Dissipation Kinetics of Chlorpyriphos in Citrus Fruit, Juice and Soil

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Abstract

To study the dissipation kinetics of chlorpyriphos in citrus fruit a field experiment was conducted during Khariff 2013. The insecticide chlorpyriphos 20 EC (Dustban) is applied @ of 500 g a.i./ha on citrus plants initiating at fruit formation stage and follow by two more sprays at an interval of 10 days. The citrus fruit samples collected at an time interval of zero hours, 1, 3, 5, 7, 10, 15 and 20 days after last spray. The citrus fruit samples extracted, cleanup and final extract was analyzed on Gas Chromatography with Electron Capture Detector. The limit of quantification of chlorpyriphos found to be 0.05 mgkg^{-1}. The initial deposits recorded in citrus fruit was 4.121 mgkg^{-1} and dissipated to 0.058 mgkg^{-1} on 15 days day. And chlorpyrphos residues were below detectable level 20 days after last application. The waiting period was worked out 6.2 days after last application of chlorpyriphos. The citrus juice samples at harvest and soil samples at harvest not recorded chlorpyriphos residues and were below detectable levels.

Keywords: Chlorpyrphos – residues – GC- ECD, Citrus fruit, juice and soil.

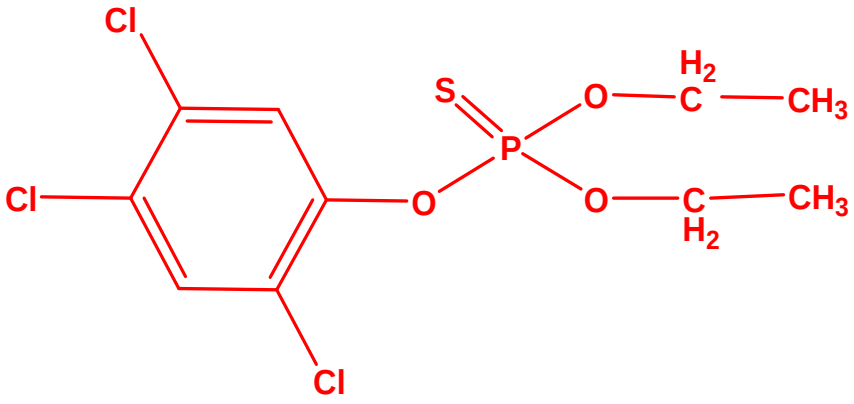
Introduction

Chlorpyrphos belongs to pyridine organo thio phosphate insecticide and its IUPAC name is O,O-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate ($C_9H_{11}Cl_3NO_3PS$) with a molecular weight of 350.6. The molecular structure of the Chlorpyrphos is depicted in Fig.1. This molecule is a broad spectrum [1] insecticide having acaricidal, insecticidal and nematicidal activity therefore it is widely used in India. The molecule is introduced by Dow chemical company, USA. It is a hazardous chemical and colorless crystalline solid with Mild mercaptan odor. The pesticide acts on the nervous system[2] of insects by inhibiting acetyl cholinesterase. The chemical Chlorpyrifos is moderately toxic to humans, and exposure has been linked to neurological effects, persistent developmental disorders and autoimmune disorders. Chlorpyrifos is very highly toxic [1,3] to aquatic invertebrates, freshwater fish, and other estuarine and marine organisms. Exposure to agriculture workers[4] during pregnancy retards the brain damage in children, and mostly home use was banned in the U.S. But still it is widely used in India on many crops and also other purposes . The study carried out in Kashmir valley on apples when chlorpyriphos applied on apples[5] recorded initial deposits 1.61 to 1.98 mgkg^{-1} and degraded after 30 days and chlorpyriphos treated gherkin[6] also recorded residues and half-life calculated 1.65 days. Therefore it is essential to study the residues levels of chlorpyriphos in citrus fruit, hence this studies. Chlorpyriphos is applied on the citrus crop at different doses and different stages i.e., soil drenching and spraying on crop.

Material and Methods

An experimental laid out in citrus plants field at Manikayapur of Karimnagar district. Telangana, India for conducting the Chlorpyriphos residues studies . And dissipation and persistence

pattern were studied by conducting the experiment during Kharif seasons 2013 by applying the pesticide Chlorpyriphos 20% EC (Dusban) at the rate 500 g a.i./ha on citrus plants. Three application chlorpyrphos was given at an intervals 10 days interval initiation at fruit formation stage. The citrus fruit samples were drawn at zero hours, 1,3, 5, 7, 10, 15 and 20 days time intervals after last application and samples were brought to laboratory further processing for the analysis Chlorpyriphos residues studies.



CHLORPYRPHOS

Fig.1 Chemical structure of Chlorpyriphos

To standardization of analytical method for chlorpyrphos residues in citrus , the Certified Reference Materials (CRMs) of chlorpyriphos purity of 99.0% procured from Dr. Ehrenstorfer (Germany). Primary standards, intermediary and working standards were prepared from these CRMs using Acetone: n-:Hexane (1:9) as a solvents. The working standards of chlorpyriphos was prepared in the range of 0.01 mg kg^{-1} to 0.5 mg kg^{-1} in 10 ml calibrated graduated volumetric flask using n-Hexane as solvent. All the standards were stored in deep freezer maintained at -20^0C.

Extraction and Cleanup

The citrus plants applied three time with chemical formulation chorpyriphos (Dusban 20EC) formulation were collected at regular intervals i.e., from zero hours to 20 days and were analyzed. Before the treated of citrus plants the control untreated citrus samples were analysed and the method is validated by spiking with standard of chlorpyriphos of different concentrations into untreated control citrus fruit, citrus juice and soil samples. Two kg of citrus fruits plucked from untreated citrus plants were pooled and mixed well and 200 g of representative citrus fruit sample was drawn by quartering method for analysis. Citrus fruits were homogenized with robot coupe blixer.

The citrus samples treated with chlorpyrphos followed the standard procedure for extraction and cleaned up[7]. The representative citrus fruit samples of 2 kg collected after application were chopped into small pieces and from those 25 grams of sample obtained after quartering was macerated in a wearing blender for 30 seconds with anhydrous 10 grams of sodium sulphate. The macerated citrus fruit samples were extracted[5] with acetone by shaking for 1 hour on mechanical shaker.

Table 1. Gas Chromatographic parameters for Chlorpyriphos analysis

Instrument	Gas Chromatography Varian - 3800
Column	Varian Factor Four capillary column 30 m length. 0.23 mm internal diameter 0.25 mill micron film thickness, 1% methyl siloxane
Column Oven Temperature (^{0}C)	Initial temperature 150^{0}C for 2 min. – increase @ 10^{0}C/min. up to 240^{0}C – final hold time 20 min.
Detector Temperature (^{0}C)	280
Injector Temperature (^{0}C)	260
Injector status	Split less mode
Carrier Gas	Nitrogen Iolar II, purity 99.99%
Carrier Gas Flow (ml/min)	1 ml
Make up flow (ml/min)	Nitrogen gas 30 ml
Retention time (min)	**Chlorpyrphos 10.085 minutes**
Total run time	31 minutes

The contents were filter through Filler paper No. l using suction pump. The acetone extract was concentrated and diluted with 4% sodium chloride solution (100 ml) and partitioned with 3 x 50 ml of n-Hexane in a separatory funnel shaking vigorously for 1 – 2 minutes each time. The upper n-hexane layer collected over sodium sulphate. All the three fractions of n-hexane layer were pooled and evaporated to dryness. The dried residues were dissolved in mixture of acetone: hexane 1:9 (10 ml) and passed through C18 SPE cartridge for cleanup. Finally the cartridge was eluted with 10 + 10 ml n-hexane. The finally eluted extracted was evaporated to dryness. The dried residues are dissolved in 5 ml n-Hexane and analysed on Varian 3800 Gas Chromatography instrument using Electron Capture Detector (GC-ECD). The instrument parameters were presented in Table 1. The same analytical technique mentioned above is followed for citrus juice and soil samples. In case of juice, 10 ml of citrus juice samples and for soil 25 grams soil sample is taken for extraction. The cleanup procedure followed as in citrus fruit samples.

Results and Discussions

Linearity, recoveries of Chlorpyriphos in citrus fruit, juice and soil

Prior to samples analysis the instrument linearity and method validations experiments were conducted. The Linearity of chlorpriphos was tested on instrument Gas Chromatography Varian CP 3800 using Electron Capture Detector (ECD). The linearity was determined by analyzing the standard solution of different chlorpyriphos concentrations i.e, 0.05 mgkg^{-1}, 0.1 mgkg^{-1} , 0.25 mgkg^{-1} , 0.5 mgkg^{-1} , 0.75 mgkg^{-1} and 1.0 mgkg^{-1} . The each concentration of the chlorpyriphos standardized six time on GC-ECD and average of the areas taken to draw linearity curve. The linearity curve of chlorpyrphos is depicted in Fig.2. The regression equation and R^2 values were calculated for instrument sensitivity. The GC chromatograms of different concentrations of Chlorpyriphos were depicted in Fig. 3 to 6.

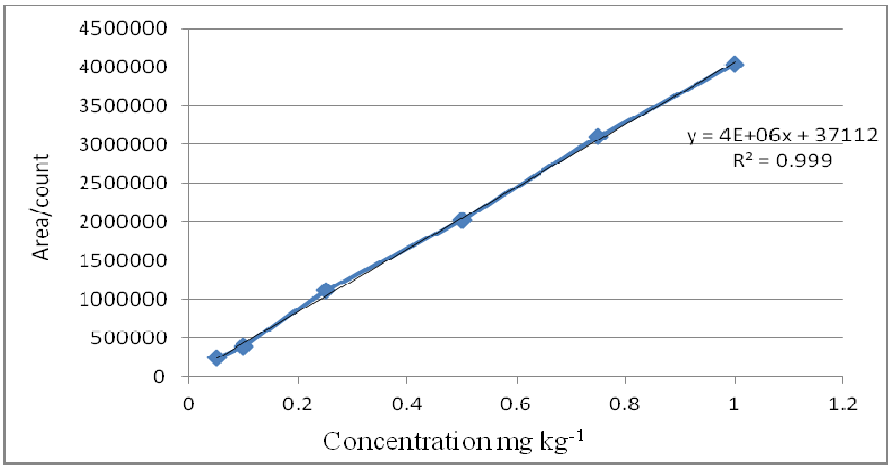


Fig. 2 Linearity curve of Chlorpyriphos

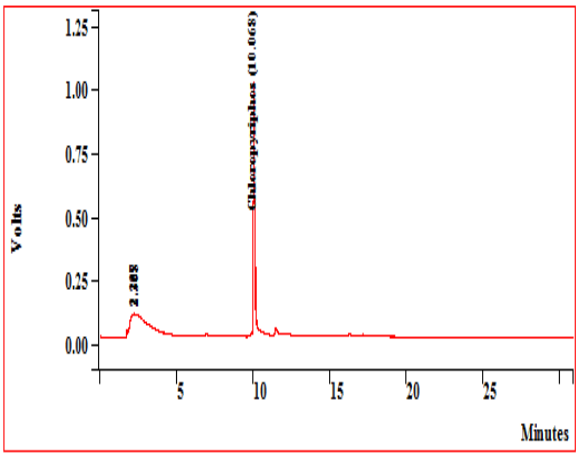


Fig. 3 Chlopryrphos1.0 mgkg^{-1}

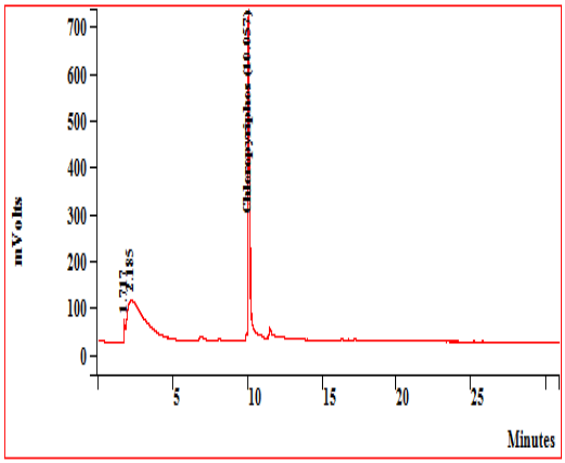


Fig.4 Chlorpyrphos 0.5 mgkg^{-1}

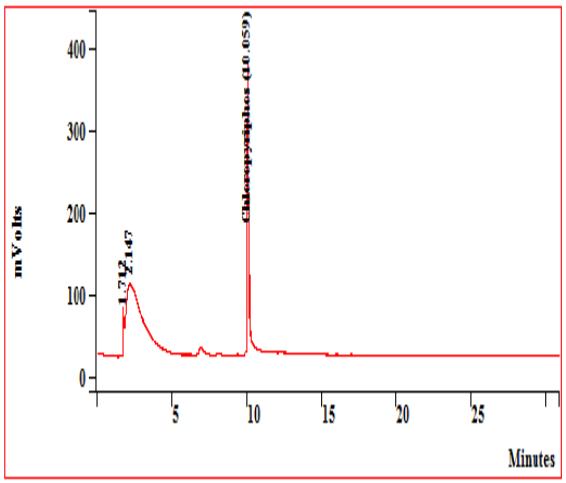


Fig. 5 Chlorpyrphos 0.1 mgkg^{-1}

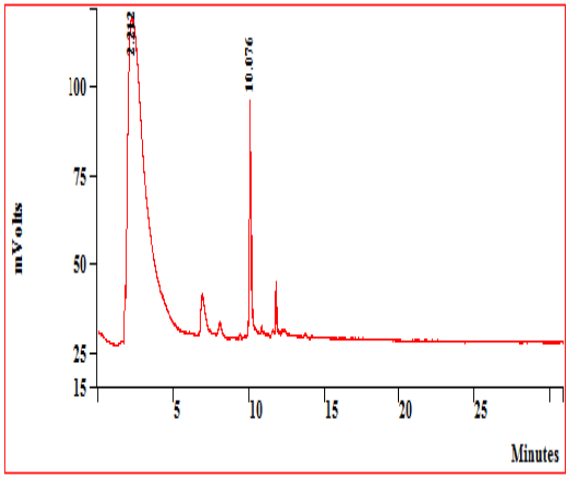


Fig.6 Chlorpyrphos 0.05 mgkg^{-1}

GC Chromatograms of Chlorpyriphos Standards

Recoveries of Chlorpyriphos in Citrus fruit, citrus juice and soil

The control untreated citrus fruit samples were collected for method validation and recoveries studies of chlorpyriphos . The citrus fruit samples were extracted and cleaned as per standard procedure. The recovery of chlorpriphos was carried out to investigate the accuracy and precision of the extraction and cleanup method. For recoveries studies the control Citrus samples spiked with chlorpryiphos concentration viz., 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg^{-1} and extraction and cleanup as per the procedure discussed earlier. The mean recovery of the residues calculated for applying recovery factor while calculating the residues in samples. The precision of instrument parameters, for repeatability and reproducibility was studied for finalization method. Fortification and recovery studies data was presented in Table 2 and the validated was method followed for qualitative and quantitative estimation of chlorpyrphos in citrus fruit. The recoveries obtained were 90.0 to 94% with an average of 91.3% at 0.05 $mgkg^{-1}$ level , while at 0.25mg kg^{-1} fortification level the recoveries were 96.0 - 97.2% with an average percentage of 96.7% and the recoveries ranged 92.5 – 96.0 0% with an average of 94.5% at 0.5 $mgkg^{-1}$ level. The Limit of Quantification (LOQ) was worked out 0.05 $mgkg^{-1}$ in citrus matrix.

The Perusal of table 3 indicates that percent recoveries of Chlorpyriphos and in control citrus fruit juice samples ranged 88.0% to 92.0% with an average of 90.0% at 0.05 $mgkg^{-1}$ level , the recoveries 92.8% t0 96.0% with an average of 94.0% at 0.25 mg kg^{-1} level and found the recoveries 91.0% to 894.5% an average of 92.5% at 0.5 mg kg^{-1} levels. The Limit of detection (LOD) i.e., sensitivity of the instrument/Detector and Limit of Quantification /determination (LOQ) in citrus juice were found to be 0.01 and 0.05 $mgkg^{-1}$ respectively.

Table 2. Recoveries of Chlorpyriphos at various fortification levels in citrus fruit samples

Replication	Chlorpyriphos spiked levels in citrus fruit					
	Recovery of Chlorpyriphos ($mgkg^{-1}$)					
	0.05 $mgkg^{-1}$	% recovery	0.25 $mgkg^{-1}$	% recovery	0.5 $mgkg^{-1}$	% recovery
R1	0.047	94.0	0.242	96.8	0.475	95.0
R2	0.045	90.0	0.240	96.0	0.462	92.5
R3	0.045	90.0	0.243	97.2	0.480	96.0
Average	0.046	91.3	0.241	96.7	0.472	94.5
SD±	0.001	2.309	0.002	0.611	0.009	1.803

Table 3. Recoveries of Chlorpyriphos at various fortification levels in citrus fruit juice samples

Replication	Chlorpyriphos spiked levels in citrus fruit juice					
	Recovery of Chlorpyriphos ($mgkg^{-1}$)					
	0.05 $mgkg^{-1}$	% recovery	0.25 $mgkg^{-1}$	% recovery	0.5 $mgkg^{-1}$	% recovery
R1	0.045	90.0	0.232	92.8	0.455	91.0
R2	0.042	88.0	0.240	96.0	0.472	94.5
R3	0.046	92.0	0.233	93.2	0.460	92.0
Average	0.044	90.0	0.235	94.0	0.462	92.5
SD±	0.002	2.00	0.004	1.744	0.009	1.803

Table 4. Recoveries of Chlorpyriphos at various fortification levels in soil samples

Replication	Chlorpyriphos spiked levels in soil					
	Recovery of Chlorpyriphos ($mgkg^{-1}$)					
	0.05 $mgkg^{-1}$	% recovery	0.25 $mgkg^{-1}$	% recovery	0.5 $mgkg^{-1}$	% recovery
R1	0.042	82.0	0.242	96.8	0.480	96.0
R2	0.045	90.0	0.240	96.0	0.465	93.0
R3	0.046	92.0	0.243	97.2	0.476	95.0
Average	0.044	88.0	0.242	96.7	0.474	94.7
SD (±)	0.002	5.292	0.002	0.611	0.008	1.528

The control soil samples spiked with chlorpyriphos for recoveries studies. The chlorpyriphos concentrations viz., 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg^{-1} spiked in control soil samples and analyzed. And the mean recovery of the residues calculated for applying recovery factor while calculating the residues in samples. Fortification and recovery data was presented in Table. 4 And qualitative and quantitative estimation of chlorpyriphos in done in soil samples. The recoveries obtained were 82.0 to 92% with an average of 88.0 % at 0.05 $mgkg^{-1}$ level , while at 0.25mg kg^{-1} fortification level the recoveries were 96.0 - 97.2% with an average percentage of 96.7% and the recoveries ranged 93.0 – 96.0 0% with an average of 94.7 % at 0.5 $mgkg^{-1}$ level. .The residues were detected below 0.05 mg kg^{-1} were mentioned as the Below Determination Levels are (BDL) for chlorpyrphos.

Residues of Chlorpyriphos in Citrus fruit, Citrus juice and soil

A dissipation and persistence pattern was studied by conducting the field experiment during 2013-14 Kharif seasons. The pesticide Chlorpyriphos 20% EC (Dusban) applied at the rate 500 g a.i./ha on citrus plants so as to recommend MRLs, pre harvest intervals based on the risk analysis of the treatments. The Citrus plants are treated as per recommended dose of Chlorpyrphos concentration. The spray application was given thrice initiating at fruit formation stage with an interval of 10 days. The citrus fruit samples analysis was carried out in the citrus fruits collected at 0 (2 h after application) hours, 1, 3, 5, 7, 10, 15 and 20 days after the third spray. Approximately 2 kg of citrus fruit sample were collected and pooled together, packed in plastic bags and transported to the laboratory for further processing. Extraction and clean up of the samples were carried out as per the validated method as described earlier.

The citrus fruit samples were collected from Zero hours to 20 days after last application and extracted and cleanup as per procedure. The final concentrated extract was analysed on Varian CP3800 GC- ECD. The Citrus fruit samples GC- Chromatograms were depicted in Fig 7 to 13. The results obtained were presented in Table. 5 . The date in the table indicated that the fruit samples collected at zero hours (2 hours after last spray) recorded initial deposits 4.121 $mgkg^{-1}$, which dissipated to 3.871 $mgkg^{-1}$ in 1^{st} day sample and 2.090 $mgkg^{-1}$ in 3^{rd} day sample, in 5^{th} day citrus fruit recorded the chlorpyriphos residues were 1.812 $mgkg^{-1}$. The citrus samples collected at 7^{th}, 10^{th} and 15^{th} day recorded residues ranged 1.076 $mgkg^{-1}$, 0.514 $mgkg^{-1}$ and 0.058 $mgkg^{-1}$ respectively. The residues were Below Detectable Level after 15^{th} on wards in citrus fruit . And dissipation percentage of chlorpyriphos residues degraded to 6.07% , 49.28%, 56.03%, 73.89%,87.53 and 98.59% at 1^{st} day, 3^{rd} day, 5^{th} day, 7^{th} day, 10 the

day and 15th day samples respectively. The chlorpyriphos residues were below the determination level (BDL) after 20th day. The dissipation bar graph is depicted in Fig.14.

Table. 5 Dissipation and persistence of Chlorpyriphos in citrus fruit, citrus juice and soil

Days after spray	Residues of Chlorpyriphos ($mgkg^{-1}$)				
	R1	R2	R3	Means	% Dissipation
0	4.108	4.130	4.125	4.121±0.012	-
1	3.791	3.851	3.971	3.871±0.092	6.07
3	2.120	2.078	2.072	2.090±0.026	49.28
5	1.772	1.830	1.834	1.812±0.035	56.03
7	1.103	0.995	1.130	1.076±0.071	73.89
10	0.512	0.496	0.534	0.514±0.019	87.53
15	0.069	0.055	0.054	0.058±0.008	98.59
20	BDL	BDL	BDL	BDL	-
Citrus juice at harvest	BDL	BDL	BDL	BDL	
Soil at harvest	BDL	BDL	BDL	BDL	
Regression equation	Y= -0.275x+3.549				
MRL value	1 $mgkg^{-1}$				
Waiting period	6.2 days				

BDL : Below Detectable Level,
MRL : Maximum Residue Limit SD : Standard Deviation

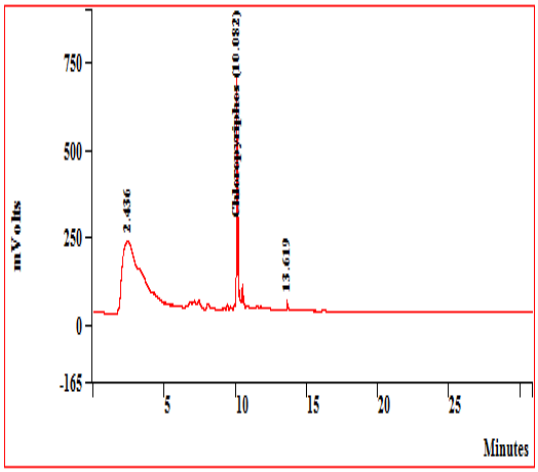


Fig. 7 Zero hours citrus fruit sample

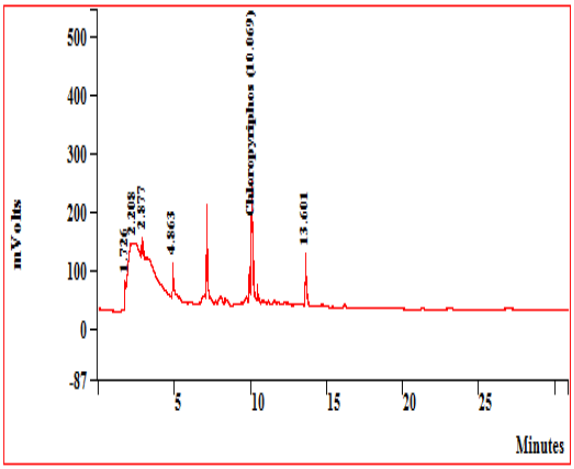


Fig. 8 One day citrus fruit sample

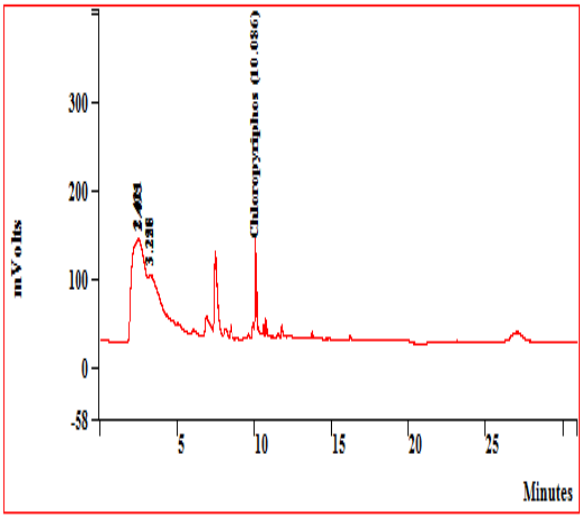


Fig. 9 Three days citrus fruit sample

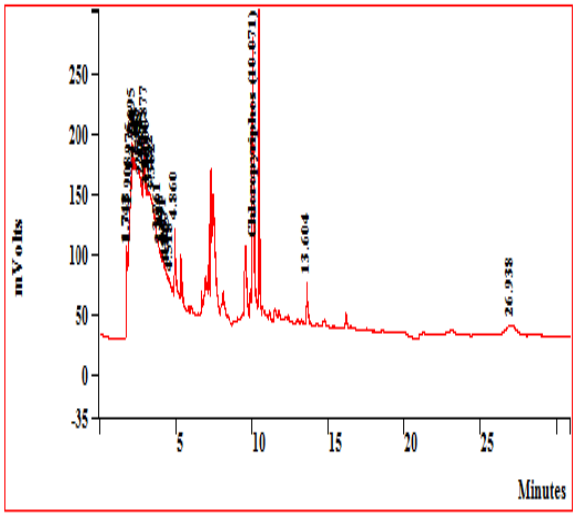


Fig.10 Five days citrus fruit sample

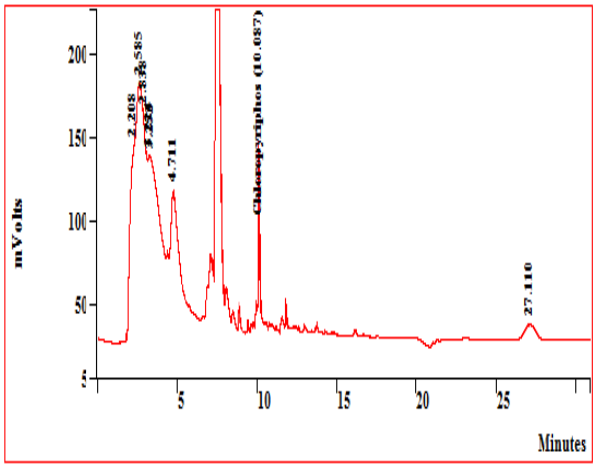


Fig. 11 7 days citrus fruit sample

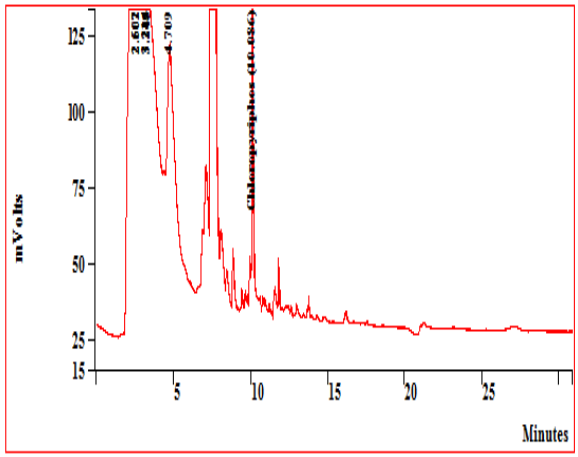


Fig.12 10 days citrus fruit sample

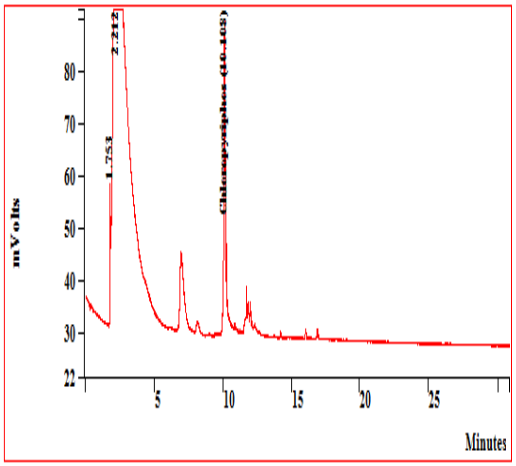


Fig.13 15 days citrus fruit sample

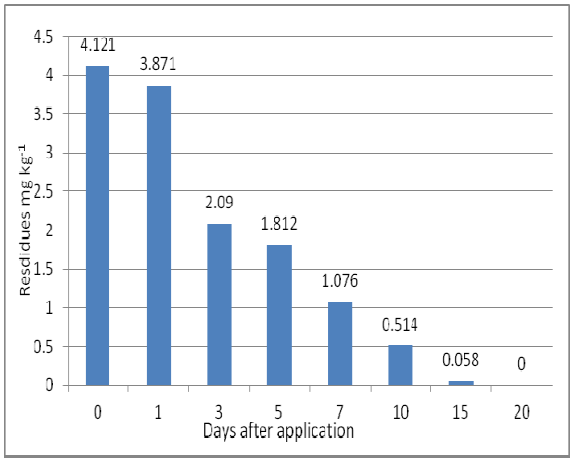


Fig. 14 Dissipation bar graph of Chlorpyrphos in citrus fruit

The Citrus fruit juice samples and soil samples collected at harvest time were analyzed for chlorpyrphos residues and the residues Below Detectable Levels.

The regression equation was derived as $y = - 0.275 x + 3.549$. As the Maximum Residue Limit (MRL) of Chlorpyriphos was 1.0 mg kg^{-1}, the waiting period for safe consumption of Citrus fruit was calculated as 6.2 days. By these findings , it can be recommend that the apply chlorpyriphos @ 500 g a.i./ha on citrus plants initiating at fruit formation stage followed another two application has to wait 6.2 days for safe consumption of Citrus fruit .The citrus juice extracted from citrus fruit at harvest time and soil does not detected the chlorpyriphos residues and are below detectable levels (BDL).

The residues data of chlorpyriphos on citrus was not much available, but another crops it is there. The crop apple[5] applied with chlorpyriphos @ 200 g a.i./ha and 400 g a.i./ha recorded initial deposits of chlorpyriphos recorded initial deposits 1.61 µg g^{-1} and 1.98 µg g^{-1} dissipated to 0.09 µg g^{-1} after 15 days. The Chlorpyriphos applied @ 100 , 200 and 300 g a.i./ha on okra[8] recorded initial deposits ranged 0.389 mg kg^{-1} to 0.874 mg kg^{-1} which dissipated to below detectable level after 15 days. The brinjal[9] crop applied with chlorpyripphos @ 100, 200 and 300 g a.i./ha recorded residue ranged 0.362 to 0.876 mg kg^{-1} and dissipated below detectable level at 13 to 17 days after application. The Chlorpyriphos @ 0.05% applied on cardamon[10] and analysed on Gas Chromatography found initial deposits 1.63 mg kg^{-1} and after curing the deposits were 4.86 mg kg^{-1} and 4.86 mg kg^{-1} after processing, this is because of loss of weight during curing and processing. The data obtained in present investigation was similar and nearer to the data reported by the other researchers.

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