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PROPOXUR RESISTANCE OF HOUSEFLY, *MUSCA DOMESTICA* L., IN TAICHUNG[1]

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T. C. Wang, H. L. Kao and K. S. Kung (1985) Propoxur resistance of housefly, *Musca domestica*, L., in Taichung. *Bull. Inst. Zool., Academia Sinica* **24**(1): 139-146. Investigations on housefly resistance to propoxur in Taichung were reported. Propoxur is one of the most widely used insecticides for housefly control in Taiwan. Housefly in Taichung area was found ca. 200 times more resistant to propoxur than a standard resistant strain, *Rut*; *bwb*; *ocra*. Biochemical mechanisms conferring the propoxur resistance here at least included altered acetylcholinesterase, altered aliesterase and high microsomal mixed-function oxidase. Genetic factor for the resistance was probably multiple. Parathion and diazinon significantly increased the insecticidal effect of propoxur on local housefly. Methyl parathion, however, was found antagonistic.

Housefly, *Musca domestica* L., is one of the pest insects with medical importance in Taiwan. Control of this insect completely relied on insecticides in this island. Propoxur, a cholinesterase inhibitor with fast knockdown and long residual effects, has dominated the chemical control list for many years. Recently, control failure of this insecticide was reported from Taichung area. Development of insecticide resistance in pest insect is one of the reasons which generally cause the failure of chemical control. Unfortunately, the information in Taiwan was absent to do any judgement.

In 1971, Brown and Pal pointed out that the housefly at least is found resistant to 11 organochlorines, 18 organophosphates, 5 carbamates, 7 pyrethroids and 2 growth inhibitors. In their paper, first report of insecticide resistance of housefly in Taiwan also was included. A more complete report was later made by Hayashi and Hatsukade in 1974. In their results, housefly is resistant to malathion and tolerant to fenitrothion but susceptible to other insecticides including DDT, γ-BHC, diazinon, Baytex, DDVP, pyrethrins and allethrin.

Our studies reported here were aimed to investigate the propoxur resistance in housefly of Taichung area. With this paper, we described the resistance levels and two of major resistance mechanisms, i. e., altered ali-esterase (AliE) and altered acetylcholinesterase (AChE). Primary investigation on the possibility of resistance due to high microsomal mixed-function oxidase also was included.

MATERIALS AND METHODS

Housefly Strains

Normal Susceptible Strain (*NS*): Suscep-

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tible, wild type in phenotype.

Aristapedia, Carnation Eye Strain (*ar, car*): Susceptible, arista tarsal segment-like and compound eyes carnation in color. Those 2 mutant characters are controlled by 2 recessive genes on chromosome II.

White Eye, Brown body, Point Wing Strain (*w,bwb,pw*): Susceptible, compound eyes white and body brown in color, wings pointed. Mutant characters are controlled by 3 recessive genes on chromosome III.

Rutgers; Brown Body; Ocra Eye Strain (*Rut;bwb;ocra*): Resistant to diazinon and other insecticides due to high DDT dehydrochlorinase, altered ali-esterase, high microsomal mixed-function oxidase and high glutathione transferase. Phenotypes differ from wild type fly in having brown body and yellow compound eyes, which are controlled by 2 recessive genes on chromosome II. Genes responsible for all resistance mechanisms described above are located on chromosome II.

Paration Resistant, Carnation Eye, Classical Wing Strain (*PR,car,clw*): Resistant to methyl and ethyl parathion due to altered ali-esterase, which is controlled by a semidominant gene on chromosome II. Another semidominant gene on chromosome II also is responsible for high DDT dehydrochlorinase and causes DDT resistant in this strain. Two recessive genes on chromosome II make fly in this strain having carnation compound eyes and 2 wings perpendicular to the body axis.

Tripathi-Cholinesterase Strain (*Tri-ChE*): Resistant to tetrachlorvinphos and other insecticides due to the altered acetylcholinesterase and 4 other mechanisms as those in *Rut*; *bwb*; *ocra* strain. Fly is wild type in phenotype.

Kaohsiung 4 Strain (K_4): Isofemale line collected from Kaohsiung, Taiwan in December, 1981.

Taichung Strains: *T-C A* and *T-C B* were collected from Garbage Dumping Area in Taichung on January 22 and February 17, 1982, respectively, and left unselected by any insecticide ever since. *T-C 5*, *6* and *9* were selected from *T-C A*.

Insecticides

Diazinon: *0, 0*-Diethyl *0*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate, 95% technical grade, supplied by Shell Company Branch in Taiwan.

Methyl Parathion: *0, 0*-Dimethyl-*0*-*p*-nitrophenyl phophorothioate, 80% technical grade, supplied by Ming-Feng Chemical Company in Taiwan.

Parathion: *0, 0*-Diethyl *0*-*p*-nitrophenyl phosphorothioate, 95% technical grade, supplied by Shell Company Branch in Taiwan.

Piperonyl Butoxide (pb): alpha-(2-(2-Butoxyethoxy) ethoxy)-4, 5-methylenedioxy-2-propyltoluene, 97% technical grade, supplied by Chi-Cheng Chemical Engineering Company in Taiwan.

Propoxur: 2-(1-Methylethoxy) phenyl methylcarbamate, 99% technical grade, supplied by Shell Company Branch in Taiwan.

Triphenylphosphate (TPP): purchased from Merck, W. German.

Bioassay

Tested insecticide was diluted in acetone to prepare a series of 5 to 8 different concentrations. Residue film method proposed previously (Hoyer *et al.*, 1965; Plapp and Casida, 1969) was used to test the toxicity of insecticide to housefly. Exact 24 hours later, the mortality was counted and transferred into LD_{50} by probit analysis.

In the study of synergistic effect, synergist of maximum dose that killed no housefly at all was used to pretreat the insect for 24 hours before the insecticide was used for bioassay. All bioassay and housefly rearing were performed at 25±2°C. Calculations of synergistic effect were as follows:

$$\text{Synergistic Effect (SR)} = \frac{LD_{50} \text{ of insecticide alone}}{LD_{50} \text{ of insecticide with the pretreatment of synergist}}.$$

***In vitro* assay of acetylcholinesterase activity**

Method developed by Ellman *et al.* (1961)

and modified by Motoyama *et al.* (1980) was used. Tested housefly included *NS*, *Tri-ChE* and *T-C* 9 strains. Four to 7-day old female adult was decapitated on dry ice. Enzyme was prepared by homogenizing each head with .5 ml phosphate buffer (.1 M, pH 7.0) in ice bath. Homogenate was filtered through glasswool and activated in 17-19°C water bath for 10 minutes before assayed. Enzyme preparation of .2 ml, after the additions of 1.3 ml phosphate buffer (.1 M, pH 8.0), 1.0 ml dithiobisnitrobenzoic acid and .5 ml acetylcholine iodide (4×10^{-4} M), was used to measure the absorbance change continuously at 412 nm by using a Jasco UVIDEC-1 DB spectrophotometer. The enzyme activity, being expressed as *mole substrate hydrolyzed/min./housefly head*, was calculated as follow:

$$\text{Enzyme Activity (R)} = 1.1\times10^{-3}\times\frac{\text{absorbance change/minute}}{\text{numbers of housefly head/ml homogenizing buffer}}.$$

In vitro inhibition of acetylinesterase

Same method as described above was used here to measure the acetylcholinesterase activity except that the enzyme had been exposed to propoxur before assay was started. Propoxur was prepared to a series of 7 to 8 different concentrations in acetone. Fifty μl of each concentration was added into the test tube. Air then was blown into the tube to dry the residual acetone in the bottom. After the dry was complete, .4 ml enzyme preparation was added into the test tube and consistently shaken in a 17-19°C water bath for 10 minutes. The enzyme then was measured for its activity as described previously. The inhibition percentage was calculated as follow:

$$\text{Inhibition Percentage (I\%)} = 100\% - \frac{\text{Activity after Inhibition}}{\text{Activity without Inhibition}}\times100\%.$$

Probit analysis was later used to translate the I% values into I_{50} value for propoxur.

In vitro assay of ali-esterase activity

Hestrin's method modified by Robbins *et al.* (1958) and Bigley and Plapp (1960) was used to measure the ali-esterase activity in 12 strains of housefly in this experiment. For each 2 female adults of 4 to 7-day old, 1 ml phosphate buffer (.05 M, pH 7.4) was used to homogenize in the ice bath. After filtered through glasswool, 2 ml of enzyme preparation was added into each test tube and incubated in 32°C water bath for 15 minutes. One ml methyl butyrate (.04 M in acetate buffer and phosphate buffer) was used as substrate and incubated with enzyme preparation in 32°C for another 30 minutes. Two ml alkaline hydroxylamine (2 M hydroxylamine hydrochloride-3.5 M sodium hydroxide, 1:1) then was added to stop the reaction. One ml 4N HCl and 1 ml ferric chloride (.37 M in .1N HCl) were laterly used to react with the product. Whatman No. 40 paper was used to filter the whole mixture before the absorbance was measured at 540 nm in DB spectrophotometer. Enzyme activity was transferred from the absorbance values by ploting to a standard curve and expressed as μmole/mg/30 min.

Field investigation of resistance

Collection of housefly in Garbage Dumping Area in Taichung, Taiwan was made in August, 1982. Twenty-nine isofemale lines were established in the laboratory from the collection. F_1 females were bioassayed with 1 mg/jar propoxur. The mortality were recorded and compared with those of laboratory strains to understand the resistance of housefly in field.

RESULTS AND DISCUSSION

Susceptibility to propoxur

Susceptibility of 12 housefly strains to propoxur was presented in Table 1. The *w, bwb,pw* strain was the most susceptible among all. Its LD_{50}, .0077 mg/jar, therefore, was used as the denominator to measure the resistance ratio (RR) for other strains. The other 2 susceptible strains, *ar*, *car* and *NS*, were about 2 times less susceptible than *w,bwb,pw*.

Rut;bwb;ocra and *Tri-ChE* strains were

24.7 and 10.1 times more resistant than *w, bwb,pw*, respectively. *PR,clw,car*, with only one resistant mechanism, was the most susceptible among 3 resistance strains tested. The 6-fold resistant level raised in *PR,clw,car* indicated that the detoxification mechanism of altered ali-esterase might cause the cross resistance to propoxur in housefly.

TABLE 1
Susceptibility of housefly to propoxur

Strain	Slope	LD_{50}[1]	RR[2]
w, bwb, pw	2.24	.0077	1.0
ar, car	2.10	.015	1.9
NS	2.86	.018	2.3
PR, clw, car	2.82	.047	6.1
Tri-ChE	.80	.078	10.1
K_4	1.17	.13	16.9
Rut; bwb; ocra	2.57	.19	24.7
T-C B	.95	.71	92.2
T-C A	.89	.77	100.0
T-C 5	.44	4.49	583.1
T-C 6	.73	23.46	3046.8
T-C 9	.42	38.51	5001.3

1) mg/300 ml jar.
2) RR (Resistance Ratio)
$$= \frac{(LD_{50} \text{ of each strain})}{(LD_{50} \text{ of w, bwb, pw strain})}.$$

Local strains established from the collection in Taichung were found to be the most resistant. Strains without selection, i. e., *T-C A* and *T-C B*, were about 100 times more resistant than *w,bwb,pw*. After the selection, the resistant levels increased to ca. 58-, 305- and 500-fold higher than those of original strains in *T-C 5*, *T-C 6* and *T-C 9*, respectively. The changes of the resistant level during the selection course was shown in Fig. 1.

The single strain collected from Kaohsiung, the K_4, was less resistant than those from Taichung. No selection has been made on this strain since collection. However, its resistant level was higher than all resistant strains but *Rut;bwb;ocra*.

Stability of resistance in Taichung strain

Resistance levels in housefly strains were

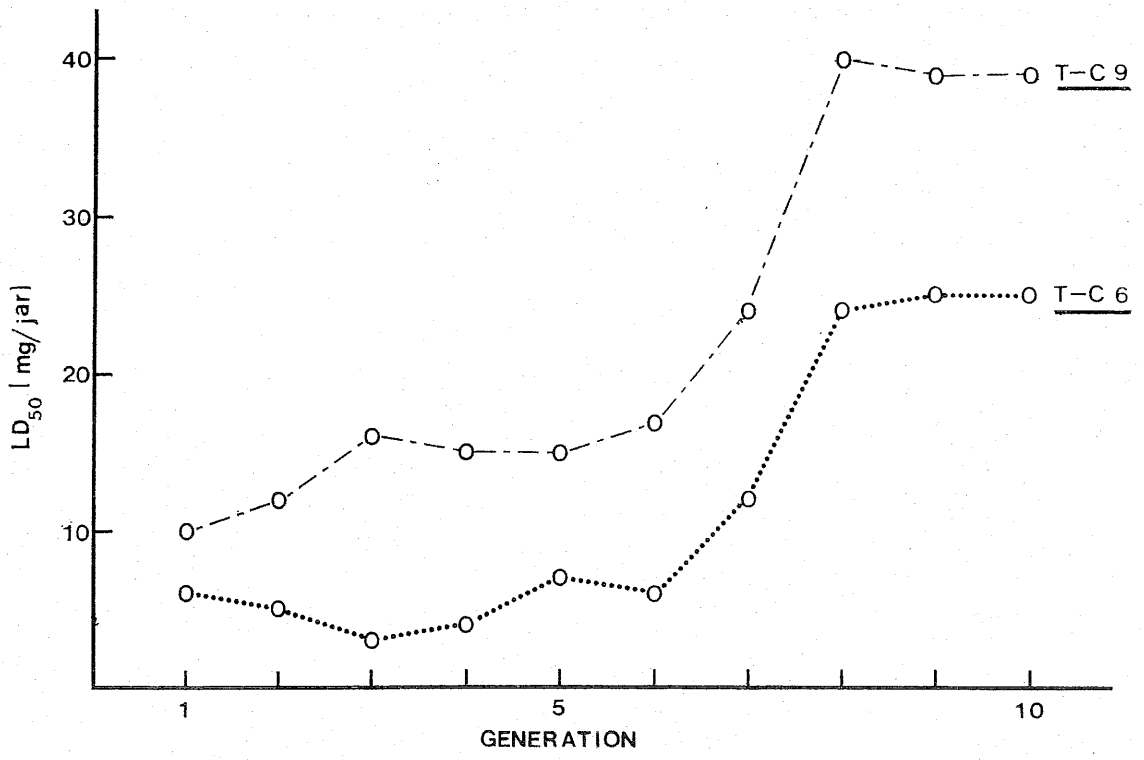

Fig. 1. Establishment of T-C 9 and T-C 6 local resistant strains.

stable after the 8-generation consecutive selection with propoxur (Fig. 1). However, after the selection pressure had removed, the resistance level in *T-C 9* strain dropped immediately (Table 2). But even at the most susceptible level, i. e., LD_{50} is .68 mg/jar, *T-C 9* was still 3- to 4-fold more resistant than *Rut;bwb;ocra*.

The slope values of DM-curve, which used to represent the heterogenesis of the test population to insecticide, ranged from .42 to .95 in strains collected from Taichung. In all strains but *Tri-ChE*, slope values of DM-curve were higher than those of Taichung strains, ranging from 1.17 to 2.86. It seemed that the Taichung strains were more heterogenous to propoxur treatment than others. However, continuous selection with purpoxur in Taichung strains for more than one year failed to increase the slope values of DM-curve (Table 2).

TABLE 2
Fluctuation of LD_{50} and slope of dosage-mortality curves after the selecting pressure of propoxur removed

Date	LD_{50}[1)]	Slope
Jun. 4th, 1982	38.51	.42
Aug. 23rd, 1982	.68	.57
Nov. 22nd, 1982	14.29	.38
Jan. 26th, 1983	1.24	.52
Mar. 31st, 1983	1.68	.36

1) mg/300 ml jar.

In 3 resistant strains we tested, *Tri-ChE* was the only one similar to Taichung strains with a very low slope value. *Tri-ChE* strain differs from other 2 resistant strains in having 2 gene loci located distantly on chromosome II for insecticide resistance (Plapp and Wang, 1981, 1983). *PR,clw,car* and *Rut;bwb;ocra*, on the other hand, were known for having either one single gene or several genes closely linked for insecticide resistance. Therefore, the slope values of DM-curve in Taichung strains probably reflected that the genetic factor for propoxur resistance in those strains were multiple and apparently not closely linked or even dispersed on different linkage groups.

Field investigation of resistance

Results of field investigation in garbage dumping area of Taichung city were presented in Fig. 2. Over 83% flies collected, i. e., 24 out of 29 strains, were more resistant than the

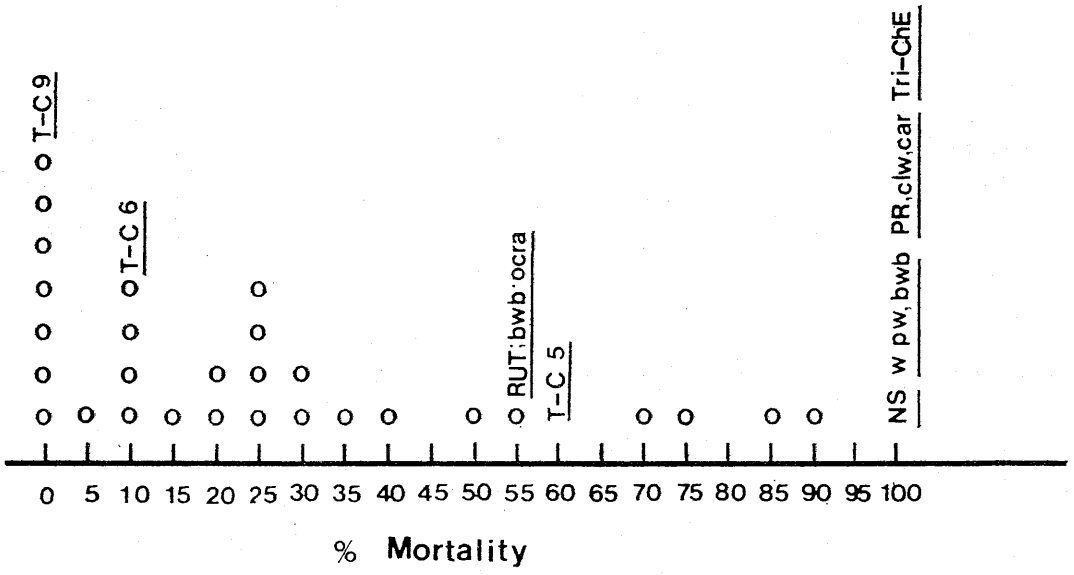

Fig. 2. Percent mortality of housefly at a given propoxur dose of 1 mg/jar. "O" on the graph represents F_1 flies of each strain established from field investigation.

TABLE 3
In vitro acetylcholinesterase activity of housefly and its inhibition by propoxur

Strain	Activity[1]	AR[2]	I_{50}[3] (M)	IR[4]
NS	6.70	1.0	3.93	1.0
Tri-ChE	6.05	.9	73.60	18.7
T-C 9	18.90	2.8	3958.05	1007.1

1) micromole hydrolyzed/min./housefly head.
2) AR=activity ratio=(activity of each strain)/(activity of NS strain).
3) Values in the column represent 10^{10} times actual values.
4) IR=inhibition ratio=(I_{50} of each strain)/(I_{50} of NS strain).

most resistant strain, *Rut*; *bwb*; *ocra*. *Tri-ChE*, *PR*, *clw*, *car* as well as 3 susceptible strains showed more susceptible than all Taichung flies collected. *T-C 9* strain was among the most resistant group which 1 mg/jar killed no fly at all (Fig. 2). Mortality of *T-C 6* and *T-C 5* strains were 10% and 60% at this dose, respectively. This result also indicated that the *T-C 9* strain was qualified to represent the resistant fiies in the flied.

Acetylcholinesterase activity

In vitro acetylcholinesterase (AChE) activity of *NS*, *Tri-ChE* and *T-C 9* strains and their inhibition by propoxur were presented in Table 3. AChE activity in *T-C 9* strain was about 3 times higher than those in *NS* and *Tri-ChE* strains. The resistance of AChE to the inhibition of propoxur, which expressed as I_{50} in the Table 3, was one thousand times higher in *T-C 9* than that in *NS* and 54 times higher than that in *Tri-ChE* strain. The results indicated that the *T-C 9* strain not only had more AChE activity but also had altered AChE for its resistance to propoxur.

Ali-esterase activity

The ali-esterase (Ali-E) activity of 8 strains were shown in Table 4. Three susceptible strains, including *ar,car,w,bwb,pw* and *NS*, had the highest Ali-E activity among all. K_4 strain was lower than *Tri-ChE* and higher than other 2 resistant strains. *T-C 9* strain was almost equal to *PR,clw,car*, the typical low Ali-E strain, in Ali-E activity. In other words, the altered Ali-E was one of the resistance mechanisms in *T-C 9* for propoxur resistance.

TABLE 4
Ali-esterase activity of housefly

Strain	Activity[1]	R[2]
ar, car	54.40	20.0
w, bwb, pw	51.85	19.1
NS	50.05	18.4
Tri-ChE	30.93	11.4
K_4	27.29	10.0
Rut; bwb; ocra	10.68	3.9
PR, clw, car	3.45	1.3
T-C 9	2.72	1.0

1) micromole methyl butyrate hydrolyzed/mg/30 min.
2) $R=\text{ratio}=\frac{(\text{activity of each strain})}{(\text{activity of T-C 9 strain})}$.

The correlation between propoxur resistance and the Ali-E activity was negative (Fig. 3). This confirmed the fact described previously by others that higher the Ali-E activity, lower the resistance level in housefly (Oppenoorth 1959, Bigley and Plapp 1960).

Synergistic effect

Synergistic effects of pb, the microsomal mixed-function oxidase inhibitor, and TPP, the esterase inhibitor, on propoxur in *Rut*; *bwb*;*ocra* and *T-C 9* strains were presented in Table 5. The higher synergistic ratio (SR) of pb than that of TPP in *Rut*;*bwb*;*ocra* confirmed that this strain was resistant mainly because the high oxidase activity. In *T-C 9*

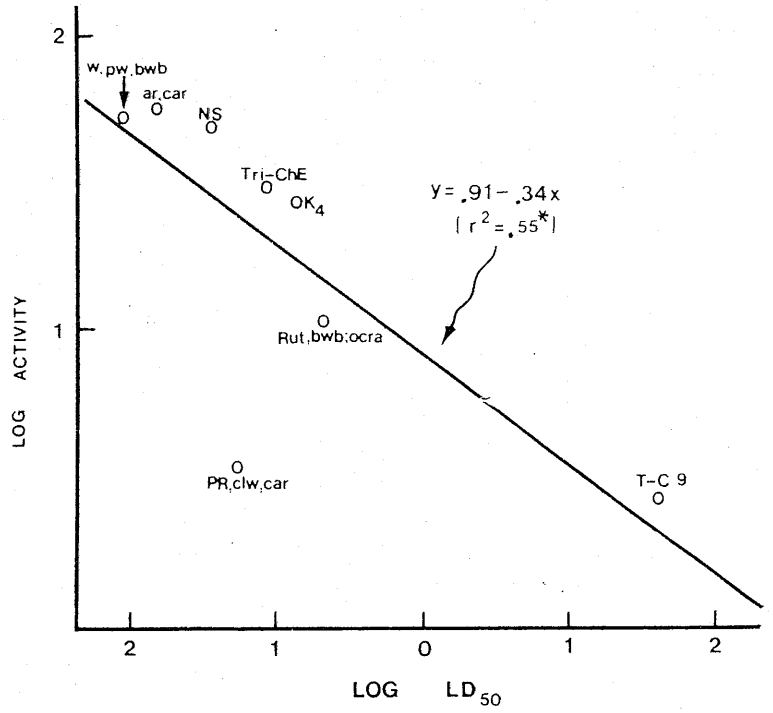

Fig. 3. Correlation of propoxur resistance and ali-esterase activity in housefly strains.

TABLE 5
Synergistic effect of pb and Tpp to propoxur in Rut; bwb; ocra and T-C 9 strains of housefly

Treatment	Rut; bwb; ocra		T-C 9	
	LD_{50}[1]	SR[2]	LD_{50}[1]	SR[2]
Propoxur	.19	—	10.11	—
Propoxur+pb	.016	11.9	1.87	5.4
Propoxur+TPP	.037	5.1	3.83	2.6

1) mg/300 ml jar.
2) SR=synergistic ratio= $\frac{(LD_{50} \text{ of insecticide alone})}{(LD_{50} \text{ of insceticide and synergist used combinedly})}$.

TABLE 6
Synergistic effect of 3 dialkyl phosphorothioate insecticides to propoxur in housefly

Treatment	LD_{50}[2]	SR[3]
Propoxur	14.29	—
Propoxur+Methyl Parathion[1]	54.43	.26
Propoxur+Parathion[1]	.77	18.56
Propoxur+Diazinon[1]	.70	20.41

1) 10^{-3} mg/300 ml jar.
2) mg/300 ml jar.
3) SR=synergistic ratio
=(LD_{50} of propoxur)/(LD_{50} of propoxur+dialkyl phosphorothioate).

strain, both pb and TPP increased the insecticidal effect of propoxur significantly. However, SR of pb was about twice that of TPP, indicating that the oxidase also was more significant for propoxur resistance in *T-C 9* strain.

Oppenooth in 1972 discovered that the diethyl phenyl phosphorothionate (SV_1) is synergistic to organophosphates and carbamates (Eto 1976). In our results, 2 organophosphates with similar chemical structure to SV_1, i. e., parathion and diazinon, showed satisfactory effect when used combinedly with

propoxur (Table 6). In the results, .1% of those 2 dialkyl phosphorothionates increased the SR to 18.56 and 20.41, respectively. However, methyl parathion was found antagonistic to propoxur in *T-C 9* strain.

CONCLUSION

In Taiwan, housefly is still one of the most serious pest insects with medical importance. Due to the absence of detailed studies in its insecticide resistance, chemical control had been no strategies whatsoever. Our reports indicated that the propoxur resistance in this insect was quite serious. Besides, housefly in Taichung area has developed a versatile system for detoxifying the insecticide, including at least 2 metabolic mechanisms and one target site change. Control failure with chemical tools in this area, therefore, should not be surprised. In order to get the status of housefly control improved, more researches on illucidating the insecticide resistance are needed. Our report here is thus not a conclusion but a start.

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臺中地區家蠅對安丹之抗藥性

王清澄 高慧蓮 貢穀紳

本研究之目的在瞭解臺中地區家蠅對安丹之抗藥性，安丹爲本省防治衞生昆蟲上主要的藥劑之一。以國際上最常用的抗性品系，Rut; bwb; ocra 爲例，臺中地區家蠅對安丹的抗性比其高出約兩百倍左右。根據我們的研究，這個地區之家蠅對安丹之所以產生抗性，在生化的機制上，至少包括轉變型的乙醯膽鹹脂酶，轉變型的水解脂酶與高活性的氧化酶，控制臺中地區家蠅抗性的遺傳因子可能是多因子，而且不似世界其他地方之家蠅一般緊密地連鎖在一起。巴拉松與大利松對抗丹之家蠅有良好的協力作用，而甲基巴拉松則呈拮抗作用。