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HPLC analysis of Deltamethrin after 24 hours treatment of *Callosobruchus analis*

Samina Arif, Naim ul Hassan Naqvi, Muhammad Arshad Azmi, Abid Raza, Nadia Fatima, Sadaf Tabassum

Abstract
Toxicity of Deltamethrin essential oil against *Callosobruchus analis* by three methods Direct Application method (DAM), Glass film method (GFM) and Filter paper impregnation method (FIM) after 24 hours of treatment. The LC_{50} value calculated as 0.3352 µl/cm^2 by DAM, 0.043901 µl/cm^2 by GFM and 0.03472 µl/cm^2 by FIM. HPLC analysis shown the activity of Deltamethrin (standard) against *C. analis* by (DAM) shown by peaks 89765, 6785, 3365 and in Deltamethrin (sample) peaks results shown as 79918, 2672, 1348 µm, similarly by (GFM) Deltamethrin (standard) shown as one peak 7449, peak (2) and (3) were absent and Deltamethrin (sample) observed as 89885, 10689, peak 93) was absent, similarly by (FIM) Deltamethrin (standard) found as one peak 59784, peak (2) and (3) were absent and Deltamethrin (sample) shown as two peaks which was 73809, 2115 µm.

Keywords: Hplc, Deltamethrin, *Callosobruchus Analis*

1. Introduction
Ledwick AGL [1] worked on simple and sensitive technique for detecting organo chlorine pesticides on thin layer chromatogram. Sherp GJ *et al.* [2] reported extraction, clean-up and chromatographic determination of organophosphate, pyrethroid and carbamate Insecticides in grain and grain product. Mattern GC *et al.* [3] worked on various commodities (apples, tomatoes, potatoes) by GC- CIMS (Chemical ionization mass spectrometry). Darwish A *et al* [4] worked on wool after pour- on or plunge dipping application by reverse phase (RP) HPLC. Chen ZM *et al.* [5] worked on chromatographic method for determination of pyrethrin and pyrethroid pesticides residues in crops foods and environmental sample. Lekic M *et al.* [6] reported thin layer chromatography of pesticides. Sherma J *et al.* [7] reported thin layer chromatography of pesticides a review of application for 2002-2004. Cao H *et al.* [8] worked on HPTLC determination of imidacloprid, Fentrothin and parathion in Chinese cabbage. Guo-Fang P *et al.* [9] worked on simultaneous determination of 405 pesticide residues in grainby accelerated solvent extraction then gas chromatography-massspectrometry and liquid chromatography-tandem mass spectrometry Tache F *et al.* [10] reported specificty of an analytical HPLC assay method of Metformin Hydrochloride. Shishovska MA *et al.* [11] reported the sample HPLC method for determination of permethrin residues in wine. Shishovska MA *et al.* [12] reported the Fast and Universal HPLC method for determination of Permethrin in formulations using 1.8µm practical packed column and performance comparasion with other columns. Ravikumar CH *et al.* [13] Determination of pyrethroid residue in rice by gas chromatography tandem mass spectromet.

2. Material and Methods
2.1 Performance Liquid Chromatography (HPLC):
During the recent years chromatography technique has undergone a rapid development and high performance liquid chromatography (HPLC) is one the achievement. The detection by this techniques is simple, rapid and sensitive. HPLC have been used for the separation of pesticides, by using a packed column (Zorbax TMNH2) a polar bounded phase with particle size of about 7 HM in diameter. The columns are packed to uniform bed density by using a high pressure slurry loading technique. The column was used with fractionated n-hexane as mobile phase with a flow rate of 2.5 ml/min. A UV detector was used with a wavelength of

205 nm, pressure 200 kg/cm^2 and the absorbance 0.08 with chart speed 10 mm/ min for detection of pesticide. Standard sample of Deltamethrin as well as the samples from animal tissues (strain of*Callosobruchus analis* treated with Deltamethrin were prepared by following method.Before the application of sample to HPLC, pesticides accumulated with fats were extracted from the animal tissue (adult *Callosobruchus analis*) and initial separation of interfering organic residue such as fats, pigments and other organics must be carried out. For each step involved in the method experiments were carried out in the following manner.

2.2 Quantitative Analysis of Pesticide Residues by High Performance Liquid Chromatography (Hplc)

During the recent years chromatography technique has undergone a rapid development and high performance liquid chromatography (HPLC) is one the achievement. The detection by this techniques is simple, rapid and sensitive. HPLC have been used for the separation of pesticides, by using a packed column (Zorbax TMNH2) a polar bounded phase with particle size of about 7 HM in diameter. The columns are packed to uniform bed density by using a high pressure slurry loading technique. The column was used with fractionated n-hexane as the mobile phase with a flow rate of 2.5 ml/min. A UV detector was used with a wavelength of 205 nm, pressure 200 kg/cm^2 and the absorbance 0.08 with chart speed 10 mm/ min for detection of pesticide. Standard sample of *Acorus calamus* as well as the samples from animal tissues (strain of *Callosobruchus analis* treated with *Acorus calamus*) were prepared by following method.

Before the application of sample to HPLC, pesticides accumulated with fats were extracted from the animal tissue (adult *Callosobruchus analis*) and initial separation of interfering organic residue such as fats, pigments and other organics must be carried out. For each step involved in the method experiments were carried out in the following manner.

2.3 Extraction of fats from under test insect tissue

The pesticides are lipophilic in properties and accumulate with Lipid, therefore for the extraction of Deltamethrin fat must be extracted. For this purpose following procedure were adopted.

2.4 Soxhlation

For the extraction of pesticides residues from samples of *Callosobruchus analis* Pulse beetle tissue [15] method was used. A known quantity of sample (100) beetles of each strain treated with Deltamethrin separately. Weighing about mg Marcerated with anhydrous sodium sulphate (Na2So4) and was transferred into a thimble made 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 flask was then placed on a heating mantle. The process of extraction was carried out for two hours during which all the pesticide residues must have been extracted with solvent. Better recoveries were noticed by this method. The fat extracted solvent was then reduced to about 1 ml evaporation. For complete recovery of pesticides the column chromatography (sorption) was employed and material was passed three to four time through the columns given below.

2.5 Sorption

This process of sorption was carried out in chromatographic columns of alumina following Holden AV, Kadoum AM [15, 16].

2.6 Alumina and Silica columns

The alumina column was prepared as and Goternative method for the sepration of fat from pesticides which was used by [15]. The column was made by glass column having length 40-42 cm with internal diameter 6mm. The column was filled with 2 grams of alumina without calcium of 0.3 micron size already, activated at 800 °C for 4 hours inn 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 (fractional) and transferred on the surface of the alumina column. Now the pesticide adsorbed on the column were eluted with 12 ml of n-hexane and volume 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 trace of moisture 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 and then with 12 ml of 10% diethyl ether in hexane. All the fraction were concentrated and make up to / ml separated and identified by HPLC. The same procedure was adopted using the different quantities of standard Deltamethrin per ml of n-hexane which are 2µg, 4µg and 6µg in case of Deltamethrin. It was used to obtain standard peaks for comparison with the samples. Standard chromatograph were prepared by mixing standard. Deltamethrin for comparison with chromatograms obtained.

3. Results

Table 1: Toxicity of Deltamethrin against *Callosobruchus analis* after 24 hours treatment of Direct Application method showing LC_{50} value as 0.03352 µl/cm^2.

S.No.	Dose inml	Dose in µl/cm^2	Mean Mortality	S.D	S.E	Confidence limit at 95%
0	Control	-	-	-	-	-
1	0.00312	0.00693	29%	2.1095	0.9459641	3.9459104 ± 7.6540896
2	0.00625	0.01388	35%	1.00	0.448463	6.1210765 ± 7.8789235
3	0.0125	0.02777	49%	1.5	0.67264	1.3183855 ± 11.118385
4	0.0250	0.0555	66%	3.3466	1.5004484	10.259122 ± 16.140878
5	0.050	0.111	92%	0.547725	0.2456165	17.918592 ± 18.881408

Table 2: Toxicity of Deltamethrin against *Callosobruchus analis* after 24 hours treatment by Glass Film Method showing LC $_{50}$ value as 0.043901 µl/cm^2.

S.No.	Dose in ml	Dose in µl/cm^2	Mean Mortality	S.D	S.E	Confidence limit at 95%
0	Control	-	-	-	-	-
1	0.00625	0.01388	32%	4.3817	1.964	2.550 ± 10.24944
2	0.0125	0.02777	44%	5.019	2.2511	4.38384 ± 13.212156
3	0.025	0.0555	55%	1.2247	0.549	9.92396 ± 12.07604
4	0.050	0.111	84%	1.095	0.4912	15.8324 ± 17.762752
5	0.100	0.222	96%	0.77459	0.3473	17.3196 ± 20.66804

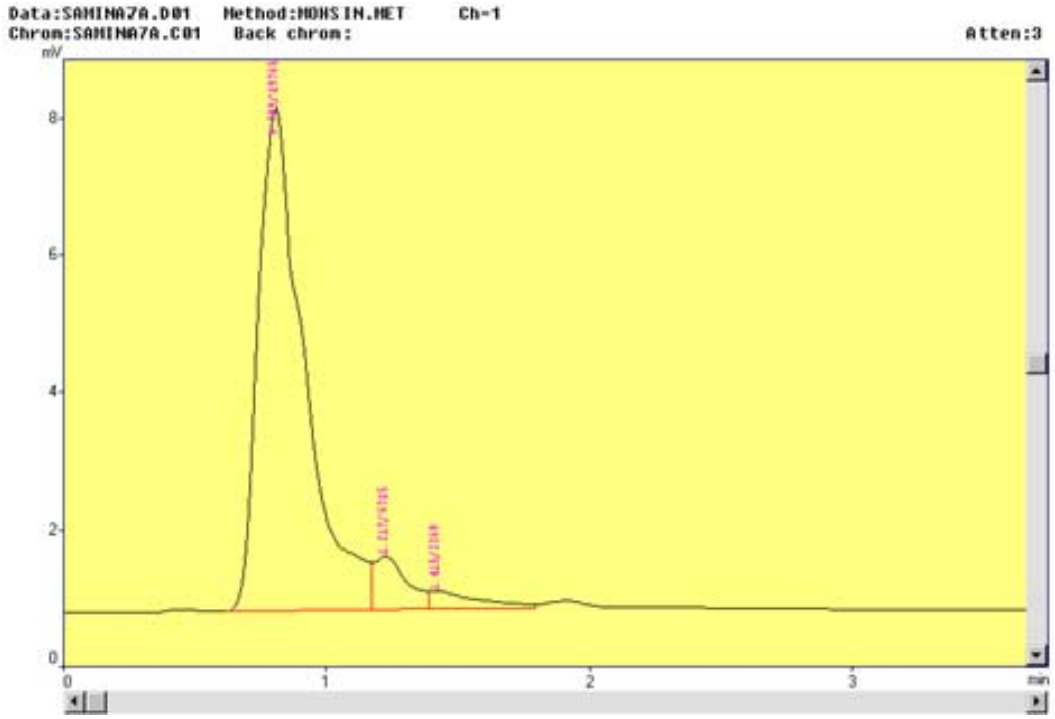
Table 3: Toxicity of Deltamethrin against *Callosobruchus analis* after 24 hours treatment by Filter Impregnation Method showing LC $_{50}$ value as 0.03472 µl/cm^2.

S.No.	Dose in ml	Dose in µl/cm^2	Mean Mortality	S.D	S.E	Confidence limit at 95%
0	Conrol	-	-	-	-	-
1	0.00312	0.006933	24%	1.3038	0.58466	3.6540664 ± 5.9459336
2	0.00625	0.01388	38%	1.5165	0.680078	6.2670472 ± 8089329528
3	0.0125	0.02777	49%	1.4832	0.665129	8.4963472 ± 11.103652
4	0.0250	0.0555	76%	1.30384	0.58466	14.054447 ± 16.345553
5	0.050	0.111	81%	0.83666	0.37518	15.46468 ± 16.935352

Sample Information

Sample name: Deltamethrin (1)

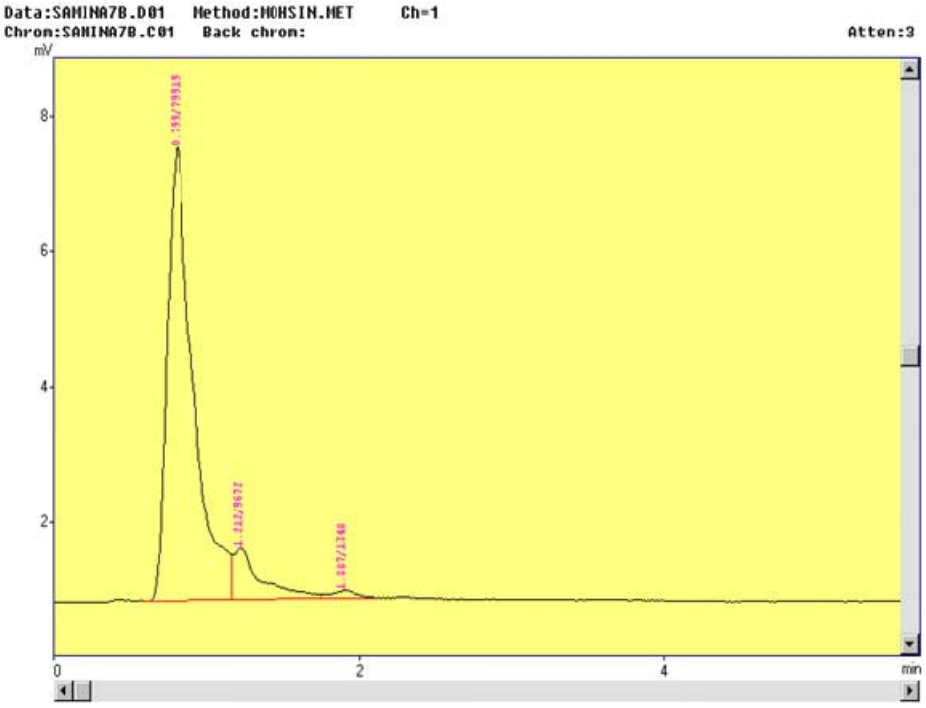
Direct application method (standard)



Peak Results

S. No.	Peak No.	RT in min	Area in µm
1.	1	0.789	89765
2.	2	1.217	6785
3.	3	1.415	3369

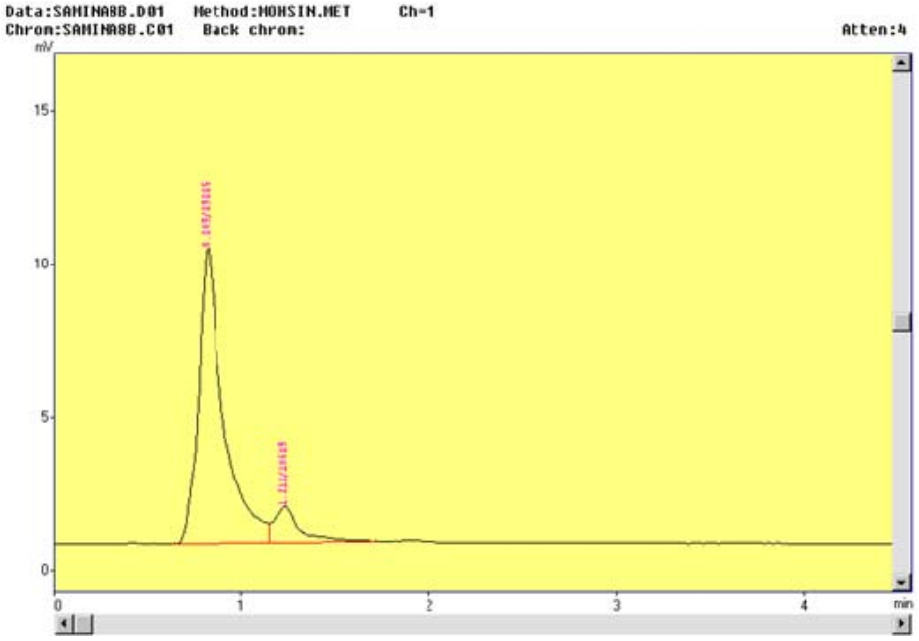
Sample Information
Sample name: Deltamethrin (2)
Direct application method (sample)



Peak Results

S.No.	Peak No.	RT in min	Area in µm
1.	1	0.796	74449
2.	2	-	-
3.	3	-	-

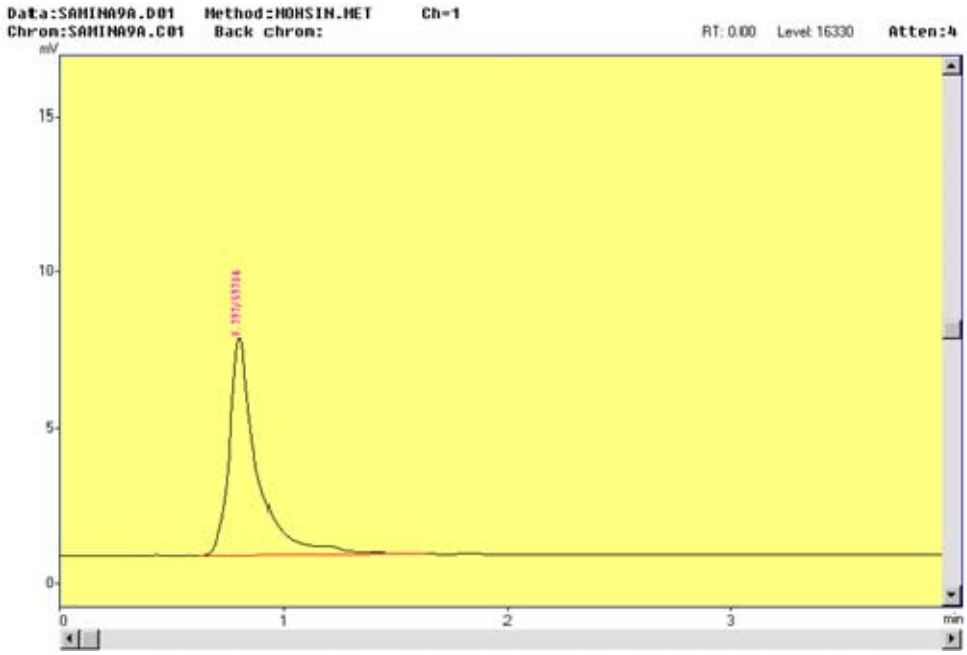
Sample Information
Sample name: Deltamethrin (4)
Glass film method (sample)



Peak Results

S. No.	Peak No.	RT in min	Area in µm
1.	1	0.809	89885
2	2	1.218	10689
3.	3	-	-

Sample Information
Sample name: Deltamethrin (5)
Filter paper impregnation method (standard)

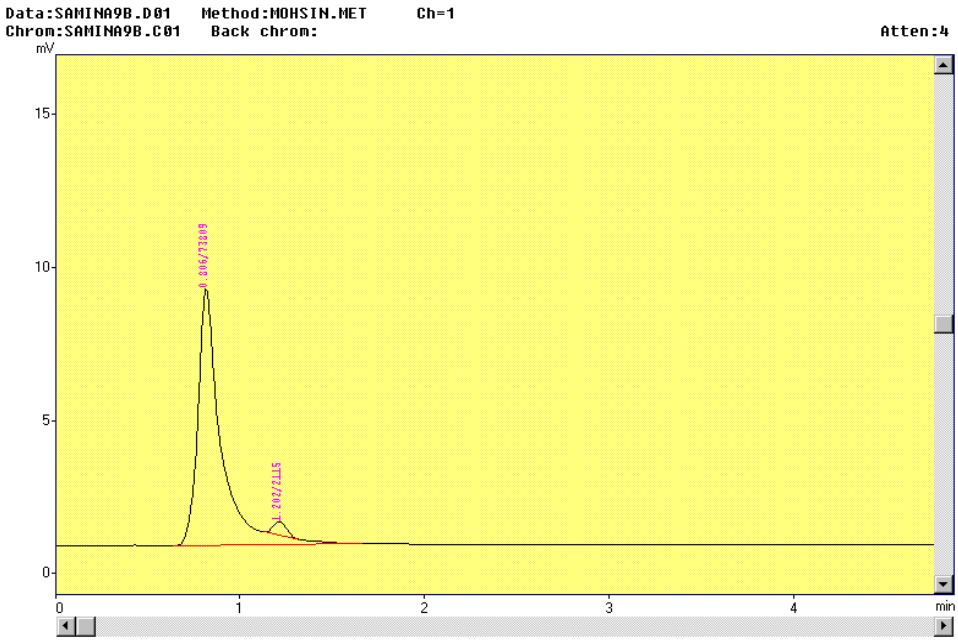


Peak Results

S.No	Peak No.	RT in min	Area in µm
1.	1	0.797	59784
2.	2	Absent	Absent
3.	3	Absent	Absent

Sample Information
Sample name: Deltamethrin (6)
Filter paper impregnation method (sample)

Peak Results



S,No	Peak No.	RT in min	Area in µm
1.	1	0.806	73809
2.	2	1.202	2115
3.	3	Absent	Absent

Toxicity of *deltamethrin* against *Callosobruchus analis* by Direct application method were observed as 29%, 35%, 49% 66% and 92% at the dose of 0.0138, 0.2777, 0.0555, 0.111 and 0.222 µl/cm^2 respectively as shown in Table 1. The LC_{50} value was calculated as 0.03352 µl/cm^2. while in Glass film method mean mortalities were observed 32%, 44%, 55%, 84%, and 96% at the dose of 0.1388, 0.02777, 0.0555, 0.111 and 0.222 µl/cm^2 respectively as shown in Table 2. The LC_{50} value was calculated as 0.04390 µl/cm^2, while in Filter paper impregnation method mean mortalities were observed as 24%, 38%, 49%, 76% and 81% at the dose of 0.00693, 0.0138, 0.02777, 0.0555 and 0.111 µl/cm^2 as shown Table 3. The LC_{50} value was calculated at 0.034721 µl/cm^2.

Chromatogram No. 1 Deltamethrin (Standard) (DAM): Market product (Pyrethroid): The first peak (1) is possibly of active ingredient (Deltamethrin) this is the major peak. After that, there (2) minor peak 3 and 4 which may be of biodegradable product or detergent.

Chromatogram No. 2 Deltamethrin (sample) (DAM): The chromatogram has a similar pattern as both the samples are from the separable product obtained from the market.

Chromatogram No. 3 Deltamethrin (Standard) (GFM): The first major peak is a major one (1) representing Deltamethrin. Peak 2 and 3 did not appear clearly, possibly due to the less concentration of the other components.

Chromatogram No. 4 Deltamethrin (sample) (GFM): There is one major peak of active ingredient. The second peak may be detergent or some other component.

Chromatogram No.5 Deltamethrin (Standard) (FIM): There is one major peak (1) of the active ingredient.

Chromatogram No. 6 Deltamethrin (Sample) (FIM): There is one major peak i.e active ingredient (Deltamethrin) and minor peak (2) which may be of biodegradable product.

HPLC analysis shown the activity of Deltamethrin (standard) against *C.analis* by (DAM) shown by peaks 89765, 6785, 3365 and in Deltamethrin (sample) peaks results shown as 79918, 2672, 1348 µm, similarly by (GFM) Deltamethrin (standard) shown as one peak 7449, peak (2) and (3) were absent and Deltamethrin (sample) observed as 89885, 10689, peak 93) was absent, similarly by (FIM) Deltamethrin (standard) found as one peak 59784, peak (2) and (3) were absent and Deltamethrin (sample) shown as two peaks which was 73809, 2115 µm.

4. Discussions

Manadas R [17] worked on reserved-phase High performance liquid Chromatography (RP-HPLC) method for simultaneous and separate determination of cis and trans- permethrin and piperonl butoxide in shampoo formulation is described and fully validated. The method entails the quantification of both component by external standard and ultraviolet detection at two wavelength (201 and 287). In the present study detection of deltamethrin by HPLC against *Callosobruchus analis* moreever method were used DAM, GFM and FIM pest in known volume 0.15 µl by Atomizer then 100 insects were released in 90 mm petridish. Haddad PR *et al.* [18] worked on acetone which is best solvent and provided extraction of the pesticides over a 48 hours period and did not give high levels of ballast material. Pyrethroid present in extract at levels in excess of 0.5 µg/ml could be determined by direct injection but at lower concentrations, clean up and pre concentration was required. Clean up of acetone extract was accomplished with either florisil or alumina pre-columns and up to a ten folds pre concentration was achieved by adsorption of pesticides on c18 pre column or by concentrating the extract through evaporation of the solvent. These approaches gave good recoveries and linear calibration plates. Detection limits were order of 0.05µg/ml. Reserved phase HPLC with detection 225nm. In the preset study pesticide known volume was sprayed on the inner surface of petridishes and HPLC analysis observed after 24 hours of treatment of deltamethrin by three different methods. Trajkovska V *et al*. [19] worked on HPLC method for simultaneous determination of the pesticides captan, Lerbumenton and deltamethrin. The calibration curves constructed in the range of 20-400 ng mass of pesticides in the column followed linear dependence in the whole range and the values of co-efficient of correlation were higher than 0.9971. In the present study detection of deltamethrin by HPLC after 24 hours treatment of *Callosobruchus analis* and peak area showed the presence of pesticide. Huiri D *et al*. [20] worked on developed a novel method for separating and enriching pyrethroid insecticide from vegetables by solvent sublation and detection of pyrethroid content is performed by (HPLC). They observed the detection values ranged from for bifenthrin1.4 β/kg and for propathrin 4.2 β/kg. The recoveries of vegetables sample were from 85.7% to 110.4% and S.D value were 1.70% to 6.19%. Present work showed the activity of deltamethrin by HPLC peaks results showed by different method (DAM, GFM, FIM) chromatogram (13), (15) and (17) were Deltamethrin standard sample showed by peaks. Similarly chromatogram (14), (16) and (18) deltamethrin (sample) by three above method showed peak (1) representing deltamethrin and peaks (2) and (3) which may be biodegradable products or detergent. Result is not comparable may be due to different pesticide and insects also because they extracted the pesticide from vegetable. Aldana-Madrid ML *et al*. [21] Detecting and quqntifing insecticide residue in stored wheat, corn, chickpeas and beans as well as to determine their mutagenic potential in Maxican grain.Grains were sampled from primary storage sites in Sonora, Maxico, Malathion, Chlorpyrifus, Deltamethrin, Cypermethrin, 4,4-DDE, 4,4-DDD and 4,4-DDT were analysed in 135 samples were not mutagenic and most pesticides level were within regulation limits. In the present study insecticides residues in mung beans Vigna radiate after 24 houre treatment of Deltamethrin by three different methods DAM, GFM, and FIM against *Callosbruchus analis*. Peak area observed in standard.

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