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THE LABORATORY EVALUATION OF DIFLUBENZURON AGAINST *AEDES AEGYPTI*

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ABSTRACT
The effects of Diflubenzuron (Technical and Wettable Powder) were studied against different larval instars of *Aedes aegypti*. Diflubenzuron was effective against all larval instars. Surprisingly, Diflubenzuron (Tech.) was found to be more active than formulated product (25WP) irrespective of concentration or exposure. IC_{50} (Inhibition concentration of 50%) of technical and formulated product were calculated for different larval instars. .

KEY WORDS: *Aedes.aegypti***,** Diflubenzuron, IGR

INTRODUCTION

Insect Growth Regulators (IGR) exercise their maximum effect at the time of metamorphosis Speilman *et al.* (1966), Speilman *et al.* (1967), Jakob and Schrof (1972), Georghiou and Lin (1974).An IGR there for does not exhibit any toxicity to target insect but shows various abnormalities (Siddall, 1976). The first chitin synthesis inhibitor introduced into the market as a new novel insecticide was benzolphenylurea Diflubenzuron (Miyamoto *et al.*, 1993). Diflubenzuron has also been effective against Coleopteran and Diptera. Tunaz and Uygun (2004) reported that role of insect growth regulators for insect pest control. The present work incorporates results of an extensive investigation into biological activity of Diflubenzuron against *Aedes aegypti* larvae.

MATERIALS AND METHODS

The mosquito colonies:

Experiments were conducted on different larval instars (II, III and IV) of laboratory reared strains of *Aedes aegypti* (L). The culture regimes followed were based on the protocols suggested by Christopher (1960). Aedes aegypti were reared in a special mosquito insectary maintained at 28+ 2^{o}C, relative humidity 70-80% and photoperiod 12:12.

The Test Chemical:

The test chemicals used for experiments in the present work Diflubenzuron (Dimlin) Technical and Diflubenzuron (Dimlin) 25WP formulation were obtained from M/S Philips-Duphar Holland Company, Netherlands.

Experimental Methods:

Diflubenzuron (Technical 97.6% pure) test chemical were dissolved in AR grade acetone, while Diflubenzuron (25WP) dissolved in distilled water and diluted to desired concentration. From the latter the required concentration was added to 50ml water as per WHO Standards (WHO Technical Report 1970). Chosen developmental stages of mosquitoes (different larval instars) were added in the above aqueous solutions as per designs described below.

Design I- Continuous exposure
In this II-IV instar larvae were used. Each stage was exposed to the test chemicals in desired concentrations right up to emergence of adults. In other word, the II instars were exposed for the complete duration of II through III, IV and pupal stages. The III instars were exposed for the duration of III-IV-Pupa while the IV instars were exposed to the test chemical for the duration of their IV instar and pupal stages only.

Design II: Discontinuous exposure
In this III and IV instar larvae of *A. aegypti* were exposed to the test chemical for specific period's viz. 30min, 60min, 8hrs and 3days separately. The test larvae were transferred to untreated water following completion of given exposure period to the test chemical.

Concentration used for experiment:

For both designs concentration range from $1x10^{-6}$ to 1 PPM were used. Larval and Pupal mortality as well as numbers of normal and/or abnormal adults emerging from the treated stages were recorded.

Types of development inhibition observed:

Since a fair variety of effects were observed as a consequence of IGR treatment of various larval stages, these are annotated below for convenience.

LM: represents death during the larval stage without initiation of pupation
PM: The pupa completely escapes from the larval cuticle, but remains partially or totally unmelonised and eventually dies.
AM: Adults emerge but are unable to fly away from the water surface and eventually die.
NA: No abnormal effect. Full grown normal mosquito adults emerge and survive.

RESULTS

Diflubenzuron (Tech.):

When II instars were treated with this chemical, larval mortality occurred even at the lowest concentration ($1x10^{-3}$ ppm). Thus 42.8% cumulative larval mortality, 21.4%pupal mortality, 28.5% normal adult emergence were recorded. Above $1x10^{-3}$ ppm up to 1ppm, 100% larval mortality was elicited. In case of II instar larvae treated at $1x\ 10^{-6}$ ppm 37.5% larval mortality, 6.2% abnormal adult mortality and 62.5% normal adult emergence were elicited. At all concentrations $1x10^{-6}$ ppm, 100% larval morality was exhibited. The IV instars did not show any larval mortality up to the $1x10^{-3}$ ppm. However, 10% pupal mortality and 90% abnormal adult emergence were recorded. Above this concentration, 100% larval mortality was obtained at all concentrations.

Diflubenzuron (25WP):

Continuous exposure of III instar of Diflubenzuron (WP) induces larval mortality from $1x10^{-5}$ ppm onwards (13.6%). As the concentration was increased there was an increase in larval mortality, pupal mortality and abnormal adult mortality were observed .At 1 ppm there was 100% larval mortality, consequently no normal adults emerged However, when III instars were exposed continuously , the larval, pupal mortality and abnormal adult mortality started appearing from $1x10^{-6}$ ppm onwards. At $1x10^{-3}$ ppm cumulative larval mortality was 32.5%, pupal mortality24% abnormal adult mortality 22.3% and normal adult 21% From $1x10^{-2}$ to 1 ppm, 100% larval mortality was obtained. When IV instars were treated with this chemical, larval mortality 8.3%, pupal mortality 12.5% and abnormal adult mortality 12.5 was obtained at $1x10^{-4.}$ As the concentration was increased there was significant increase in larval mortality resulting in decrease of adult emergence. At 1ppm 100% larval mortality was recorded.IC_{50} (50% inhibition concentration) values were calculated for various instars using statistically (Table-1).

Discontinuous Exposure:

Thirty Minutes Exposure:

Third instars were exposed to Diflubenzuron (WP), 100% adult emergence up to $1x10^{-4}$ ppm. A rest of the concentration up to 1 ppm 100% larval mortality was obtained. With Diflubenzuron (Tech.) 100% adult emergence was recorded up to $1x10^{-2}$ ppm, after which 100% larval mortality occurred at all concentrations.

In case of IV instars treated with Diflubenzuron (WP), at $1x10^{-3}$ ppm reduced adult emergence (74.3%) was observed. At $1x10^{-2}$ ppm it reduced to 19.0%.At $1x10^{-1}$
and 1 ppm 100% was recorded. Diflubenzuron (Tech.) gave 100% normal adult emergence up to $1x10^{-2}$ concentration. However, at $1x10^{-1}$ and 1 ppm concentration larval morality was 62.3% and 72.3%, respectively, while pupal mortality was 37.6% and 27.6% respectively.

Sixty Minutes Exposure:

Diflubenzuron (WP) failed to adversely affect III instar larvae up to $1x10^{-4}$ ppm. However, 100% larval mortality was recorded at subsequent concentrations. Diflubenzuron (Tech.) did not affect adult emergence up to $1x10^{-3}$ ppm. At $1x10^{-2}$ ppm 80.9% adult emergence was recorded while at the remaining two concentrations 100% larval mortality was observed. In case of IV instars Diflubenzuron (WP) produced 100% adult emergence up to $1x10^{-6}$ ppm. Larval and pupal mortality and abnormal adult's mortality were recorded at $1x10^{-3}$ ppm (18.2, 28.3 and 52.3%) At higher concentrations, 100% larval mortality was elicited. In case of Diflubenzuron (Tech.) treatments, 100% normal adults were obtained up to $1x10^{-5}$ concentration. Subsequent concentrations reduced adult emergence up to 22.3% ($1x10^{-2}$ ppm). At $1x10^{-1}$ and 1 ppm 100% larval mortality was obtained.

Eight Hours Exposure:

Diflubenzuron (Tech.) when tested on IV instar for 8 hours at $1x10^{-6}$ and $1x10^{-5}$ ppm gave rise to 100% normal adult emergence. Subsequent concentrations viz. $1x10^{-4}$ and $1x10^{-3}$ induced larval morality (18.3% and 19.2%) pupal mortality (39.2 and 58.2%0, abnormal adult mortality (18.2% and 22.5%) and normal adults (24% and 0%) $1x10^{-2,}1x10^{-1}$ and 1 ppm concentration gave 100% larval mortality. When IV instars were exposed to Diflubenzuron (WP) for 8 hrs, no significant activity was recorded up to $1x10^{-4}$ ppm concentration. At $1x10^{-3}$ and $1x10^{-2}$ ppm concentrations, normal adult emergence decreased to 89.7% and 0% respectively. Finally at $1x10^{-1}$ ppm and 1 ppm 100% larval mortality was recorded.

Three Days Exposure:
Exposure to Diflubenzuron (Tech.) for 3 days was ineffective up to 1x10-3 ppm concentration. As the concentration was increased from $1x10^{-2}$ to 1 ppm, 100% larval mortality was noted. With Diflubenzuron (WP) only two lower concentrations namely $1x10^{-6}$ and $1x10^{-5}$ ppm exhibited 100% normal adult emergence. Rest of the concentrations exhibited 100% larval mortality.

DISCUSSION

In case of Diflubenzuron insecticidal as well other effects were elicited at various concentrations on exposure at minimum as well as maximum durations. Results in the present investigation also revealed that technical Diflubenzuron is unmistakably more active than the commercial wettable powder formulation irrespective of concentration or exposure time of the treatment. Presumably deeper and faster penetration of technical Diflubenzuron through the deployed carrier solvent, acetone, as also slower and dubious contact action of Diflubenzuron as a water soluble formulation, may be the reasons. From the foregoing, it becomes apparent that II and III instar larval stages of the mosquito *A. aegypti* are not sensitive enough to be the targets for practical application of these chemicals. The IV instar stage on the other hand is obviously the most sensitive one to IGR action, and may therefore, be considered ideal for application of these chemicals.

Diflubenzuron exercise fundamentally non-hazardous growth and development regulatory or metamorphosis/ecdysis inhibitory effects, restricted largely to insect taxa. These chemicals are therefore non-hazardous (to non target species), and generally environment friendly. Limitations, of specificity, sensitive stage dependence, lesser persistence and higher cost notwithstanding, in the extant circumstances of high environmental pollution by synthetic organic insecticides and universal recognition of need for more birational pest management strategies, they deserve serious consideration as candidates of promise for incorporation in Integrated Management protocols for mosquito vectors.

Table-1. Determination of IC_{50} values (50% inhibition of adult emergence (ppm)) of the Diflubenzuron treated II, III and IV instars *A. aegypti* larvae.

Test Chemical	II	III	IV
Diflubenzuron(Tech.)	$3.47x10^{-6}$	$1x10^{-6}$	$1.59x10^{-4}$
Diflubenzuron (25WP)	$6.16x10^{-4}$	$4.70x10^{-6}$	$1.7x10^{-2}$

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