Sublethal Effects of Pyrethroid and Carbamate Insecticides on the Chemosensory Responses of the Housefly, *Musca domestica* *nebulosa* (Diptera: Muscidae)

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Sublethal effects of insecticides on the behaviour and physiology of insects are poorly understood because of a general paucity of information on this topic. However, studies on such effects are important for the understanding of neurotoxic action of insecticides (SHARMA, 1969) as well as for the assessment of overall ecological impact of insecticides, since non-target species in the periphery of the treated area often receive sublethal doses of insecticide (CHADD and BRADY, 1982).

Feeding responses of the housefly measured in terms of the tarsal response thresholds to sucrose solutions are reportedly affected by the sublethal doses of insecticides. SMYTH and ROYS (1955) were first to report that the exposure of the housefly to sublethal doses of DDT had resulted in lowering of tarsal response threshold to sucrose solutions. Similarly, such treatment also caused lowering of the labellar response threshold of the housefly (SOLIMAN and CUTKOMP, 1963). Sublethal treatment of other chlorinated hydrocarbons, like dieldrin, produced DDT-like effects on the tarsal responses of the housefly (SHARMA, 1973). In contrast to these findings with DDT, SOLIMAN and CUTKOMP (1963) found that the organophosphate parathion produced no effects on tarsal sensitivity of the housefly to sugar solutions until the hyperactive stage of toxicosis.

Evaluation of the tarsal thresholds measured by the extension of proboscis by a water-satiated fly in response to the tarsal stimulation by sugar solutions as described by DETHIER (1976) can be used as an index of the excitatory state of the central

nervous system (DETHIER et al., 1965). Using SMITH and ROY's work (1955) as a rationale, the effects of a few pyrethroid and carbamate compounds on sucrose-stimulated tarsal responses of the housefly are described in this paper.

Test flies were drawn from a local strain of *Musca domestica nebulosa*, which had been reared in laboratory for 16 y at $28 \pm 2^\circ\text{C}$ and 60–65% R.H. under 12L–12D. Adults were provided with milk prepared from a commercial brand of milk powder (Amul Sray, India) as food. Eggs laid on milk-soaked cotton pads were allowed to hatch in the same medium before being transferred to a larval medium consisting of slightly moistened, half crushed rabbit diet pellets (Gulmohar, India). Dry cotton covering the medium acted as a substrate for pupation.

Insecticides used for treatments were as follows: 3-phenoxybenzyl ester of *cis*-2,2 dimethyl-3-(2-chloro-2-methyl vinyl) cyclopropane carboxylic acid, i.e., indothrin; α -cyano-3-phenoxybenzyl ester of 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropane carboxylic acid, i.e., cypermethrin; α -cyano-3-phenoxybenzyl ester of *cis*-2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropane carboxylic acid i.e. alphamethrin (all synthesized in the Organic Division of our laboratory); 3-phenoxybenzyl ester of 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropane carboxylic acid, i.e., permethrin and 1-naphthyl *N*-methyl carbamate, i.e., carbaryl (Courtesy of Vector Control, WHO, Geneva).

Acetone was used as solvent to prepare various concentrations of insecticides. Insecticide solution was applied topically in 1 μl of acetone on the dorsal side of the housefly with the help of an Agla microsyringe. Control flies received 1 μl of acetone alone. The series of sugar concentrations used to determine tarsal thresholds were prepared from sucrose (BDH, AR quality) dissolved in glass distilled water.

LD₂₀, LD₅₀ and ED₅₀ doses were estimated against 0–16-h-old starved, water-satiated flies drawn from several batches from different rearings. Insecticide-treated flies were held at $25 \pm 1^\circ\text{C}$, fed with distilled water and mortality count was taken after 24 h. LD₂₀ was chosen as the sublethal dose to study its effect on the tarsal response of the housefly. LD₂₀ treated flies were held for 2 h at 25°C and fed on water, for acclimatization and recovery from knockdown effect, if any. These flies were

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mounted on waxed glass rods by their wing tips and allowed to acclimatize to this condition for 1–2 h. Tarsal response bioassays were carried out between 08.30–16.00 h in an air-conditioned lab maintained at $25 \pm 1^\circ\text{C}$. Tarsi of the mounted flies were first presented to distilled water and only those flies which did not respond to distilled water were exposed to the ascending series of sucrose solutions viz: 0.0025 M, 0.005 M, 0.01 M, 0.025 M, 0.05 M, 0.1 M, 0.25 M, 0.5 M and 1 M held in watch glasses. A tarsal response was considered positive if there was full uninhibited extension of the proboscis immediately after dipping of the tarsi into a given concentration of sucrose solution. After presentation of each sugar solution, the tarsi were repeatedly washed in distilled water so as to remove traces of sugar solution that might be adhering. An interval of 15 min was allowed between the presentation of each successive sugar concentration. Distilled water was offered before each sucrose test solution to eliminate the possibility of response to water alone. Percent tarsal response was calculated as the percentage of flies displaying positive tarsal response out of total number of flies examined.

Percentage mortality, and percent tarsal response were converted into probits and plotted against log dose and log sucrose concentrations to determine LD_{50} , LD_{20} , and ED_{50} values, respectively. ED_{50} is the effective dose/concentration of sucrose solution which, when offered to housefly tarsi, elicits the proboscis extension response from 50% of the test population. The data were subjected to probit analysis, and from the regression equations LD_{50} and ED_{50} values were calculated together with fiducial limits (95% confidence) for these values (BUSVINE, 1957).

On the basis of fiducial limit values, given in parentheses for respective ED_{50} values (Table 1, ED_{50}), it can be observed that sublethal or LD_{20} application of pyrethroid and carbamate insecticides (Table 1, LD_{20}) to the houseflies had caused a significant increase in their ED_{50} values (Table 1, ED_{50} : A, B versus C) as compared to the ED_{50} of the acetone-treated control batch. In other words, sublethal treatment with these insecticides had inhibited the proboscis extension response or the tarsal response in the housefly. Further examination of ED_{50} values (Table 1, ED_{50} : A, B) shows that permethrin had caused a maximal increase in the ED_{50} value followed by indothrin, cypermethrin, alphamethrin, and car-

baryl, in decreasing order.

An examination of the relative toxicities of pyrethroids on the basis of fiducial limit values of LD_{50} values (Table 1, LD_{50}) and their corresponding ED_{50} values (Table 1, ED_{50} : A) reveals that permethrin is the least toxic but induces the highest level of tarsal response inhibition. On the other hand, alphamethrin, comparatively the most toxic pyrethroid, produces relatively the lowest level of tarsal response inhibition. Indothrin and cypermethrin show intermediate levels of tarsal response inhibition paralleling the intermediate toxicity levels. Carbamate and carbaryl, relatively less toxic than pyrethroids (Table 1, LD_{50} : A versus B), also inhibited the tarsal response (Table 1, ED_{50} : B versus C).

Results of the present work indicate that treatment of flies with sublethal doses of pyrethroids and carbamates, regardless of their different modes of insecticidal action (BAILLIE, 1985), produce similar inhibitory effects on the tarsal response. Most reports on the effects of pyrethroids on sense organs indicated that they cause an increase in sensory input (CLEMENTS and MAY, 1977; HOLAN et al., 1980). Thus sublethal pyrethroid treatment would be expected to increase the fly's tarsal response, but the present data suggest the opposite, i.e., an elevation of the tarsal threshold, or of ED_{50} . However, sublethal permethrin treatment of the tsetse fly, *Glossina austeni*, also produced similar inhibitory effects on its probing responses, but failed to affect the proboscis extension responses of the blowfly, *Protophormia terraenovae* (CHADD and BRADY, 1982). Therefore, "unexpected" elevation in the tarsal threshold, i.e., ED_{50} in houseflies after sublethal pyrethroid treatment may be due to the reduced responsiveness of the central nervous system (CNS) to sensory inputs (BURT and GOODCHILD, 1971; BURT et al., 1971). In fact, DETHEIR et al. (1965) described the tarsal response, or the proboscis extension reflex, as a fairly reliable index of the excitatory state of the CNS.

Nonetheless, the exact mechanism of how pyrethroids affect the sense organs is still not clearly understood. More relevant are the results of KAISSLING's (1980) work: it showed that after application of 1R-trans-permethrin to *Antherea polyphemus*, extracellular recordings from pheromone stimulated olfactory hair exhibited an initial burst of increased action potentials, which were eventually blocked, although the generator poten-

Table 1. Comparison of ED₅₀ values of sucrose induced tarsal responses observed in sublethal insecticide-treated and untreated houseflies in relation to LD₅₀ values

Insecticide	Equation ^a (LD ₅₀)	LD ₅₀ ng/fly (95% conf. limit)	LD ₂₀ ng/fly	ED ₅₀ mm Sucrose (95% conf. limit)	Equation ^b (ED ₅₀)
A Pyrethroids					
Permethrin	$Y = -0.0492 + 2.673X$	7.744 (7.740-7.747)	3.8	85.29 (84.94-85.63)	$Y = -0.0014 + 1.7064X$
Indothrin	$Y = 0.0016 + 2.90X$	5.282 (5.280-5.287)	2.7	76.75 (76.45-77.01)	$Y = -0.013 + 1.7375X$
Cypermethrin	$Y = 0.001 + 3.677X$	2.288 (2.287-2.289)	1.4	54.86 (54.82-54.87)	$Y = 0.0038 + 1.8239X$
Alphamethrin	$Y = 0.0009 + 2.609X$	0.8243 (0.8243-0.8244)	0.39	51.12 (51.09-51.14)	$Y = 0.0033 + 1.8447X$
B Carbamate					
Carbaryl	$Y = 0.006 + 1.94X$	375.1 (373.4-376.8)	138.4	50.69 (50.47-50.90)	$Y = -0.002 + 1.8492X$
C Control	—	—	—	23.90 (23.79-23.99)	$Y = -0.0034 + 2.1021X$

^a $Y = \% \text{ Mortality}$; $X = \text{Insecticide Dose}$; ^b $Y = \% \text{ Tarsal Response}$; $X = \text{Sucrose Concentration}$

tials from sensory hair remained unaffected. We do not know why there is an apparently negative correlation between the toxicity, i.e., LD₅₀, of the pyrethroid and its ability to elevate tarsal threshold, i.e., ED₅₀.

Carbamates are "centrally acting" insecticides which tend to produce convulsive bursts of variable length separated by relative inactivity (MILLER and ADAMS, 1977). This inactivity may be linked to the inhibitory action on tarsal responses of the carbaryl-treated housefly.

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Control of *Epinotia granitalis* (Lepidoptera: Tortricidae) with *Paecilomyces* spp. on Cotton Bands Wrapped on the Trunks of *Cryptomeria japonica*¹

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Larvae of *Epinotia granitalis* (Lepidoptera:

Tortricidae) attack the phloem of Sugi (*Cryptomeria japonica*) and Hinoki (*Chamaecyparis obtusa*), and are becoming increasingly serious pests. Spraying of chemical insecticides, trimming or thinning, and planting of resistant races of *cryptomeria* have been attempted as control methods, but have not always been effective in the field. Development of effective control methods are still needed.

Two species of fungal genus *Paecilomyces* (YAMAZAKI and KURANAGA, 1988) and a nuclear polyhedrosis virus (MITSUHASHI and SHIMAZU, 1988) were discovered to be *E. granitalis* pathogens. We have confirmed these agents as virulent to the larvae of this insect in the laboratory (unpublished), and have suggested the possible use of these pathogens for the control of *E. granitalis*. Infecting larvae with fungi under coverings on the trunk of *cryptomeria* appears promising because the larvae

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