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Tracing Monooxygenase Level by Synergistic Bioassays in Field and Sequential Generations of House Flies (Diptera: Muscidae)*

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Abstract. Carbaryl, parathion, and diazinon bioassays were conducted in field through fourteen sequential generations of house fly, *Musca domestica* (L.) to trace monooxygenase level and role in the detoxification process. Parathion was more toxic to susceptible (S) strain (LC_{50}0.013μg/vial) than diazinon (LC_{50} 0.33μg/vial). Carbaryl showed the lowest toxicity against the flies (LC_{50} 35μg/vial). The toxicities of carbaryl, parathion, and diazinon against S strains in relation to the field strains have been increased by 11, 45, and 346-folds respectively. Pretreatment of insects with piperonyl butoxide (Pb) inhibited the monooxygenase system, depriving the insects of a key defense mechanism against the three insecticides used in this study. Almost similar percent dependency values were obtained for the field strains (64, 62, and 61%) and S strains (71, 60, and 53%) on monoxygenases for detoxification of carbaryl, parathion, and diazinon respectively.

Introduction

Tolerance of insects to pesticides is a very known phenomenon. It can finally be due to any of several factors acting alone or in combination. In many cases, detoxification of insecticides by the metabolic action of enzymes is a major factor in insecticide tolerance. There is evidence, however, that monooxygenase detoxification mechanism is of far greater importance than others [1].

Biological assays are recommended for the detection and confirmation of insecticide tolerance or resistance. They can be conducted with insecticides and synergists to estimate relative detoxification role of the various enzyme systems. Brattsten and Metcalf [2,3,4], have suggested that the use of Pb which inhibits monooxygenases, could be used to alter the LD_{50} of carbaryl, which is metabolized by monooxygenases to indicate *in vivo* monooxygenase level in insects. Brindley [5] has indicated that synergist difference (SD) and percent dependency (%D) were more useful parameters in estimating monooxygenase or other enzyme system *in vivo* than synergist ratio.

Bioassays could effectively be used if the physiological and environmental factors are standardized [6]. Previous work, however, confirmed the effectiveness of the insecticide bioassay and revealed population differences in susceptibility and detoxification potential in field populations of black grass bugs [7] and pea aphids [8].

It is well established that monooxygenase activity shows considerable variation with respect to the physiological factors such as species, strain, sex, age, stage of development, and endogenous or exogenous factors and the environmental factors such as pesticide pressure, diet, and secondary plant substances [9,10,11].

Therefore, this study was organized to test the ability of the synergistic contact bioassy for tracing monooxygenase level in field through sequential generations of house fly and estimating its role in carbaryl, parathion, and diazinon detoxification. The data of the present study are interpreted by calculations of SD and %D values [5].

Materials and Methods

Insects

Laboratory strain of house flies was reared on natural diet of milk and sugar as described by Al-Rajhi *et al.* [12]. Pupae were collected from decayed matter and organic wastes located in the farm of Animal Production Department, Oleisha, Riyadh. Adults of the first generation were considered as a field strain, those of the fourteenth generation were considered susceptible strain.

Chemicals

In addition to the synergist, Pb, three insecticides were also used in this study: Carbaryl (1-naphthyl N-methylcarbamate (analytical grade, 99.4%). Parathion (0,0-diethyl -0-p-nitrophenyl phosphorothioate (analytical grade, 99.9%) and diazinon [0.0-diethyl-0- (2-isopropyl 4-methyl -6- pyrimidly) phosphorothiolate] (analytical grade, 99.9%). These chemicals were supplied by the United States Environmental Protection Agency.

Bioassay procedures

Chemicals and insects were introduced into the bioassay vials as follows:

Parathion, diazinon, carbaryl, and Pb were dissolved in acetone and diluted to make several concentrations of insecticides and 100 μg Pb per vial. A volume of 0.5 ml of each concentration was transferred to each vial. The solutions containing the

insecticides or the synergist were then allowed to evaporate by placing each vial side ways on a breadboard rolling the vials as evaporation occurred. This action permitted the solution inside the vials to leave a residue evenly distributed on the inner walls of the vials. Each bioassay included at least 5 different concentrations of insecticide and each concentration was replicated 4 times. The insects were pretreated with Pb by placing them in vials containing 100 μg residues of Pb. The insects were then transferred after 4h into vials containing several concentrations of insecticides. The vials were covered with cheesecloth. Five insects were used in each treated vial. The bioassay temperature was held at 25 + 0.5 C. Several precautions were taken to insure that insects were alive and healthy during the 24h time interval of each experiment.

Results

Figures 1-3 show the susceptibility of house fly generatins to carbaryl, parathion, and diazinon with and without Pb. The figures clearly indicate that the susceptibility of house fly generations to the three insecticides increased considerably as house flies advanced in generation. The toxicities of carbaryl, parathion, and diazinon have increased by 11, 45, and 346-fold respectively for the S strain compared to field strain (Fig. 4-6). Parathion was more toxic to the S strain (LC_{50} 0.013 μg per vial) than diazinon (LC_{50} 0.33 μg per vial). Carbaryl showed the lowest toxicity

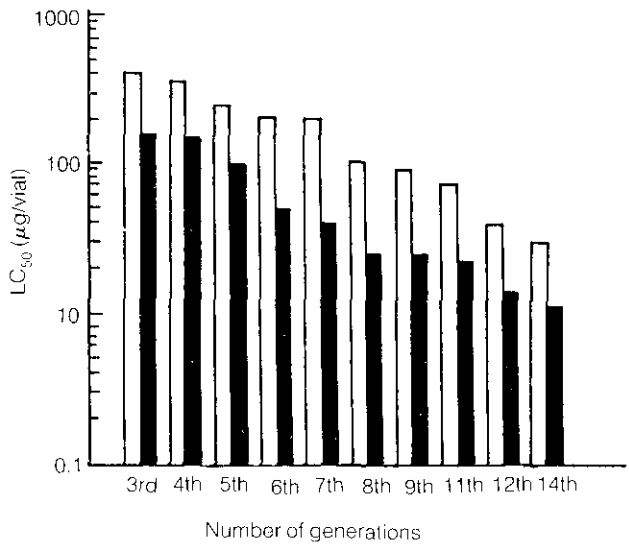

Fig. 1. Susceptibility of house fly generations to carbaryl and carbaryl + piperonly butoxide

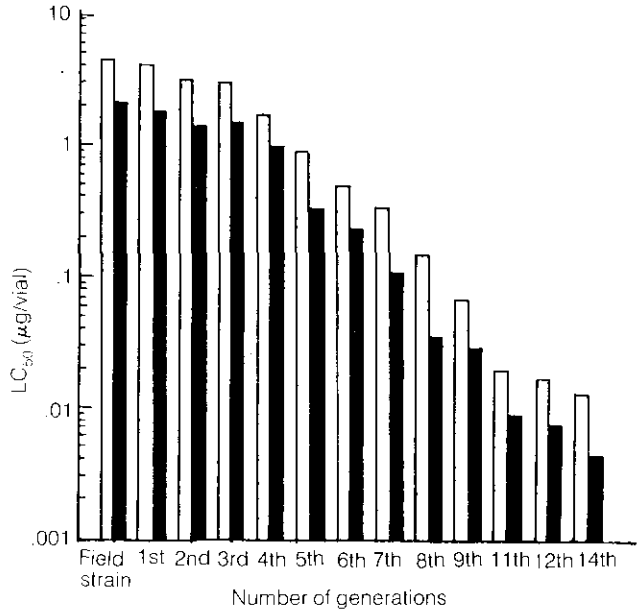

Fig. 2. Susceptibility of house fly generations to parathion + and parathion + piperonly butoxide

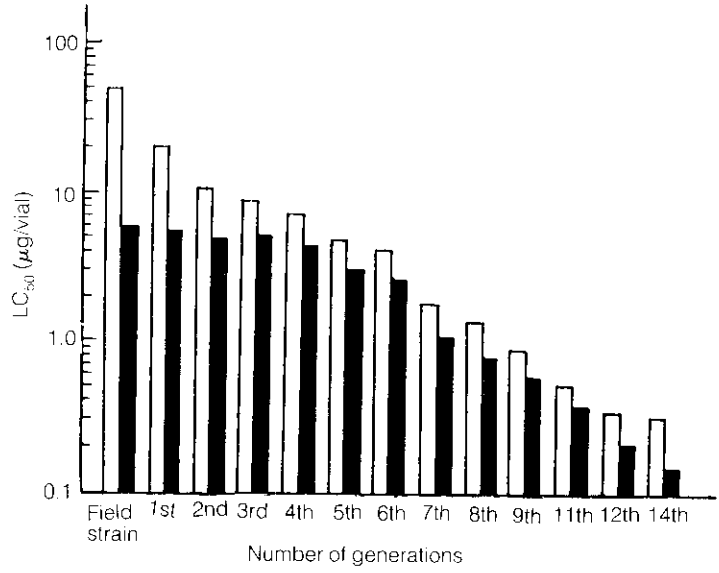

Fig. 3. Susceptibility of house fly generations to diazinon and diazinon + piperonyl butoxide

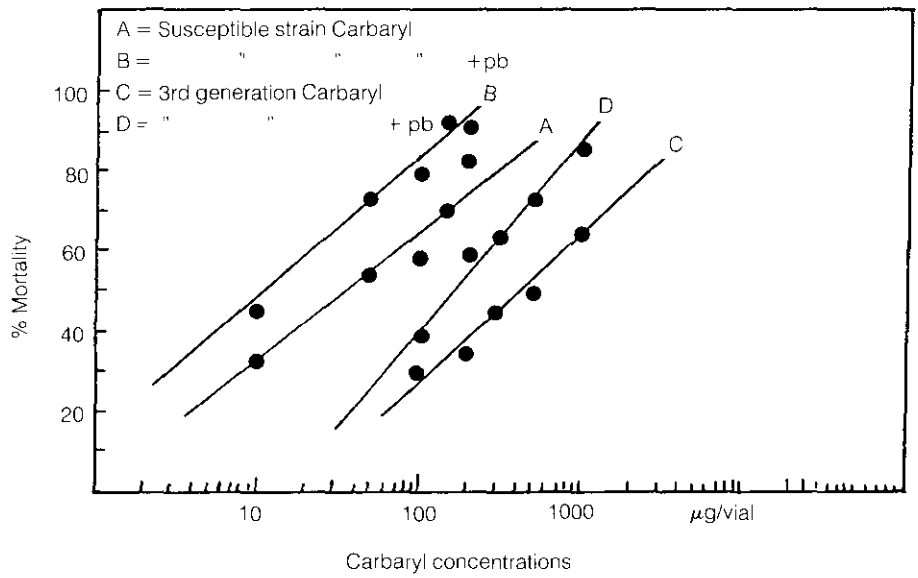

Fig. 4. Toxicity of carbaryl on susceptible strain and 3rd generation of house flies with and without piperonyl butoxide

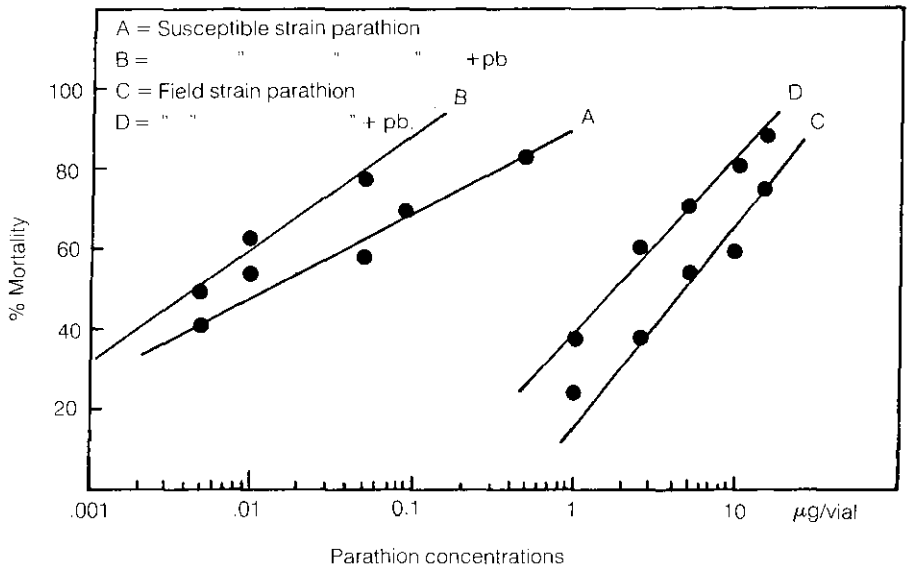

Fig. 5. Toxicity of parathion on susceptible and field strains of house flies with and without piperonyl butoxide

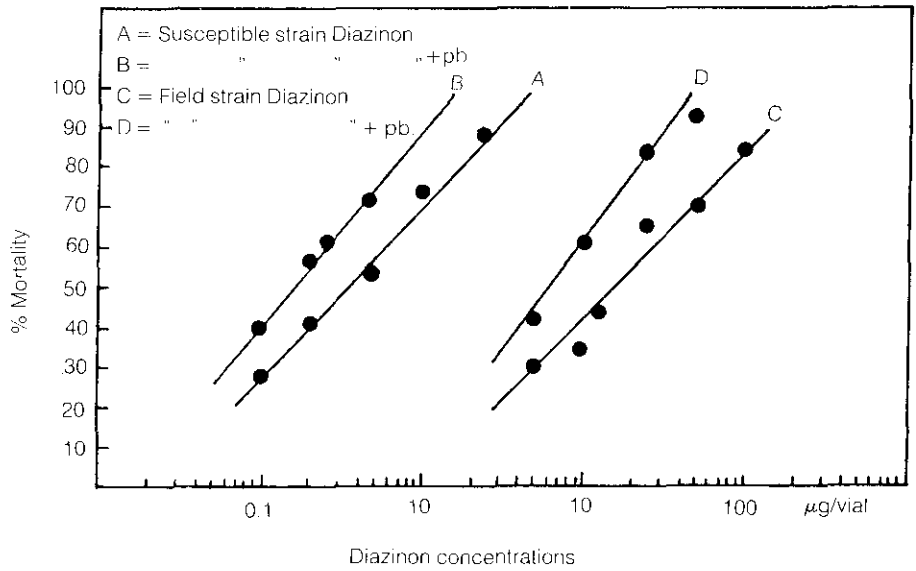

Fig. 6. Toxicity of diazinon on susceptible and field strains of house flies with and without piperonyl butoxide

against the flies (LC_{50}35 µg per vial). No mortality among field strain occurred in treatment with up to 2 mg carbaryl per vial, the highest carbaryl amount that could be dissolved in 0.5 ml acetone.

Table 1. Toxicity of synergized or unsynergized carbaryl, parathion, and diazinon to field and S strains of house fly.

Insecticide	Field strain			Susceptible strain		
	LC_{50} (µg/vial)	Synergized LC_{50}	%D on MFO	LC_{50} (µg/vial)	Synergized LC_{50}	%D on MFO
Carbaryl	400*	160	65	35	11	71
Parathion	4.5	1.7	63	0.015	0.005	63
Diazinon	15	6	62	0.44	0.23	47

*for the third generation
LC_{50} Values are calculated by liner regression
CSD is calculated from the equation: log LC_{50} = 1.014 log (CSD) – 0.009.

$$\%D = \frac{\text{Unsynergized } LC_{50} - \text{Synergized } LC_{50}}{\text{Calculated Synergist Difference (CSD)}}$$

Treatment with Pb enhanced the toxicity of carbaryl, parathion, and diazinon with similar degrees. The table shows the %D values of the flies on monooxygenase for detoxification of the three insecticides used in this study.

Discussion

The enhancement of carbaryl, parthion, and diazinon toxicities by Pb treatment revealed the relative importance of the flies monooxygenases in detoxification of the three insecticides. Metcalf and Fukuto [13] reported great enhancement of carbaryl toxicity against house flies when Pb was used.

Osman and Brindley [7] found that grass bug's monoxygenase detoxification was less important than that of the alfalfa leafcutting bee, where monooxygenases have an important role [14]. On the other hand, Al-Rajhi and Brindley [8] indicated that Pb did not enhance carbaryl toxicity against pea aphids. They concluded that aphids may be dependent on detoxification mechanism(s) other than monooxygenase.

The relative tolerance of the house fly field strains to carbaryl toxicity may be attributed to penetration or detoxification mechanism(s) other than monooxygenase.

The estimated %D values for monooxygenase detoxification of carbaryl, parathion, and diazinon were similar for the field and S strains indicating that environmental factors have little or no effect on flies monooxygenase level.

The data of this study confirmed the effectiveness of the synergistic bioassays in revealing monooxygenase level and role in house flies and obviate the possible alternative detoxification mechanism (s). It also indicated that environmental factors have little or no effect on monooxygenase level in house flies, under such limited and homogeneous population of insect inhabiting the location.

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الكشف عن مستوى النشاط الأنزيمي للمونو أكسجينيز بواسطة اختبارات التقويم الحيوي المنشط على سلالات حقلية وحساسة من الذباب المنزلي

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ملخص البحث. أجريت اختبارات التقويم الحيوي للمبيدات: كرباريل ـ باراثيون، ديازينون على سلالة حقلية وعلى مدى أربعة عشر جيلاً متتابعًا من سلالة معملية من الذباب المنزلي *Musca domestica* (L)، لقياس مستوى النشاط الأنزيمي للمونوأكسجينيز، وكذلك لبيان دوره في عمليات تقليل سمية المبيدات المختبرة. وقد أظهرت النتائج أن البارثيون كان أكثر سمية للسلالة الحساسة من الذباب المختبر حيث كان التركيز القاتل النصفي (ت ق ٥٠) من الباراثيون ٠,٠١٣ ميكروجرامًا/أنبوبة بينما كانت قيمة (ت ق ٥٠) للديازينون ٠,٣٣ ميكروجرامًا/أنبوبة أما الكرباريل فقد كان أضعف المبيدات الثلاثة في هذا المجال حيث كانت قيمة ت ق ٥٠ له ٠,٣٥ ميكروجرامًا/أنبوبة. كما أظهرت النتائج أيضًا أن سمية كل من كرباريل وباراثيون ودايازينون للسلالة الحساسة (المعملية) قد تضاعفت عن سميتها بمقدار ١١، ٤٥، ٣٤٦ ضعفًا على التوالي.

معاملة الحشرات بالمثبط بيبرونيل بيوتركساييد أدت إلى تثبيط إنزيمات الـ مونو أكسجينيزز حيث وجد أن نسب اعتماد السلالة الحقلية والحساسة على هذه الإنزيمات في تقليل سمية المبيدات الثلاثة تكاد تكون متقاربة حيث كانت ٦٤٪، ٦٢٪، ٦١٪ للسلالة الحقلية و ٧١٪، ٦٠٪، ٥٣٪ للسلالة الحساسة لكل من الكرباريل والباراثيون والديازينون على التوالي.