

Quantitative Analysis of DDT by GLC in Different Strains of *Culex Fatigans* L. by Using Hexane

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Abstract: Estimation of DDT by GLC (gas liquid chromatography) in K.U. strain of *Culex fatigans* L., shows that 59.68 ng DDT is found after the treatment with 0.175 ppm. In G.I. strain 18.3 ng DDT is found after treatment with 0.325 ppm whereas 4.35 ng DDT is found in L.Y. strain after the treatment with 0.425 ppm.

Key Words: *Culex fatigans*, DDT, GLC.

1. INTRODUCTION

In vivo metabolism of DDT in susceptible and resistant strains has been studied by using GLC and HPLC [2-5,9-14] etc. In this connection residue of DDT in different strains has been used as a parameter. In the present study also residue of DDT after *in vivo* metabolism has been utilized to find the resistance phenomenon in K.U. (susceptible strain), G.I. strain (resistant standard) and a newly found L.Y. strain (resistant) of *Culex fatigans* L.,

MATERIALS AND METHODS

Rearing Technique

Rearing technique described by Ashrafi *et al.* (1966) [1] was used for all strains and only the late third instar larvae were used during experiment.

Detection and Isolation

For detection and isolation of organochlorine pesticide from the animal tissue. Gas chromatograph (Pye-Unicam-204) equipped with electron capture detector (ECD) was used. Before the application of sample to GLC, Pesticides accumulated with fats were extracted from animal tissue (mosquito larvae) and initial separation of interfering organic residue (fats, pigments and other pigments and organics) was carried out. Each step involved in the experiments were carried out in the following manner.

Extraction of Fat

The pesticide is lipophilic in properties and accumulate with lipids, therefore for the extraction of DDT, fat must be extracted, for such purpose following procedures were adopted.

Soxhlation Method

The extraction carried out in a well-known soxhlet apparatus, appears to be one of the best procedures for extraction of residues from finally ground samples. For the extraction of Fat from samples of animal tissues Holden and Marsden (1969) [6] method was used. A known quantity of samples (hundred larvae) of each strain treated with DDT weighing about 50 mg was macerated with Na₂SO₄ (anhydrous sodium sulphate) and was transferred into a thimble made of filter paper.

The thimble was then placed in the extractor which was fitted to the bolt head flask containing 60 ml of n-hexane which was then fitted with condenser connected to the tap water for cooling. The apparatus was then placed on a water bath. The process of extraction was carried out for seven hours during which all the fat contents have been extracted with solvent. Better recoveries were noticed by this method. The fat extracted solvent was then reduced to about 1 ml by evaporation. For complete recovery of pesticides the column chromatography (sorption) was employed and the material was passed three to four times through the column.

Sorption

The process of sorption was carried out in chromatographic columns of alumina [6] and silica [7,8].

Alumina and Silica Columns

The alumina column was used for the separation of fat from pesticides which was used by Holden and Marsden (1969) [6]. The column was made of glass having length 40-42 cm long with internal diameter 6 mm. The column was filled with 2 grams of alumina without calcium of 0.3 micron size (already activated at 800 °C for four hours in a furnace and then partly deactivated by shaking with 5% by weight of water). The concentrated extract was re-dissolved in 1 ml of n-hexane (fractionated) and transferred on the surface of the alumina column. Now the pesticides adsorbed on column

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were eluted with 12 ml of n-hexane and column of eluted sample was then reduced to 1 ml which was passed through a new column. The new column of the same size was packed with 2 gram of silica gel for column chromatography No. 60, 0.060 millimeter size (activated at 120 °C for 2 hours, cooled and deactivated with 3.5% distilled water). For the removal of traces of moisture a layer of activated Na₂ SO₄ was set on top of the silica gel. The elution of pesticides was done by 5 ml of n-hexane. The fractions were concentrated to 1 ml, separated and identified by GLC. The same procedure was adopted using the different quantities of standard DDT per ml of n-hexane which were 20, 40, 60, 80 and 100 ng/ml. It was used to obtain standard peaks for comparison with the above samples.

For the quantitation of pesticide GLC equipped with electron capture detector with a radioactive source for supply of electrons i.e., NI-63 was used. The instrumental parameters used are tabulated below.

Parameters	Instrument
Column length	3ft
Column internal diameter	4 mm
Packing material	3% SE-30 on chromosorb-W
Column temp.	150°C
Detector temp	200°C
Injector temp.	175°C
Gas flow N ₂	40 ml/min
Chart speed	2 ml/ min
Electron source	“NI-63”

RESULTS AND DISCUSSION

Quantitative estimation of DDT was carried out by GLC in treated larvae. The peak heights obtained by the injection of the treated samples were read on calibration curve (Fig. 9), prepared by using different quantities of standard DDT (Fig. 1-5). The results are as follows.

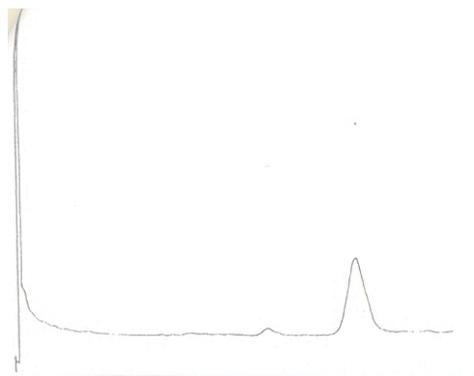


Fig. (1). Chromatogram showing amount of standard DDT after the injection of 20 ng/ml to GLC.

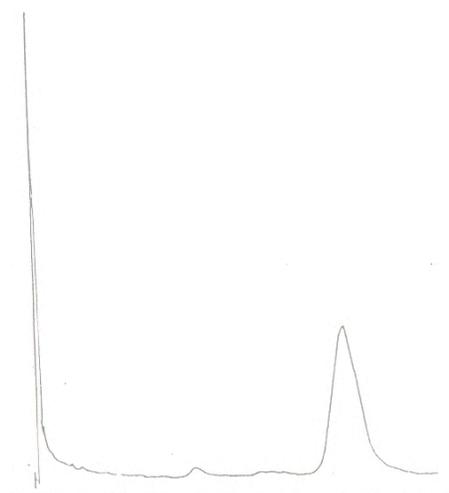


Fig. (2). Chromatogram showing amount of standard DDT after the injection of 40 ng/ml to GLC.

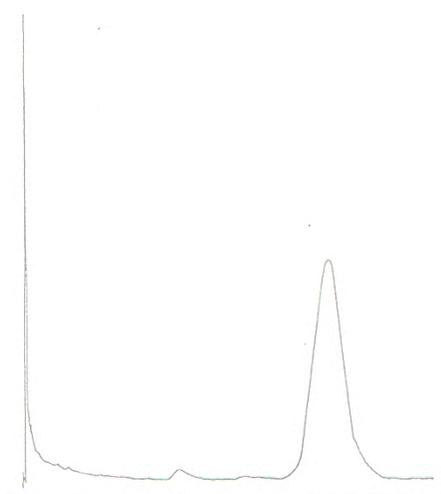


Fig. (3). Chromatogram showing amount of standard DDT after the injection of 60 ng/ml to GLC.



Fig (4). Chromatogram showing amount of standard DDT after the injection 80 ng/ml to GLC.

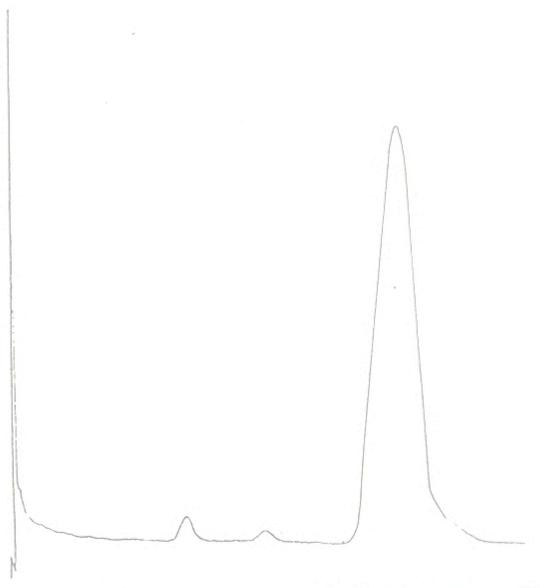


Fig. (5). Chromatogram showing amount of standard DDT after the injectin of 100 ng/ml to GLC.

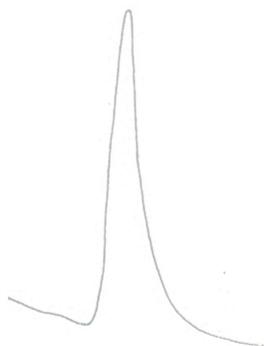


Fig. (6). Chromatogram showing amount of standard DDT nondegraded by GLC in hexane after the treatment of *Culex fatigans* (K.U. starin).

In the case of K.U. strain the quantity of DDT was found to be 59.68 ng (Fig. 6) when treated with 0.175 ppm DDT. In G.I. strain the quantity of DDT was found to be 18.3 ng (Fig. 7), after treatment with 0.325 ppm DDT, whereas in L.Y. strain the quantity of DDT was found to be 4.35 ng (Fig. 8), when treated with 0.425 ppm DDT.

Gale and Hofberg (1985) [5,6] reported a gas chromatographic (GC) procedure for the determination of chlordimeform in emulsifiable concentrate formulations containing about 45% active ingredient. Laboratory repeatability of better than 1% reproducibility was 1.2% for the formulation. During present investigations also a gas chromatographic procedure was used for the comparative quantitative determination of DDT in susceptible and resistant strains of *Culex fatigans* L. For this purpose a susceptible K.U. strain and two resistant strains, G.I. strain (standard) and L.Y.

resistant strain were used. A sample from the susceptible K.U. strain having 0.175 ppm of DDT reproduced 59.68 ng of DDT by GLC and reproducibility was found to be approximately 0.3%. The G.I. strains, which had 0.325 ppm of DDT, reproduced 18.3 ng of DDT by GLC, and reproducibility was about 0.02% while the L.Y. strain which had the maximum quantity of DDT. (0.525 ppm), reproduced only 4.35 ng of DDT by GLC and the reproducibility was 0.022%. The variation from the results of recent work and Gale and Hofberg (1985) [5,6] may be due to difference in insecticide or resistant factor.

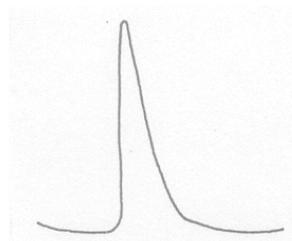


Fig. (7). Chromatogram showing amount of standard DDT nondegraded by GLC in hexane after the treatment of *Culex fatigans* (G.I. Strain).



Fig. (8). Chromatogram showing amount of standard DDT nondegraded by GLC hexane after the treatment of *Culex fatigans* (L.Y. starin).

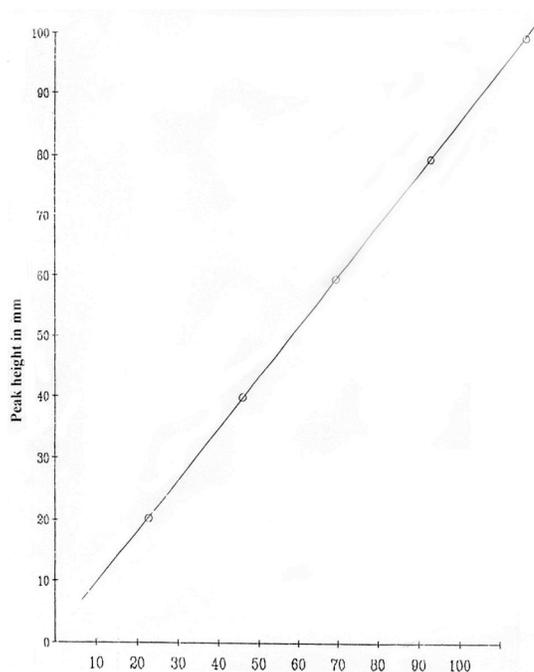


Fig. (9). Calibration curve for DDT obtained from GLC concentration ng/ml.

Syzmezynski *et al.* (1986) [13] studied the samples of adipose tissue from the abdominal cavity, which were taken at random during surgery on the abdominal cavity. The samples were taken and then analyzed for the presence of chlorinated pesticides by means of gas chromatography. All the analyzed samples contained β -BHC, γ -BHC or Lindane, HCB, PP-DDE and DDT isomers. K.U., G.I. and L.Y. strains were treated with DDT by means of contact/WHO method and the unmetabolized amount of DDT (remaining in the body in its original form) was estimated by gas chromatography.

The results show that minimum degradation takes place in the case of susceptible K.U. strain and with maximum amount of DDT in its actual form. In the case of the resistant L.Y. strain, a lower amount of DDT was detected. Isomers of DDT were also recorded in the form of peaks, but these were not identified because the standards of these isomers were not available.

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