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LABORATORY EVALUATION OF SUBSTITUTED ARYL ISOTHIOCYANATES AGAINST *AEDES AEGYPTI* (L)

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The compounds tested were phenyl, *p, m*-chlorophenyl, *p,m*-bromophenyl, *p, o*-methoxyphenyl and *o, m, p*-tolyl isothiocyanates. They proved moderately toxic to the 4th instar larvae of *Aedes aegypti* (L). Examination for the structure - activity relationship showed that the compounds substituted in *para* - position were slightly more toxic, while *ortho* - substituents gave minimum kill. Larvae were more susceptible to test chemicals than pupae. The LC_{50} ranged from 1.5 to 4 ppm for the larvae while the pupal mortality was 75 – 100% at 10 ppm concentration.

The longer exposure of the pupae not only induced mortality but also caused the abnormal emergence patterns. The emerging adults lacked vigour and most of them could not emerge completely at higher doses and died as feeble adults.

INTRODUCTION

Some organothiocyanate pesticides have been used for the last 30 years while others have been tested as insecticides including 3 - thiocyanopropyl phenyl ether [1], aliphatic thiocyanates [2], isobornyl thiocyanoacetate [3] and *p* - dimethyl aminophenyl thiocyanoacetate [4]. Bakry *et al.* [5] studied the mode of action of benzyl thiocyanates, benzyl isothiocyanates, phenyl thiocyanates and alkyl thiocyanates as insecticides and as synergists for the carbamate insecticide carbaryl. Thiocyanates have also been tested as fungicides [6], nematicides [7] and insect fumigants [8].

Data on the comparable toxicity of pure thiocyanates (–SC–N) and isothiocyanates (–N–C–S) have not been found in the literature. The present paper describes the toxicity of ten aryl isothiocyanates against larvae and pupae of *Aedes aegypti* (L). The results on dosage - mortality and structure - activity relationship have been compared with the pure thiocyanates already reported. The paper also includes the effects of longer exposure of test compounds on the pupae and emerging adults.

MATERIAL AND METHODS

The candidate compounds tested were prepared by the method of Dains [9]. The chemical composition of the test compounds is as follows:

Compound	Chemical composition	Desig.
1	Phenyl isothiocyanate	AIT 1
2	*p* - Chlorophenyl ,,	AIT 2
3	*m* - Chlorophenyl ,,	AIT 3
4	*p* - Bromophenyl ,,	AIT 4
5	*m* -Brompphenyl ,,	AIT 5
6	*p* - Methoxyphenyl ,,	AIT 6
7	*o* - Methoxyphenyl ,,	AIT 7
8	*o* - Tolyl ,,	AIT 8
9	*m* -Tolyl ,,	AIT 9
10	*p* - Tolyl ,,	AIT 10

Larval Treatment. The compounds were dissolved in acetone with traces of emulsifier (triton 180, 0.02% in stock solution) and diluted to appropriate concentrations. One ml of the prepared solution in acetone was added to 249 ml of water for each concentration of the candidate compounds (WHO standard test [10] for measuring resistance in mosquito larvae)

In each experiment early 4th instar laboratory-reared larvae were exposed to various concentrations of the candidate compounds at 83+5 ^{o}F and 74+4% RH. The mortality was noted after the exposure time of 24 hr. In each test, six or seven concentrations of the compounds were tried. Larvae of the same age were treated with acetone and traces of emulsifier (< 1 ppm) in control beakers. Each treatment was repeated four times in replication on different days. The percent mortality was plotted on the probit scale and the points fitted visually with a straight-line.

Pupal Treatment. For pupal treatment the method described by Roberts *et al.* [11] was modified for testing the candidate compounds against pupae. In each replicate 20 pupae of only one age group (0 – 24 hr) were exposed to different concentrations of compounds in 100 ml beakers. Mortality of pupae, percentage of incomplete

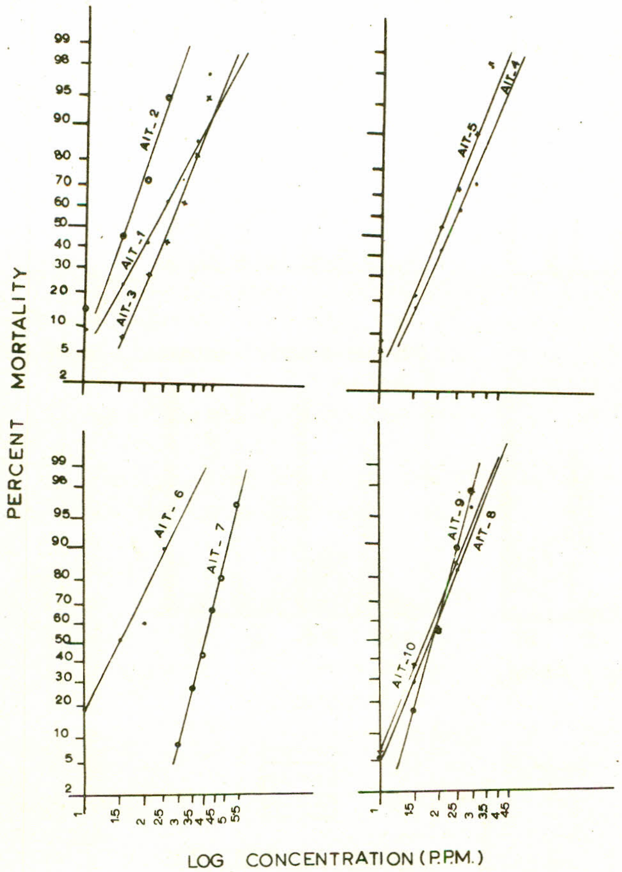


Fig. 1. Dosage-mortality regression lines of ten aryl isothiocyanates against the 4th instar larvae of *Aedes aegypti* (L). AIT. 1 (phenyl isothiocyanate) AIT. 2 (*p*-chlorophenyl), AIT. 3 (*m*-chlorophenyl), AIT. 4 (*p*-bromophenyl), AIT. 5 (*m*-bromophenyl), AIT. 6 (*p*-methoxyphenyl), AIT. 7 (*o*-methoxyphenyl), AIT. 8 (*o*-tolyl), AIT. 9 (*m*-tolyl), AIT. 10 (*p*-tolyl).

emergence, and feeble adults were recorded after 72 hr. The 72-hr period provided sufficient time for emergence or for the pupae to succumb to the candidate compounds. Pupae were considered dead when they could not emerge from the pupal exuvium. Appropriate controls were maintained by using specimen of the same age from the same rearing pan in 100-ml beakers with the traces of emulsifier (< 1 ppm)

RESULTS AND DISCUSSION

The dosage-mortality regression lines for the candidate compounds against the larvae are given in Fig.1. All the test compounds were moderately toxic to larvae, and their LC_{50} ranged from 1.5 to 4 ppm. The mortality induced by the compounds varied slighty, the examination of structure-activity relationship showed that compounds with substitution in *para*-position were slightly more toxic than other compounds of the series. The compound with methoxy group at *ortho*-position showed the minimum activity. In the same way the presence of Cl atom in *para*-position resulted in slightly more toxic compound than the one substituted in *meta*-position.

Bakry *et al.* [5] reported the LC_{50} range of 8 benzyl thiocyanates between 0.94 – 6 ppm when tested against *Culex pipien* larvae. They reported that compound substituted with Cl, NO_2 and CH_3 in *ortho*-position resulted in nontoxic thiocyanates due to steric. hindrance effect of the substituents. The superior activity of compounds with substitution in *para*-position, as observed in case of test compounds, is in line with the observation of Bakry *et al.* [5] Basikora *et al.*[7] studied a large group of 53 isothiocyanates of aliphatic character, some benzyl type compounds and a large group of mononuclear aromatic isothiocyanates against *Turbatric aceti*. They also reported increased toxicity of the *para*-substituents.

The insecticidal activity of the test compounds against the larvae was in the order of: 4-OCH_3 > 4-Cl > 4-CH_3 > 2 - CH_3 > 3 - CH_3 > 3 - Br > H > 4 - Br > 3 - Cl > 2 - OCH_3.

According to Bakry *et al.*[5] the insecticidal activity of benzyl thiocyanates again t *Culex pipien* larvae was in the order of: H > 4 - Cl > 3 - Cl > 3 - CH_3 > 4 - NO_2 > 2 - Cl > 2 - NO_2 > 2 - CH_3.

It is difficult to compare the activities of these two non homologous series of the compounds against two different species of mosquitoes. However, the comparison can be of some interest as both the series contain sulphur and an intact organothiocyanate molecule which is reported to be essential for the toxicity of thiocyanates [5].

The data on the relative toxicity of the test compounds against the pupae showed that the order of toxicity was different from that of larvae. The toxicity induced at various concentrations was in the order of: 4 - CH_3 > 4 - Cl > 4 - Br > 4 - OCH_3 > H > 3 - CH_3 > 3 - Cl > 3 - Br > 2 - OCH_3 > 2 - CH_3. This sequence clearly shows that the activity of compounds substituted in *para*-position is superior over other compounds in the series as observed in case of larvae. The toxicity induced by the *para*-subsituents was observed to be increased by 40–50% (Fig 2).

thiocyanates against pupae are presented in Fig 2 . These data indicate that the pupal mortality ranged from 75 to 100% at 10 ppm. The mortality was low at lower concentrations indicating that the pupal stage was relatively immune to the toxic action of isothiocyanates until metamorphosis to the adult was complete and the adult was preparing to or in process of emerging.

Figure 3 shows the incompletely emerged adults which lacked vigour to escape from the exuviae. Some pupae had a visible adult inside the pupal skin which could not split and the adults died inside the exuviae (Fig. 3). In some pupae the skin was split and the apparently normal adult was partially emerged. They failed to free themselves from the exuviae and died during emergence (Fig.3b). In some

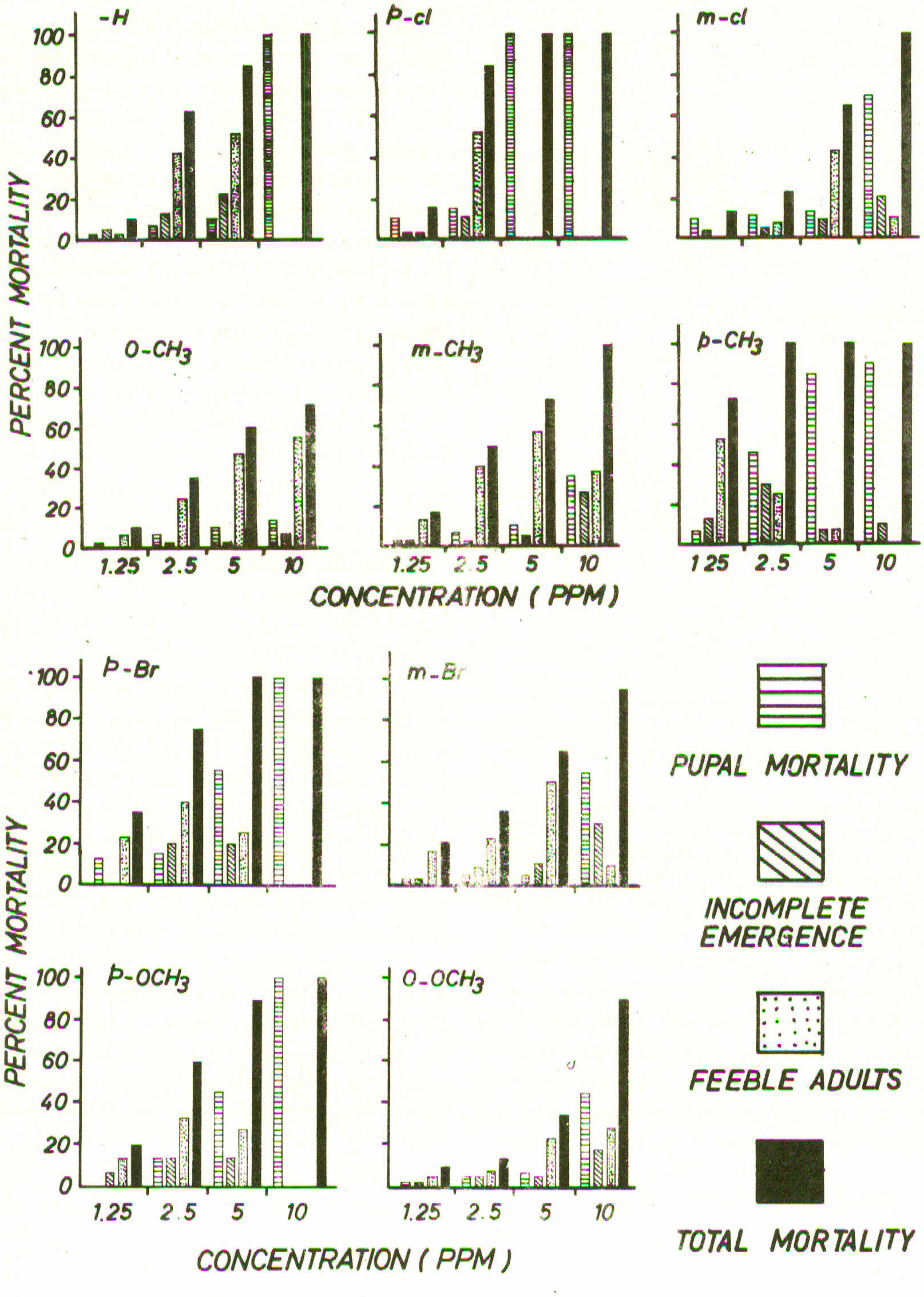
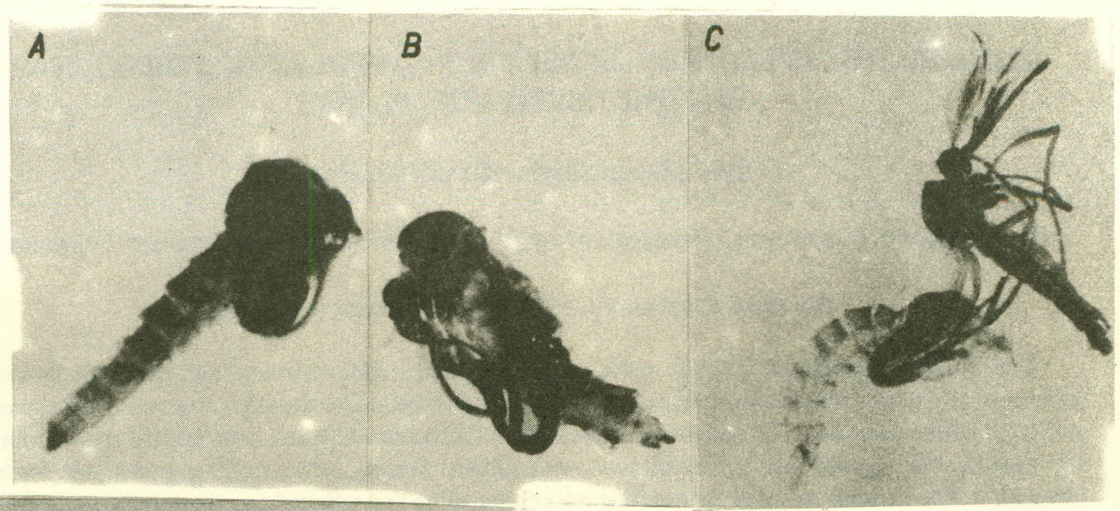


Fig. 2. Showing the pupal mortality, incomplete emergence and the percentage of feeble adults induced by various concentration of the aryl isothiocyanates. H. (phenyl isothiocyanate), *p*-Cl (*p*-chlorophenyl), *m*-Cl (*m*-chlorophenyl), *o*-CH_3 (*o*-tolyl), *m*-CH_3 (*m*-tolyl), *p*-CH_3 (*p*-tolyl), *p*-Br (*p*-bromophenyl), *m*-Br (*m*-bromophenyl), *p*-OCH_3 (*p*-methoxyphenyl), *o*-OCH_3 (*o*-methoxyphenyl).

partially emerged mosquitoes only the head could emerge while the abdomen remained inside the exuviae, in others only the head and thorax emerged and not the abdomen. Sometimes they escaped almost completely but the tarsi of the hind pair of legs remained struck to the pupal exuviae (Fig. 3c). Some adults emerged completely, but they were unable to fly from the water surface; where they remained trapped until death (Fig. 3d).

Most of these incomplete emergence patterns were similar to those described by Busvine *et al.* [12] after trea-



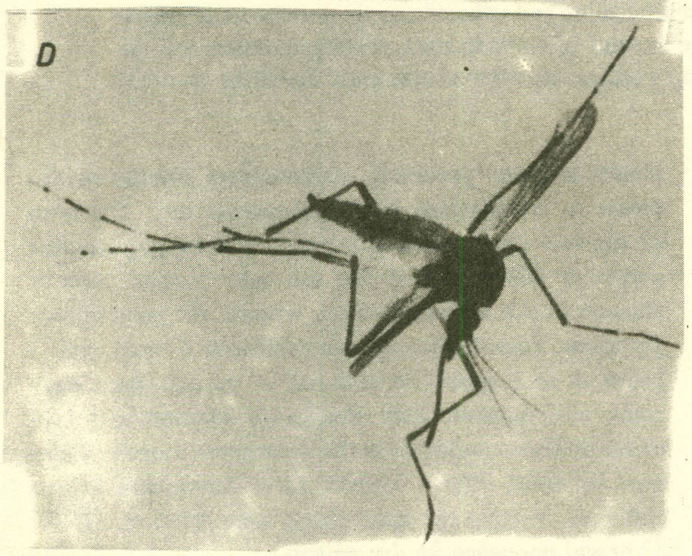


Fig. 3. Categories of lethal action on pupae treated with aryl isothiocyanates. (A) Apupa with visible dead adult inside the pupal skin. (B) An apparently normal adult partly emerged. (C) An adult almost completely emerged with the hind pair of legs struck to pupal exuvium. (D) A feeble adult.

ting larvae of *Culex pipiens fatigen, Anopheles gambiae, Aedes aegypti* and *Anopheles quadrimaculatus* with certain insect development inhibitors.

The lack of vigour in the emerging adult may be due to various factors. It is possible that the feebleness is due to suppression of the uptake of oxygen by the treated pupae. Harvey and Brown [13] noticed marked decrease in oxygen consumption in insects during the whole stages of intoxication by thiocyanates. Coon [14] has suggested that thiocyanates like hydrogen cyanide, may act as respiratory poison. Lord and Potter [15] have also reported that insecticidally active thiocyanates depress respiration in insects.

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