strain, while were ranged between 0.01-1.0 ppm against field strain. The mortality% of Deltamethrin ranged between 18-99%, while ranged between 16-98% for Cypermethrin and ranged between 15-99% in case of Cyfluthrin against laboratory strain. On the other hand, the mortality% ranged between 25-92%, 25-91 and 25-89% for Deltamethrin, Cypermethrin and Cyfluthrin against field strain, respectively. The results indicated that Cyfluthrin was the most effective insecticide (LD_{50} 0.0133 ppm) against the adult females as well as, Deltamethrin (LD_{50} 0.0185 ppm), while Cypermethrin gave (LD_{50} 0.0223 ppm) against laboratory strain. Against field strain, Cyfluthrin gave (LD_{50} 0.0846 ppm), while Deltamethrin gave (LD_{50} 0.0759 ppm), whereas Cypermethrin gave (LD_{50} 0.0645 ppm). When comparison between the three insecticides using LD_{90} level; the same trend was noticed. Slope of laboratory strain in case Deltamethrin, Cyfluthrin and Cypermethrin were 0.9995, 0.0.9594 and 0.9663, respectively, while in filed strain were 0.9817, 0.9248 and 0.9425, respectively. Results indicated that the tabulated X^2 $(Chi)^2$ were 9.5 in the three insecticides while calculated X^2 $(Chi)^2$ of laboratory and field stains of adult females using Deltamethrin was 25.15, whereas was 25.45 and 15.55 when using Cypermethrin and Cyfluthrin, respectively against laboratory stain. In other ward, reached 13.47, 6.63 and 15.55 in case Deltamethrin, Cypermethrin and Cyfluthrin, respectively against field stain. Concerning the population of Jeddah Governorate, resistance ration in each tested pyrethroid insecticides were 4.10, 2.89 and 6.36 fold for Deltamethrin, Cypermethrin and Cyfluthrin, respectively.

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1. Introduction

The common housefly, *Musca domestica* Linnaeus (Diptera: Muscidae) is actually a companion animal of livestock and humans and is not actually an ectoparasite, but it is far more important economically in many instances than any of the flies associated with livestock and poultry. The housefly becomes economically important to confined livestock by virtue of its biotic potential and synanthropic behaviour. These factors, coupled with the ability of houseflies to exploit different developmental habitats on feedlots and dairies, have made this pest economically important **(Barson *et al.*, 1994).** *M. domestica* is found in all countries of the world but is more adaptable in warm areas. It is worldwide in distribution and is a pest in homes, barns, poultry houses, food processing plants, dairies, and recreation areas. It has a tremendous breeding potential and during the warmer months can produce a generation in less than two weeks. House fly eggs are laid in almost any type of warm organic material. Animal or poultry manure is an excellent breeding medium. Fermenting vegetation such as grass clippings and garbage can also provide a medium for fly breeding. Housefly undergo complete metamorphosis, and have distinct egg, larval, pupal and adult stages **(Sanchez-Arroyo and Capinera, 2008)**. The action of pyrethroids on insects; sodium channels, and the correlation of these effects with insecticidal activity, has been reviewed extensively **(Narahashi, 1996).** Pyrethroids are axonic excitoxins the toxic effects of which are mediated through preventing the closure of the voltage-gated sodium channels in the axonal membranes. The sodium channel is a membrane protein with a hydrophilic interior. This interior is a tiny hole which is shaped precisely to strip away the partially charged water molecules from a sodium ion and create a favorable way for sodium ions to pass through the membrane, enter the axon, and propagate an action potential. When the toxin keeps the channels in their open state, the nerves cannot repolarize, leaving

the axonal membrane permanently depolarized, thereby paralyzing the organism **(Soderlund *et al.*, 2002).**

The objective of this study is to evaluate the three pyrethroid insecticides 'Deltamethrin, Cypermethrin and Cyfluthrin' against field and laboratory strains of the adult females of the housefly, *M. domestica* L. using topical application bioassay technique under laboratory conditions.

2.Materials and Methods

Mass rearing of housefly, *Musca domestica* L.:

The colonies of *Musca domestica* L. originated from adults collected from Slaughterhouse sheep in Jeddah Governorate, using a sweeping net. They were transferred into a small cage (16×16×16 cm) and transported to the Laboratory of Public Health Pests, Jeddah Amana, Jeddah Governorate, KSA for identification and colonization.

M. domestica colonies established from Laboratory of Public Health Pests, Jeddah Amana, Jeddah Governorate, KSA (generation > F25) and maintained under a 14:10 (light:dark) and maintained at 268±2°C and 70–80% humidity were used. Houseflies were reared using standard conditions **(Sawicki, 1964)** to generate similar-sized individuals. Adult houseflies were reared in wire-frame cages covered with wire-gauze (75x75x75 cm), with mesh screening on top, provided a 10% sugar solution; adults were laid their eggs in artificial diet containing from 600 gm powdered milk, 1000 gm wheat bran and 30 gm yeast then mixed together with one liter of water and put in plastic tray (25x10X10 cm) also the artificial diet was developed according (**Saleem *et al.*, 2009 and** Cickova et al 2012**)**.

Insecticides used:

Commercial formulations of insecticides were used in this study representing the four main groups of insecticides commonly applied on mosquito control. These insecticides include synthetic pyrethroide (Deltamethrin, Cypermethrin and Cyfluthrin).

2.1. Synthetic pyrethroids:

a-Common name: Cypermethrin 10%.
Trade name: Exit 100 EC.
Chemicalname:

(RS)-alpha-cyano-3-phenoxybenzyl-(1RS,3RS,1RS, 3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate.

b- Common name: Deltamethrin 2.5%.
Trade name: Agrodelta 25 EC/ULV.
Chemicalname:

(*S*)- -cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibrom- ovinyl)-2,2-dimethylcyclopropanecarboxylate.

c- Common name: Cyfluthrin 5%.
Trade name: Wopro-Cyfluthrin 5%EC.
Chemical name: (*RS*)- -cyano-4-fluoro-3-phenoxybenzyl (1*RS*, 3*RS*; 1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate.

Adult bioassay:

Contact toxicity by topical application:

A hand micro applicator (Burkard Manufacturing Co, Ltd., Middlesex, UK) fitted with a 1-ml hypodermic glass syringe and a size-27 gauge needle was used to topically apply acetone [Chimtex, Ghana] solutions of the different concentration of pyrethroid insecticides (Cypermethrin 10%, Deltamethrin 2.5% and Cyfluthrin 5%) to adult houseflies. Both laboratory and field strains of housefly were treated with 1 µl of the test solution was applied on the thorax of four-day-old adult housefly of adult females selected randomly.

Twenty insects were used for each treatment and each treatment was replicated four times. The treatment series included four groups of 20 houseflies treated with acetone alone to serve as controls. The houseflies were anaesthetized with carbon dioxide CO_2 (pressure: 20 kpa) for 20 seconds. The control flies were treated with acetone only. After treatment, the insects were kept in clean polyethylene petri dishes lined with filter paper with twenty individuals per container and provided with food and water. Mortality was scored at 24 h post treatment. Houseflies were considered dead when they were unable to right themselves to a normal posture within 2 minutes when touched on their abdomen with a pair of forceps.

Statistical analysis:

Mortality counts:

Mortality counts were made after 24 hours. The dosage mortality data were subjected to Probit analysis according to **(Finney, 1972; Bakr, 2005).** Mortality percentages were corrected according to **Abbott's (1925).** Levels of resistance in the field colony strains of the three insects were calculated as follows:

Resistant ratio (R.R) =	LD_{50} or LD_{90} of the field colony strain
	LD_{50} of LD_{90} the laboratory strain

3. Results and Discussion

1) Efficiency of pyrethroid insecticides against adults of *M. domestica* under laboratory conditions:

Pyrethroids are axonic excitoxins the toxic effects of which are mediated through preventing the closure of the voltage-gated sodium channels in the axonal membranes. The sodium channel is a membrane protein with a hydrophilic interior. This interior is a tiny hole which is shaped precisely to strip away the partially charged water molecules from a sodium ion and create a favorable way for sodium ions to pass through the membrane, enter the axon, and propagate an action potential. When the toxin keeps the channels in their open state, the nerves cannot repolarize, leaving

the axonal membrane permanently depolarized, thereby paralyzing the organism **(Soderlund *et al.*, 2002).**

In this study three pyrethroid insecticides 'Deltamethrin, Cypermethrin and Cyfluthrin' were used. The tested toxicants were evaluated against field and laboratory strains of the adult females using topical application bioassay technique. The topical bioassay method was chosen for detecting resistance as it was found to be more sensitive than the conventional WHO glass jar residue method. The evaluation of insecticidal action was compared as follow:

1.1) Comparison on basis of LD_{50} and LD_{90} values:

The used concentrations under laboratory conditions of Deltamethrin, Cypermethrin and Cyfluthrin were ranged between 0.001-0.5 ppm against laboratory strain, while were ranged between 0.01-1.0 ppm against field strain. The mortality% of Deltamethrin ranged between 18-99%, while ranged between 16-98% for Cypermethrin and ranged between 15-99% in case of Cyfluthrin against the adult females of laboratory strain, while against field strain the mortality% of Deltamethrin ranged between 25-92%, while ranged between 25-91% for Cypermethrin and were 25-89% for Cyfluthrin (Table 1 and Figs 1-3).

The required values, i.e. LD_{50}'s and LD_{90}'s are presented in Tables (1) and Fig. (4,5 and 6). Data given summarized the susceptibility of both field and laboratory strains of the adult females of *M. domestica* to the tested three pyrethoid compounds.

The results clearly showed that Cyfluthrin was the most effective insecticide (LD_{50} 0.0133 ppm) against the adult females as well as Deltamethrin (LD_{50} 0.0185 ppm), while Cypermethrin gave (LD_{50} 0.0223 ppm) against laboratory strain, while Cyfluthrin gave (LD_{50} 0.0846 ppm), while Deltamethrin gave (LD_{50} 0.0759 ppm), whereas Cypermethrin gave (LD_{50} 0.0645 ppm) against field strain. At LD_{90} level, data indicated that Cyfluthrin was the most effective insecticide (0.2821 ppm) against the adult females as well as Deltamethrin (0.3547 ppm), whereas Cypermethrin gave (LD_{90} 0.4832 ppm) against laboratory strain, while against field strain Cyfluthrin gave (1.9371 ppm) less effective than Deltamethrin (1.5345 ppm), while Cypermethrin gave (LD_{90} 1.5674 ppm).

The obtained results are agreed partially with those obtained by **Sohail *et. al.* (2004)** they indicated that cypermethrin had the lowest LD50 (183 ppm) followed by fenvalerate (247 ppm) at 48th h when tested with topical application method. Also, **Khan and Ahmed (2000)** found LD_{50} and LD_{90} values were found to be 0.44 and 3.58 ug/fly of DDVP against house flies when calculated the lethal dose values with probit analysis. Also, **Akiner and Çağlar (2005)** evaluated the resistance level of the first generation offspring against malathion, propoxur, and permethrin using the topical application method. The resistance ratio (RR) of the Balik Pulau strain house flies for propoxur and malathion ranged from 10.28 to 99.00 and 7.83 to 47.01, respectively. Permethrin appeared to be the most potent insecticide in controlling house fly in both the Balik Pulau (RR = 0.50 to 1.96) and Juru poultry farms (RR = 0.64 to 2.40). The term 'pyrethroid' is commonly used to designate a synthetic insecticide that is derived structurally from the natural pyrethrins. Pyrethroids are known to alter the normal function of insect nerves by modifying the kinetics of voltage-sensitive sodium channels, which mediate the transient increase in the sodium permeability of the nerve membrane that underlies the nerve action potential **(Soderlund and Bloomquist, 1989)**. The action of pyrethroids on insects; sodium channels, and the correlation of these effects with insecticidal activity, has been reviewed extensively **(Narahashi, 1996).**

1.2) The slope of toxicity lines:

Data in Table (1) show that the slope of laboratory and field stains of adult stage of *M. domestica* population when using Deltamethrin, Cyfluthrin and Cypermethrin. Slope of laboratory strain in case Deltamethrin, Cyfluthrin and Cypermethrin were 0.9995, 0.9663 and 0.9594, respectively, while in filed strain were 0.9817, 0.9425 and 0.9248, respectively.

1.3) X^2 $(Chi)^2$ value:

Results in Table (1) indicated that tabulated X^2 $(Chi)^2$ was 9.5 while calculated X^2 $(Chi)^2$ of laboratory and field stains of adult females of *M. domestica* population when using Deltamethrin was 25.15, whereas when using Cypermethrin, tabulated $(Chi)^2$ was 9.5 and calculated $(Chi)^2$ was 25.45, whereas in case Cyfluthrin tabulated $(Chi)^2$ was 9.5 and calculated $(Chi)^2$ was 15.55 against laboratory stains. Whereas, tabulated X^2 $(Chi)^2$ and calculated X^2 $(Chi)^2$ reached 9.5 and 13.47 in case Deltamethrin, whereas when using Cypermethrin were 9.5 and 6.63, while were 9.5 and 15.55 when using Cyfluthrin, respectively.

Pyrethroids are now offered in a variety of commercial formulations available to ordinary consumers for use in the home. It is now estimated that approximately 80-90% of households in the United States use pesticides with pyrethroids comprising a considerable percentage of total use **(Whyatt *et al.*, 2003).**

Determined the levels of resistance of Deltamethrin, Cyfluthrin and Cypermethrin on *M. domestica*; compared the adult females of the collected strain with laboratory strain, according the LD_{50} or LD_{90} values (Table 1). Concerning the population of Jeddah Governorate; the resistant ratios in the adult females to the toxicity of the three insecticides that mentioned previously at LD_{50} levels were 0.0185,0.0223 and 0.0133 Deltamethrin, Cypermethrin

and Cyfluthrin against laboratory strain, respectively, while were 0.0759, 0.0645 and 0.0846 against field strain, respectively. On the other hand, LD_{90} levels were 0.3547, 0.4832 and 0.2821 Deltamethrin, Cypermethrin and Cyfluthrin against laboratory strain, respectively, while were 1.5345, 1.5674 and 1.9371 against field strain, respectively. Results indicated that the resistance ration in each tested pyrethroid insecticides were 4.10, 2.89 and 6.36 fold for Deltamethrin, Cypermethrin and Cyfluthrin, respectively (Table 1).

The obtained results disagreed with those obtained by **Sharififard and Safdari (2003)** they determine the susceptibility or resistance of the house fly, which were collected from 3 livestock farms, to prevalent pyrethroid insecticides (delthamethrin, lambda-cyhalothrin and cypermethrin). The bioassay of the field-collected house fly using topical method confirmed that all the three house fly strains have developed very high resistance to pyrethroid insecticides in comparison to the susceptible strain. In other study **Pezzi *et al.* (2011)** evaluated toxicity of some pyrethroids by topical applications on adults of a *M. domestica* strain (OcRo) collected from an intensive chicken farm in Northern Italy, in comparison to a susceptible strain (s-DBF). The OcRo strain exhibited higher levels of resistance (RR□□) to pesticides in comparison to s-DBF strain. These results indicate that OcRo strain is now multiresistant to pyrethroids, and this should be considered for an environmentally safe pest management. On the other hand, **El-Zahrany (2012)** determined the susceptibility and resistance of house flies strains *M. domestica*. In some locations in Riyadh city to the traditional insecticides used by the Riyadh Municipality for flies control, and the effectiveness of the new bioinsecticides as an alternative to these insecticides. Four traditional insecticides were tested on these five strains, Diazinon, Deltamtherin, Lambda-Cyhalothrin and Alpha-Cypermethrin. The LD50 for north slaughterhouse strain were 1.089, 0.295, 0.132 and 0.09 g/female fly, respectively, and the resistance factor (RF) to these insecticides compared to the laboratory strain was 7, 17, 10 and 11-fold respectively. The LD50 for Azizia slaughterhouse strain were 2.737, 0.191, 0.388 and 0.357 g/female fly, respectively, and the resistance factor (RF) to these insecticides compared to the laboratory strain was 17, 11, 30 and 45-fold respectively. The LD50 for Alsaadah district slaughterhouse strain were 11.516, 0.718, 0.707 and 1.103 g/female fly, respectively, and the resistance factor (RF) to these insecticides compared to the laboratory strain was 72, 41, 54 and 138-fold respectively. LD50 for west of Riyadh slaughterhouse strain were 6.335, 0.236, 0.544 and 0.792 g/female fly, respectively, and the resistance factor (RF) to these insecticides compared to the laboratory strain was 40, 13, 42 and 99-fold respectively. Finally, The LD50s for Almowanisiyah slaughterhouse strain were 1.583, 0.359, 0.185 and 0.465 g/female fly, respectively, and the resistance factor (RF) to these insecticides compared to the laboratory strain was 10, 21, 14 and 58-fold respectively.

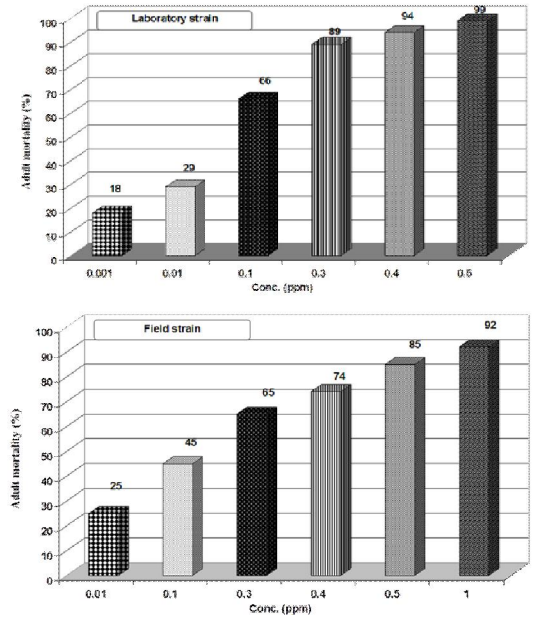

Fig. (1): %Mortality of the adult females of laboratory and field strains of *M. domestica* after treated with different concentrations of Deltamethrin 2.5% using topical application bioassay technique.

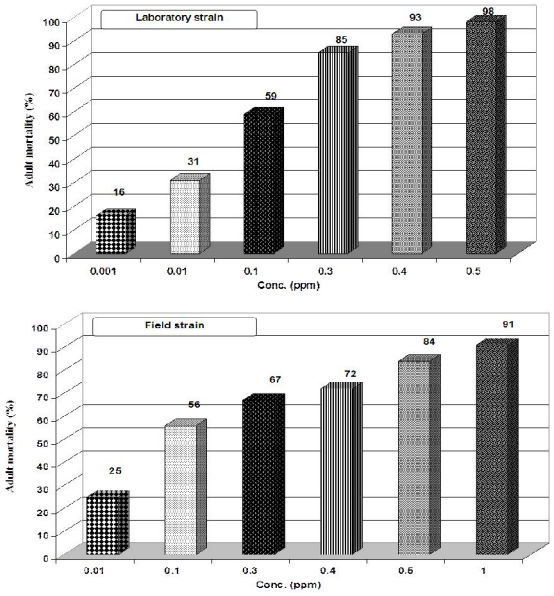

Fig. (2): %Mortality of the adult females of laboratory and field strains of *M. domestica* after treated with different concentrations of Cypermethrin 10% topical application bioassay technique.

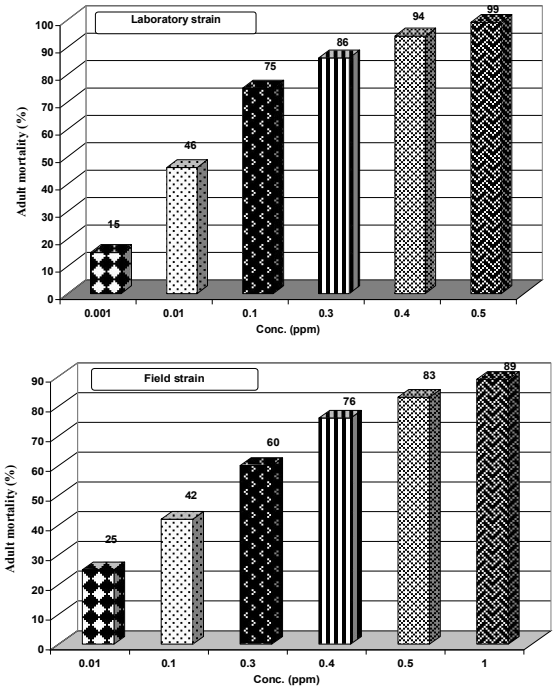

Fig. (3): %Mortality of the adult females of laboratory and field strains of *M. domestica* after treated with different concentrations of Cyfluthrin 5% using topical application bioassay technique.

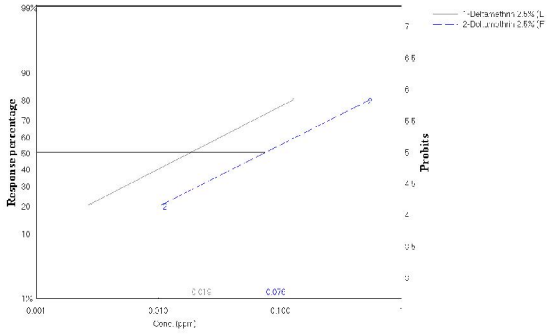

Fig. (4): Regression lines for Deltamethrin 2.5% bioassay on adults of laboratory and field strains of *Musca domestica* using topical application bioassay technique.

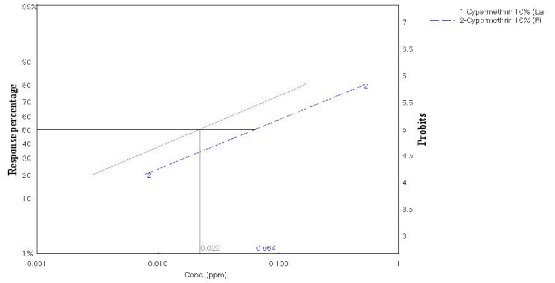

Fig. (5): Regression lines for Cypermethrin 10% bioassay on adults of laboratory and field strains of *Musca domestica* using topical application bioassay technique.

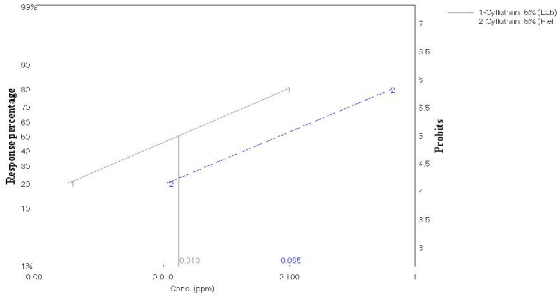

Fig. (6): Regression lines for Cyfluthrin 5% bioassay on adults of laboratory and field strains of *Musca domestica* using topical application bioassay technique.

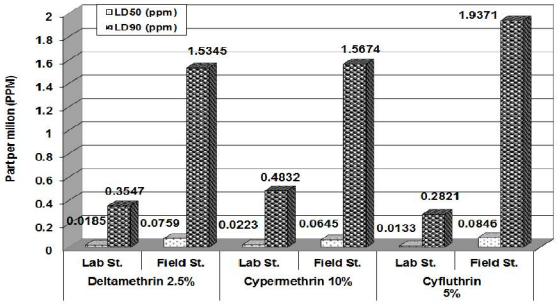

Fig. (7): Toxicity values of Deltamethrin 2.5%, Cypermethrin 10% and Cyfluthrin 20% against adults of laboratory and field strains of *Musca domestica* using topical application bioassay technique.

Table (1): Susceptibility of *Musca domestica* adults females (lab and field strains) to chemical insecticides Deltamethrin 2.5%, Cypermethrin 100 and Cyfluthrin 5% following continuous exposure for 24 h using topical application bioassay technique.

Insecticide	Housefly strain	Effective conc. (ppm)	Adult mortality (%)[a]	Statistical parameters[b]								
				LD_{50} (ppm)	LD_{90} (ppm)	Slope	X^2 $(Chi)^2$ C	X^2 $(Chi)^2$ T	P	R C	R T	RR
Deltamethrin 2.5%	Lab St.	0.001-0.5	18-99	0.0185	0.3547	0.9995	25.15	9.5	0.0	0.94	0.81	4.10
	Field St.	0.1-1.0	25-92	0.0759	1.5345	0.9817	13.47	9.5	0.0092	0.95	0.81	
Cypermethrin 10%	Lab St.	0.001-0.5	16-98	0.0223	0.4832	0.9594	25.45	9.5	0.0	0.93	0.81	2.89
	Field St.	0.1-1.0	25-91	0.0645	1.5674	0.9248	6.63	9.5	0.1567	0.97	0.81	
Cyfluthrin 5%	Lab St.	0.001-0.5	15-99	0.0133	0.2821	0.9663	8.68	9.5	0.0695	0.95	0.81	6.36
	Field St.	0.1-1.0	25-89	0.0846	1.9371	0.9425	15.55	9.5	0.0037	0.94	0.81	

a: Five replicates, 20 adults each; control mortalities ranged from 0.0%-3.0%. b: Litchfield and Wilcoxon (1949).
When tabulated $(Chi)^2$ larger than calculated at 0.05 level of significance indicates the homogeneity of results
C = Calculated; T= Tabulated; RR = Resistant ratio

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