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Gas Chromatography-Mass Spectrometer Electron Ionization (GC-MS - EI) method for the Analysis of Malathion Residue in Tomato

Abdalla Ahmed Elbashir*, Yasmin Alsadig Mohammed Elnour, Hassan Elobid Ahmed
University of Khartoum, Faculty of Science, Chemistry Department, Khartoum, 11115, Sudan, P.O box 321
Authors of correspondence
Dr. AbdallaA.Elbashir, e-mail:hajaae@yahoo.com

ABSTRACT

In this a study tomato samples were collected from Khartoum, extracted with acetone, dichloromethane and petroleum ether (1:1:1) and cleaned up by florisil column. Malathion quantitative determination is carried out by gas chromatography-mass spectrometer using the optimum ionization mode electron ionization (EI). The detection of malathion is confirmed by retention time and comparison of primary and secondary ions. Recovery studies were performed at two spikes ($0.5, 0.25 \text{ mg kg}^{-1}$) fortification levels of malathion and the recovery obtained ranged from 81% to 97%. The method showed good linearity ($R^2 > 0.995$) over the range assayed (from 0.05 to 7.0 mg L^{-1}) and the calculated limits of detection (LOD) and quantification (LOQ) were 0.03 mg kg^{-1} and 0.11 mg kg^{-1} , respectively. These limits were lower than the maximum residue levels (MRL) established by European legislations (0.5 mg kg^{-1}).

Keywords: Tomato; Malathion; Gas chromatography-mass spectrometer; Electron impact ionization; Florisil.



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INTRODUCTION

During the last two decades there have been growing social concerns over issues related to public health, environmental quality, and food safety. One of the major controversies inciting these concerns involves the production and consumption of fresh fruit and vegetables. Research has shown that diets with greater proportions of fruit and vegetables can prevent or delay a number of life threatening diseases. At the same time, public acceptance and adoption of these findings is being discouraged by ongoing re-evaluations of the possible health risks associated with minute amounts of pesticide residues sometimes found in or on these foods. The application of pesticides is essential in modern agricultural practices to control pest and diseases that damage fruit and vegetables. However, it has the drawback of pesticide residues which remain on fruit and vegetables, constituting a possible risk to consumers. [1] Therefore, governments and international organizations (FAO, WHO) have established maximum residue levels (MRLs), limiting the amount of pesticides in foods. Currently organophosphates, carbamates and pyrethroids are mostly used while some organochlorine insecticides have been banned because of their toxicity, persistence and bioaccumulation in the environment [2].

A wide variety of techniques have been used to extract and to purify pesticides from fruit and vegetables, including liquid-liquid extraction (LLE) [3] solid-phase extraction (SPE) [4] accelerated solvent extraction (ASE) [5], gel permeation chromatography (GPC) [6], and supercritical fluid extraction (SFE) [7].

The most frequently used technique for analysis of pesticide residues in fruit and vegetables is gas chromatography with different selective detectors. Such as flame photometric (FPD) [8], nitrogen-phosphorus (NPD) [9], and electron-capture detectors (ECD) [10,11]. Numerous methods use gas chromatography coupled with mass spectrometry (GC-MS) due to the possibility of confirming pesticide identity in these matrices [12, 13]. Liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS) [14, 15] has lately become a powerful analytical technique for the identification and quantification of residues in fruit and vegetable. A critical review of literature showed that different solvents such as n-hexane, petroleum ether, methylene chloride and acetone or ethyl acetate have been used for extraction of pesticide residue from fruit and vegetables [16]. As more polar pesticides, such as organophosphate and phenoxyacetic acid, polar solvents such as chloroform, acetone, acetonitrile and methanol were found to be good [17]. Ethyl acetate is found to be a good solvent as compared to other solvents for the extraction of residues of several pesticides from fruit and vegetables because its polarity is high and it is a less volatile and thermally labile compound [18].

Malathion is a broad spectrum, non-systemic organophosphorous insecticide that is used on a wide variety of crop sites and on various non-crop sites, including greenhouses, nurseries, home and garden, and public health. The chemical structure of malathion is shown in Figure 1. It is very highly toxic to fish and aquatic invertebrates but does not appear to be toxic to plants. Some residential and agricultural uses can have rather high application rates and resulting exposure.

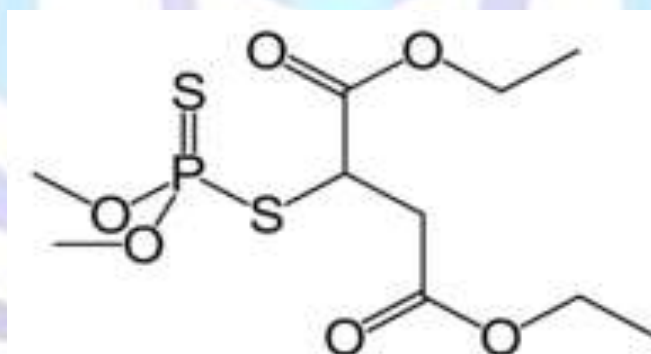


Fig. 1. Malathion or 2-(dimethoxyphosphinothioylthio) butanedioic acid diethyl ester

The present work is designed to study the residues of malathion pesticides in tomato, a sample is extracted with simple and effective procedure using low volume of organic solvent, cleanup is carried by florisil columns and residue levels were determined by gas chromatography (GC) with mass spectrometer detector (GC-MSD).

EXPERIMENTAL

Reagents and Chemicals

Acetone, dichloromethane, petroleum ether and n-hexane, of special grading for the pesticide residue analysis, were obtained from Scharlau Company.

INSTRUMENTATION

A Shimadzu GC-2010 gas chromatograph with a QP-2010plus mass spectrometer (Japan) was used. The GC system, with an electronic ionization (EI), was equipped with AOC 500 Auto injector autosampler and a splitless injection port.



Chromatographic separation was performed on column RTX-5MS (5% phenyl-95% polydimethylsiloxane; 30 m x 0.25 mm ID, 0.25 μ m).

Chromatographic conditions

Helium was used as carrier gas at a constant flow-rate of 0.9 mL min⁻¹. The column temperature was programmed as follows: 90 °C for 6 min, 20 °C /min to 200 °C (6 min.) and 20 °C /min to 260 °C (5 min). The solvent delay was 2.5 min. The total analysis time was 20 min. The injection port was maintained at 200 °C and 1 μ L, sample volumes were injected in splitless mode. The data were acquired and processed using Shimadzu GC Solution software. The eluent from the GC column was transferred via a transfer line heated at 280 °C and fed into a 70 eV electron impact ionization source, also maintained at 280 °C. Table 1 lists the pesticides along with their retention times, molecular mass, the target and qualifier ions, and their qualified target abundance ratios. The target abundances were determined by injection of pesticide standards under the same chromatographic conditions using full scan mode with the mass/charge ratio ranging of the m/z 10 to 400. In these evaluations, the characteristic ions were chosen, and the MS system was then programmed in selective ion monitoring (SIM) mode for quantification of pesticide. The choice of the ions for SIM acquisition was based on the best S/N ratio as m/z 100, 125 and 127. Values of m/z in bold type correspond to the quantification ion for analyte.

For the extraction of samples, a Polytron PT2000 homogenizer (Kinematical AG, Lucerne, Switzerland) was used. An Eppendorf model 5810R centrifuges (Hamburg, Germany) and a Büchi model R-200 rotavapor (Flawil, Switzerland) was used in the centrifugation and evaporation to dryness of samples, respectively.

Table 1: Retention time (RT, min), molecular weight (MW), target (T), qualifier ions (Q1, Q2) (m/z) and abundance ratios (%) of qualifier ion/target-ion (Q1/T, Q2/T) of the Malathion pesticides.

Malathion	RT	MW	T	Q1	Q2	Q1/T	Q2/T
	12.622	330.4	127	125	100	44.66	25.30
	12.613	330.4	127	125	100	46.54	25.75
	12.617	330.4	127	125	100	49.67	29.40
	12.600	330.4	127	125	100	46.59	24.35
	12.606	330.4	127	125	100	46.16	23.47
	12.611	330.4	127	125	100	46.21	23.17
	12.614	330.4	127	125	100	43.26	30.20
	12.619	330.4	127	125	100	55.17	18.96
	12.620	330.4	127	125	100	59.63	15.36

Stock and Standard Solutions

Pesticides stock solutions (1381 mg L⁻¹) of malathion pesticide standard was prepared by dissolving 0.1381 g of the pesticide in 100 mL of petroleum ether. A pesticide intermediate standard solution (13.81 mg L⁻¹) was prepared by transferring 1 mL from pesticide solution to a 100 mL volumetric flask and diluting to volume with petroleum ether to obtain a concentration of 13.81 mg L⁻¹. Several standard solutions, with concentrations of (0.05-7.0 mg L⁻¹), were injected to obtain the linearity of detector response and the detection limits of the pesticides studied.

Sample preparation

Two different weights (49.70 g, 52.69 g) of sample was sprayed by formulation (10 mg L⁻¹) by different volumes (1.25 mL, 2.5 mL), respectively, then was left until they were dry, extracted, cleaned up and determined. Real sample was carried out by taking sample without spraying and then was extracted, cleaned up and determined.

Extraction

Each sprayed sample was cut and put in blender and homogenized for (30 sec) with 30 mL of acetone. 60 mL of dichloromethane and petroleum ether (1:1) were added and the mixture was homogenized for (1 min) then centrifuged at 4000 rpm for (5 min), the volume of extract was concentrated in rotary evaporator with water bath at 35 °C and then was cleaned up by florisil column and determined [19].

Cleaned up

All samples were cleaned up by florisil column before analysis by GC-MS. Florisil (20g) in hexane was allowed to settle in a chromatographic column (45 cm x 20 mm) by tapping the column. To the top of florisil, a layer of 1 to 2 cm deep anhydrous sodium sulphate was added. Then the column was eluted with 200 mL of hexane and the liquid was discarded. Concentrated sample of tomato extract (1 mL) in hexane was transferred to the column, and then the column was eluted

with 200 mL of 15% diethyl ether in hexane followed by 200 mL of 50% diethyl ether in hexane. The solution was evaporated to 5 mL and injected in GC-MS.

RESULTS AND DISCUSSION

Gas chromatographic determination

Pesticides residue levels were determined by GC-MS. Representative mass spectrum and chromatograms of a standard pesticide are shown in (Figure 2 (a), (b)), and that for a tomato sample spiked with the formulation of the malathion solution and real sample are shown in (Figure 3 (a), (b)), respectively.

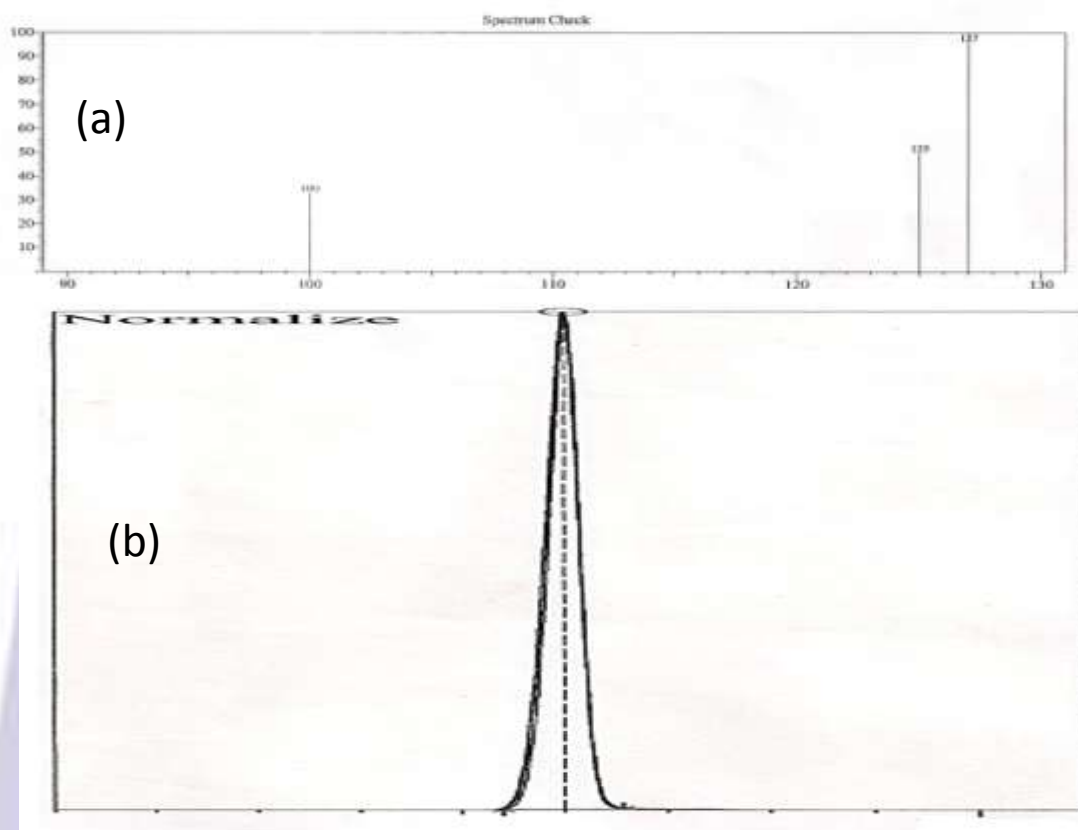


Fig. 2. (a) Mass spectrum (GC-MS-EI) of malathion fragment, (b) Chromatogram (GC-MS-EI) of standard malathion.

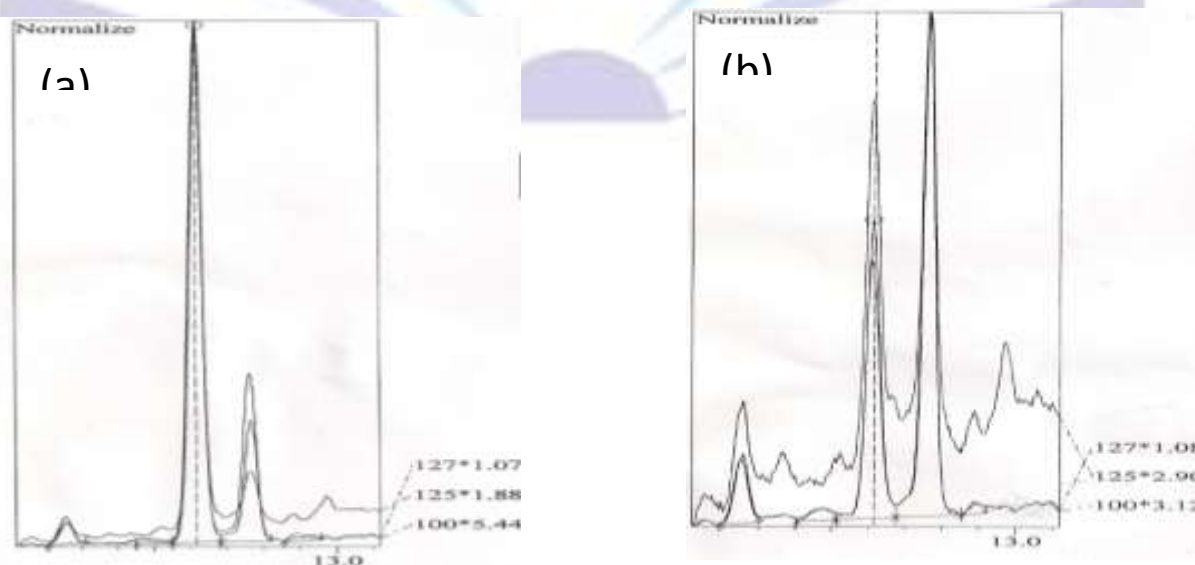


Fig 3 (a) Chromatograms (GC-MS-EI) obtained for (a) (spike 0.25 mg kg⁻¹) (b) Areal tomato sample.



Method validation

The MS response for pesticides was linear in the concentration assayed ($0.05\text{--}7.0\text{mg L}^{-1}$) with determination coefficients >0.995 for pesticides. The results are shown in Table 2.

Table 2: Calibration range ($0.05\text{--}7\text{mg L}^{-1}$)

Number	Conc. (mg L^{-1})	Mean Area
1	0.05	834
2	0.1	1718
3	0.5	3051
4	3	812336
5	5	1362964
6	7	1721302

Limit of Detection and Quantitation

The limit of detection (LOD) of the method was determined at a signal-to-noise ratio of 3 for the pesticides in tomato by GC-MS, whereas the limit of quantification was obtained at a signal-to-noise ratio of 10. The LOD is 0.03 mg kg^{-1} and the LOQ is 0.11 mg kg^{-1} .

Recovery

A study of recoveries for pesticide at two different fortification levels was carried out in order to assess the extraction efficiency of the method. For that, two tomato samples were spiked with ($0.5, 0.25\text{ mg kg}^{-1}$) of pesticide and processed as described. A recovery data obtained are shown in Table 3.

Table 3: Recovery of pesticides from spiked Tomato samples

Pesticide	Fortification level (mg kg^{-1})	recovery
Malathion	0.5	81%
	0.25	97%

Analysis of real samples

Tomato samples were analyzed following the extraction methods described above. Pesticide concentration levels in the real samples were found to be 0.3mg kg^{-1} . Analysis of samples showed the validity of method used, which allowed the determination and identification of pesticides present in the samples.

CONCLUSION

The results of this study show that the method to determine residues of pesticides in tomato is rapid, simple, sensitive and uses small volumes of solvents for sample extraction, reducing the risk for human health and the environment. Good recovery and low detection through method were obtained for the pesticides studied, including new generations of pesticides, since their decompositions quicker and has a less damaging effect on the environment. The method shows advantages compared with other conventional methods in that, the use of a low volume of organic solvent in the sample extraction, it avoids the use of a chlorinated hydrocarbon, and the time of extraction is short.

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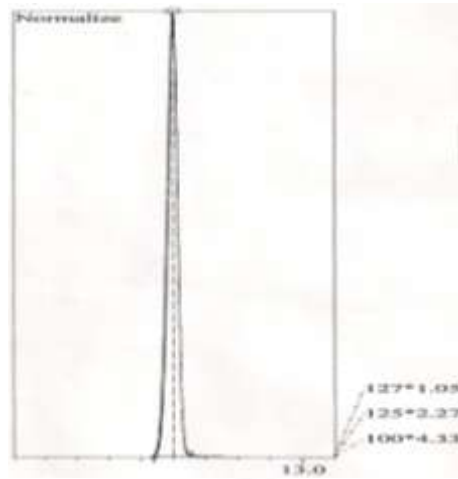


Fig. 2b: Chromatogram (GC-MS-EI) obtained for: standard malathion

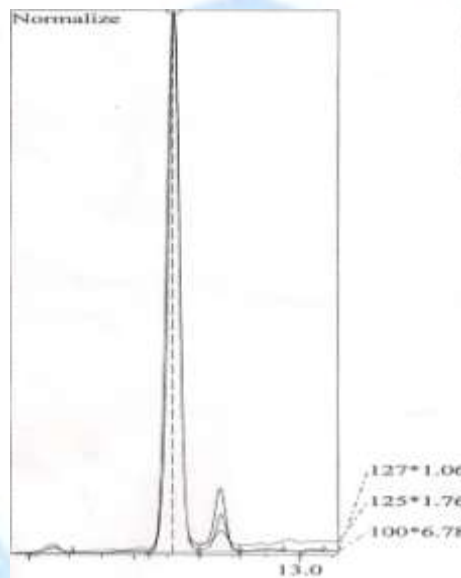


Fig 3a: Chromatograms (GC-MS-EI) obtained for: (spicke 0.5 mg kg⁻¹)

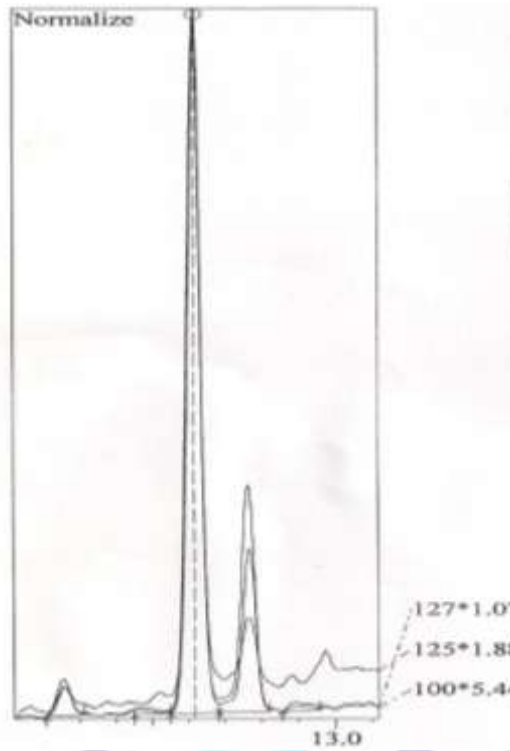


Fig 3b: Chromatograms (GC-MS-EI) obtained for: (spicke0.25 mg kg⁻¹).

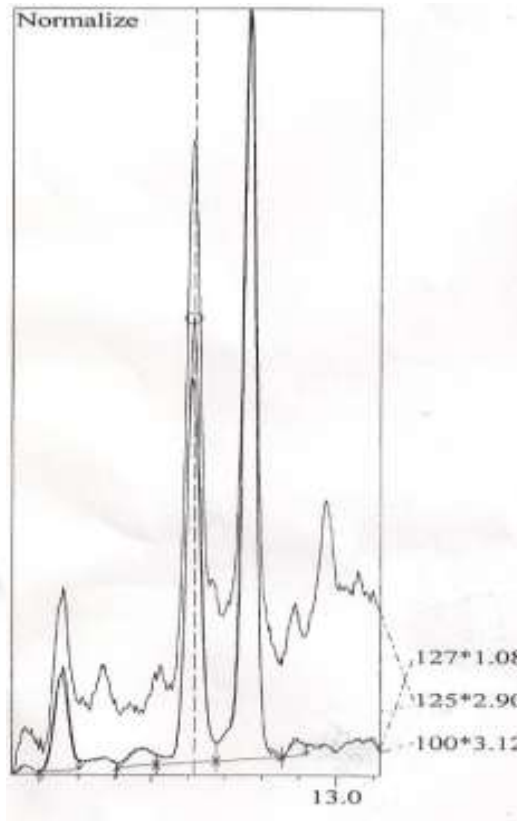


Fig 3c: Chromatograms (GC-MS-EI) obtained for: Areal tomato sample.

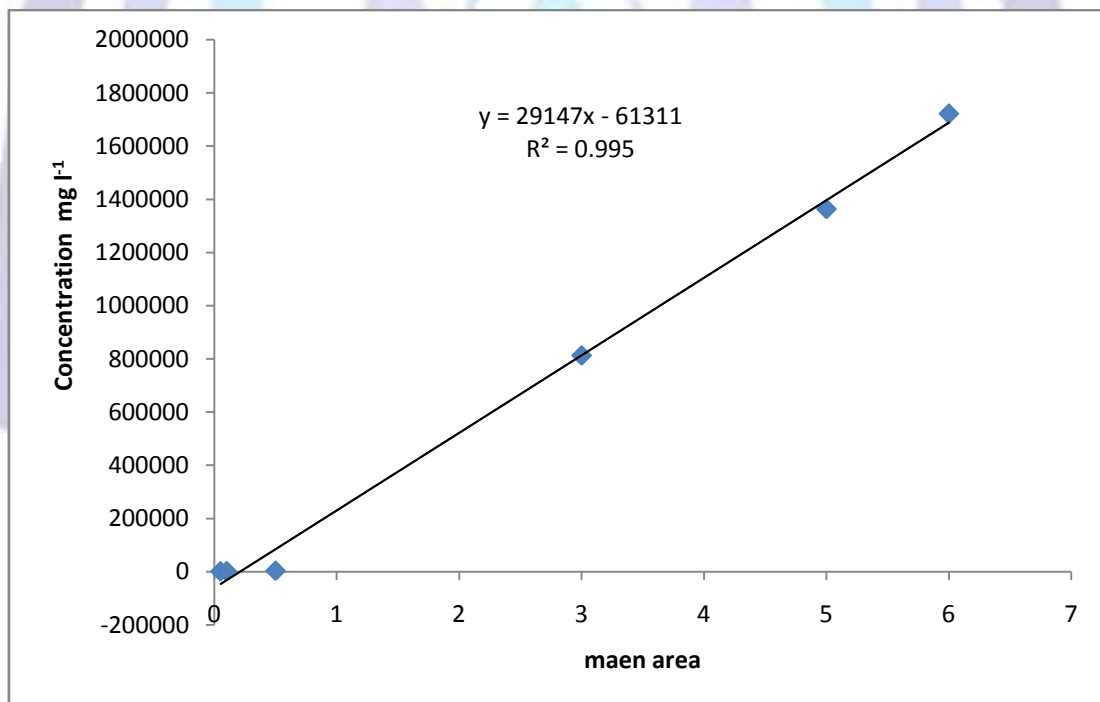


Fig.4: Calibration curve of malathion standard.