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Research Article

DISSIPATION KINETICS OF ACEPHATE ON/ IN CITRUS FRUIT, JUICE AND SOIL

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ABSTRACT

Field experiment was conducted to study the dissipation kinetics of acephate in citrus fruit. The acephate @ 560 g a.i/ha applied three times at an interval of 10 days initiating at fruit formation stage. The The citrus fruit samples were collected at an interval of zero hours, 1,3,5,7,10, 15 and 20 days after last application and citrus juice, soil at harvest. The collected citrus fruit samples were analysed by QuEChERS method after validations. The initial deposits recorded 0.414 mgkg^{-1} and same dissipated to Below Detectable level 15 days after last spray. The waiting period was calculated 13.16 days safe consumption of citrus fruit after last application. The citrus juice and soil at harvest were not detected acephate residues and are below detectable levels.

Keywords: Citrus, acephate residues, QuEChERS method, citrus juice and soil.

INTRODUCTION

Acephate belongs to an Organo phosphosphate pesticide applied for plant protection for the control of pests and insects. Its chemical formula is $C_4H_{10}NO_3PS$ and IUPAC name N-(Methoxy-methylsulfanylphosphoryl) acetamide. The molecular structure is depicted in Fig. 1. It is an organophosphate group systemic insecticide[1] used for control of sucking and biting insects. Acephate was moderate persistence which remains in environment for 10–15 days when applied recommended doses. It is used primarily for control of aphids, blackfly[2] in citrus, vegetables and in horticulture crops. It also controls leaf miners, caterpillars, sawflies and thrips. The chemical was available different forms viz., soluble powder, emulsifiable concentrates and granular formulations . This chemical sprayed and also injected in the tree/plants stem systems. In 2012, the USEPA cancelled the usage of acephate on green beans[3] grown in the United States. Acephate can cause cholinesterase inhibition[4] in humans, it can over stimulate the nervous system causing nausea, dizziness, confusion, and at very high exposures, respiratory paralysis[5] and death. But still is widely used in India on most of the cropping system. Therefore it is essential to study the toxic residue levels in Citrus.

For plant projection acephate was applied on many crops such as vegetables, citrus plants, fruit, potatoes, sugar beets, vine plants, mint, cotton, tobacco, cranberries, and other berries. The Forest industry uses it on pine trees and Christmas trees. Acephate is applied in the golf courts and turf, as well as, to horticulture crops. It can also be applied outdoors in residential areas and is often used to control fire ants. As a general use insecticide, it has a residual effect of 10 - 15 days. Acephate breaks down quickly in the environment as it is absorbed from the soil by plant roots. The roots then transport the Acephate to other parts of the plant causing toxic residue on food and in drinking water. Therefore an attempt was made to study the toxic acephate residues in citrus fruit, juice and soil.

MATERIAL AND METHODS

The field experiment was laid out to conduct the trial during Kharif 2013 at Farmers citrus farm (sweet orange variety) in the Manikayapur village of Karimnagar district, India. The field was laid out in randomized block design with acephate treatment single recommended dose i.e., acephate (Pace 75% WP) @ 560 g a.i./ha applied three times initiating at fruit formation stage on citrus plant at an interval of 10 days after first application. The citrus fruit samples were drawn at different time intervals for analysis of acephate residue in Citrus fruit, citrus juice and soil.

To standardized the analytical procedure of Acephate residues in citrus , the Certified Reference Materials (CRMs) of Acephate with a purity of 99.5% procured from Dr.Ehrenstorfer (Germany) . Primary standards, intermediary and working standards were prepared from these CRMs using Methanol (HPLC Grade) as a solvent. The working standards of acephate 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 Methanol as solvent. All the standards were stored in deep freezer and maintained temperature at -20^{0}C. The working standards of Acephate 0.01 to 0.5 mg kg^{-1} were injected in Liquid-Chromatography with Mass Spectrophometer (LC-MS/MS) consisting LC-MS Lab solution software fitted with Kinetex C18 Column, 2.6µ Particle size, 100cm Length, 3.0mm I.D For estimating the lowest quantity of these pesticides which can be detected under standard operating parameters of LC-MS/MS are presented in Table 1. The Nebulizing Gas (Argon gas) was set flow rate of 2 lits/min, Drying Gas (Nitrogen gas) flow rate was kept 15 lits/min, Desolvation Line Temperature set as 250^{0}c, Heat block temperature 300^{0}c, interface voltage 4.5 – 5.0 kv and dwell time were investigated with the aim of obtaining an intense peak for compound. First the acephate was monitored in full scan mode in mass by charge *(m/z)* range from 100- 500. By this method first identified the precursor ions of acephate in this run (Table). Then, the precursor ion was selected for collision-induced dissociation (CID) basing on the highest *m/z* ratio. The CID is performed with argon gas at ranging from 17 Kpa to 230 Kpa. A minimum of two MS/MS transitions was selected for acehate chemical. Finally, the MS/MS parameters for both the ions were optimized in full scan mode. The multi reaction monitoring (MRM) mode applied in QqQ analyzers provides a high increase in selectivity since the analyzer is focused on selected in first (Q1) and third quadrupoles (Q3). In consequence, a sensitivity gain results from the greater signal-to-noise ratio measured, in spite that the ion transmission diminishes due to path length between the ion source and the electron multiplier. The MRM transition was performed simultaneously and identified the qualifier and quantifier ions and retention times was identified by attaching the column for compounds. The QqQ analyzer is having the high scan speed it can permit to monitor up to 200 transitions in the programmed time

Extraction and Cleanup of Citrus fruit samples by adopting QuEChERS technique

The citrus plants applied three time with chemical formulation acephate were collected at regular intervals i.e., from zero hours to 20 days and are analyzed for chlorpyriphos residues by adopting the AOAC official method[6] of Analysis 2011. (QuEChERS). Analytical grade pesticide standards of acephate were procured from Dr. Ehrenstorfer (Germany), and the solvents used for extraction and analysis were viz., Acetonitrile (HPLC grade), n-Hexane (HPLC grade) and Sodium chloride (NaCl), anhydrous sodium sulphate (Na_2SO_4*)*, and anhydrous magnesium sulphate ($MgSO_4$*)* all analytical grade chemicals procured from Merck India. Before use of anhydrous sodium sulphate and anhydrous magnesium sulphate were baked for 4 hours at 600◦C in muffle furnace to remove possible phthalate impurities. Primary secondary amine (PSA - Ethylenediamine N-propyl bonding with silica gel base) was procured from Agilent Technologies. Before acephate treated of citrus plants the control untreated citrus fruit samples were collected and the method is validated by spiking the pesticide in untreated control citrus samples on LC-MS/MS-TQD in the laboratory. Two kg of citrus plucked from all the trees were pooled and mixed well and 200 g of representative sample was drawn by quartering method for analysis. Citrus fruits were homogenized with robot coupe blixer. The small quantity of homogenized 15±0.1 g sample was taken in 50 ml centrifuge tube and added with 30±0.1 ml acetonitrile. The sample was homogenized at 14000-15000 rpm for 2-3 min using Heidolph silent crusher. The samples were then added with 3±0.1 g sodium chloride and mixed by shaking gently followed by centrifugation for 3 min at 2500-3000 rpm to separate the organic layer. The top organic layer of about 16 ml was taken into the 50 ml centrifuge tube and added with 9±0.1 g anhydrous sodium sulphate to remove the moisture content. From the extract 8 ml was taken into another 15ml Centrifuse tube containing 0.4±0.01 g PSA sorbent (for dispersive solid phase d-SPE cleanup) and 1.2±0.01 g anhydrous magnesium sulphate. The sample tube was vortexed for 30 sec, followed by centrifugation for 5 min at 2500-3000 rpm. The extract of 1 ml acetonitrile was transferred into 2 ml vial by filtering through 0.22µm filter paper for the analysis on LC-MS/MS-TQD for acephate analysis.

RESULTS AND DISCUSSIONS

Linearity and Limit of Quantification of Acephate

For linearity curve the Acephate concentrations are 0.01 mg kg^{-1}, 0.025 mg kg 0.05 mg kg-1, 0.1 mg kg-1, 0.25 mg kg-1, 0.5 mg kg-1 were injected for method standardization. The retention time of Acephate was identified at 1.218 minutes and taken the area at that point for drawing a Linearity against concentrations. The linearity curve obtained was depicted in Fig.2 and R^2 value found to b e 0.9982 and the Chromatograms of acephate concentrations were depicted in Figure 3 to Figure. 6.

Recoveries studies of acephate in Citrus fruit, Citrus juice and soil

The control / untreated Citrus fruit samples were collected control citrus plants and fortified with different concentrations viz., 0.05, 0.25, and 0.50 mg kg^{-1} levels by adding required quantity of Acephate Standard solution.

The Citrus fruit samaples fortified with acephate standards were homogenized separately with robot coupe blixer, and homogenized 15±0.1g sample was taken into 50ml Centrifuge tube and for extraction and cleanup validated QuEChERS method as followed discussed earlier. The final extract of about 1 ml was filtered through 0.22microns Filter paper into auto sampler Vial for analysis on LCMS-MS under standard operational conditions. The final extract was analyzed on LC-MS/MS TQ by adopting instrumental parameters as mentioned earlier. The recovery percentage were calculated from areas of LC-MS/MS chromatograms. The data is presented in Table 2. The data indicates that recoveries obtained at 0.05 mgkg^{-1} at fortification level were ranged 80.2 to 83.5 percentage with average of 82.2%, while recoveries ranged 84.1 – 85.7% with an average of 85.5% at 0.25 mg kg $^{-1}$, and at 0.5 mg kg^{-1} level recorded ranged 87.8 to 92.8% with an average of 90.4%. These results showed that the method is suitable for the analysis of Acephate residues up to 0.05 mg kg-1, and the limit of quantifition (LOQ) was 0.05 mg kg-1.

The control un treated Citrus juice samples extracted from untreated citrus fruit. The citrus juice samples were fortified with acephate different concentration viz., 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg^{-1} and are extracted and clean up, analyzed by adopted method as in Citrus fruit and the mean recovery of the residues calculated for obtaining the recovery factor for quantification in acephate residues in citrus juice samples. Fortification and recovery studies results were presented in Table 3 and the method followed for qualitative and quantitative estimation of acephate residues. The recoveries obtained were 82.1 to 84.60% with an average of 88.2 % at 0.05 mgkg^{-1} level, while at 0.25mg kg^{-1} fortification level the recoveries were 87.8 % - 88.9 % with an average percentage of 83.3% and the recoveries ranged 92.6 – 96.50% with an average of 94.1% at 0.50 mgkg^{-1} level. The residues of acephate in citrus fruit juice can be identified and quantified up to 0.05 mgkg^{-1} and this value is mentioned as the Below Determination Levels (BDL) for acephate residues in Citrus fruit juice.

The soil samples collected from the citrus farm where the pesticide was not applied. The soil samples dried in shade and pulverized with porcelain pestle and mortar. The sieved soil samples was of 10 grams was taken in 50 ml centrifuge tube, and spiked with acephate standardard concentration viz., 0.05 mg kg^{-1}, 0.5 mg kg $^{-1}$ and 1.0 mg kg^{-1}. To that 20 ml of acetonitrile is added and rest of the procedure is adopted as in citurs fruit. The final extract is analyzed on LC-MS/MS. The recoveries data obtained was presented Table.4. The soil sample fortified with 0.05 mgkg^{-1} concentrations obtained recoveries ranged 85.6 % to 89.5% with an average of 88.0%, while at 0.25 mgkg^{-1} levels the recoveries 92.1 - 94.2% with an average of 93.2% and recoveries are range 95.8 % to 97.8 % with an average of 96.80 % at 0.5 mg kg^{-1} spiked level. The limit of detection in soil found to be 0.05 mg kg^{-1} in soil.

The recoveries of acephate at different fortification levels i.e., 0.05 mgkg^{-1} , 0.25 mgkg^{-1} and 0.5 mg kg^{-1} in citrs fruit, citrus juice and soil was depicted in Fig. 7 as bar graph.

Residues of Acephate in Citrus fruit, Juice and soil

The field trial was conducted during Kharif Season August 2013 at Farmers Field Manikyapur Village of Karimnagar district inTelangana state in order to determine the dissipation of Acephate residues of Acephate in/on Citrus fruit, Citrus juice resulting from application of the Acephatae formulation i.e., Pace 75%SP was sprayed @ 560 g a i./ha initiating at fruit formation stage followed by second and third sprays at an interval of 10 days interval . The citrus fruits samples were collected after three sprays at regular intervals i.e Zero hours, 1, 3, 5, 7, 10, 15, 20 days and labeled poly bags and brought to the laboratory. The collected samples were extracted and clean-up as per validated procedure and analyzed for Acephate residues on Liquid Chromatography with mass spectrometry detector. The residues data of Acephate was presented in Table. 4 . The data indicate that the initial deposits 0.414 mg kg^{-1} of Acephate was detected in citrus fruit samples , which dissipated to 0.355 mg/kg by 1st day, dissipated to 0.308 mg/kg by 3rd day, 0.214 mg/kg by 5th day. The acephate residues, 0.174 mg/kg , 0.069 mg/kg on 7th day and 10th day samples respectively. The acephate residues are Below Determination Level after 15 days in Citrus fruit samples. The LC-MS/MS chromatograms of citrus fruit samples were depicted in Fig. to . The citrus fruit samples collected at harvest and juice at harvest was analyzed and not detected acephate residues . The percentage of dissipation was in citrus fruit 14.25%, 25.6%, 48.39%, 57.89%, 83.25% at 1, 3, 5, 7, 10 days samples respectively. The regression equation calculated $y= - 0.0335X + 0.4009$. As the Maximum Residue Limit of acephate was 0.05 $mgkg^{-1}$ and the waiting period for safe harvest worked out 13.16 days. The acephate @ 560 g a.i./ha can safely applied on Citrus plants at fruiting stage and to be harvested 13.16 days after third application.

The investigated findings were compared with other scientistic findings. The green mustard[7] applied with acephate (Impact 75EC) formulation reported 13days for safe consumption. The acephate when applied on cotton crop [8] recorded residues upto 15 days in cotton leaves. The Acephate 75 SP applied twice at fruiting stage on brinjal[9] at the rate of 560 and 1120 g a.i./ha reported initial residue 2.54 and 4.07 mg kg^{-1} in respective concentrations . The waiting period for safe consumption reported 1.0 days for both the doses. The dissipation of Acephate in okra[10] when applied at the rate of 560 g a.i./ha reported rsidues 5.377 mg kg^{-1}, which dissipated to 2.87 mg kg^{-1} with in 24 hours and the residues below detectable level after 3 days on wards. The chill[11] crop applied with acephate @ 1500 g a.i.,/ha recorded initial deposits 0.19 mg/kg and dissipated to BDL 15 days after the last spray. The mango[12] fruits applied acephate @ 0.75 kg a.i./ha and 1.5 kg a.i./ha recorded residues 0.140 and 0.26 mg kg^{-1}, and were below detectable levels after 3 days in fruit pulp. And acephate residues are detected upto 30 days in fruit peel. The present research findings were coinciding and nearer to the finding of the other researchers.

CONCLUSION

The acephate @ 560 g a.i./ha can safely applied three times at an intervals of 10 days on citrus plants initiating at fruiting stage and can safely consume the citrus fruit and citrus juice 13.16 days after last application. The citrus juice was safely consume at harvest as the acephate residue were below detectable levels.

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Table 1: LC- MS/ MS parameters for Acephate analysis

LC-MS/MS	**SHIMADZU LC-MS/MS - 8040.**
Pump	30 AD
Auto injector	30 AC
Column	Kinetex C18, 2.6 μ Particle Size, 100mm Length, 3mm ID
Column oven temperature	40^{oC}
Pump flow	0.4 ml/min
Solvents used	A: 10mM Ammonium Formate in water B: 10mM Ammonium Formate in Methanol
Gradient program	Time(Min) Pump A% Pump B% 0.01 65 35 2.00 Stop
Mass Spectrometer	Shimadzu 8040 Triple Quadrupole Mass Spectrometer equipped with ESI(Electron Spray Ionization) interface
Interface Voltage	4.5 to 5 KV
Nebulizing, Drying gas	Nitrogen
Nebulizing gas flow	2.0 lit/min
Drying Gas flow	15.0 lit/min
Desolvation line(DL) temp	250^{0c}
Heat block temperature	300^{0c}
Scanning Mode	MRM
Polarity	Positive
Precurser ion:Q1mass (amu)	Acephate : 184
Collision gas	Argon
Dwell time (milli sec)	20
Quantifier ion: Q3 mass(amu)	Acephate : 143
Qualifier ion: Q3 mass(amu)	Acephate : 49
Retention time	1.218 minutes

Table 2: Recoveries of Acephate in citrus fruit amples

Replication	**Acephate spiked levels in control citrus fruit sample**					
	Recoveries of Acephate (mg kg^{-1})					
	0.05 mg kg^{-1}	**% recovery**	**0.25 mg kg^{-1}**	**% recovery**	**0.5 mg kg^{-1}**	**% recovery**
R1	0.04	80.2	0.211	84.1	0.454	90.8
R2	0.041	83.5	0.213	85.2	0.464	92.8
R3	0.041	83.1	0.214	85.7	0.439	87.8
Average	0.041	82.2	0.213	85.5	0.452	90.4
SD (±)	0.001	1.801	0.002	0.819	0.013	2.517

Table 3: Recoveries of Acephate in citrus juice samples

Replication	**Acephate spiked levels in control citrus juice**					
	Recoveries of Acephate (mg kg^{-1})					
	0.05 mg kg^{-1}	**% recovery**	**0.25 mg kg^{-1}**	**% recovery**	**0.5 mg kg^{-1}**	**% recovery**
R1	0.041	82.1	0.222	88.9	0.463	92.6
R2	0.042	83.2	0.220	87.8	0.466	93.2
R3	0.042	84.6	0.221	88.2	0.483	96.5
Average	0.042	83.30	0.221	88.3	0.471	94.10
SD (±)	0.001	1.25	0.001	0.56	0.011	2.10

Table 4: Recoveries of Acephate at various fortification levels in soil sample

Replication	**Acephate spiked levels in control soil sa,ales**					
	Recoveries of Acephate (mg kg^{-1})					
	0.05 mg kg^{-1}	**% recovery**	**0.25 mg kg^{-1}**	**% recovery**	**0.5 mg kg^{-1}**	**% recovery**
R1	0.043	85.6	0.230	92.1	0.484	96.8
R2	0.045	89.5	0.234	93.5	0.479	95.8
R3	0.044	88.9	0.236	94.2	0.489	97.8
Average	0.044	88.0	0.233	93.2	0.484	96.80
SD (±)	0.001	2.100	0.003	1.07	0.005	1.000

Table 5: Dissipation of Acephate residues in citrus fruit, citrus juice and soil

Days after spray	**Residues of Acephate (mg kg^{-1})**				
	R1	**R2**	**R3**	**Mean±SD**	**% Dissipation**
0	0.415	0.413	0.414	0.414±0.0012	-
1	0.354	0.364	0.347	0.355±0.0087	14.25
3	0.311	0.318	0.295	0.308±0.0118	25.60
5	0.213	0.213	0.215	0.214±0.0012	48.39
7	0.167	0.174	0.182	0.174±0.0078	57.89
10	0.069	0.069	0.070	0.069±0.0006	83.25
15	BDL	BDL	BDL	BDL	-
20	BDL	BD;	BD;	BD;	-
Citrus juice at harvest	BDL	BDL	BDL	BDL	-
Soil at harvest	BDL	BDL	BDL	BDL	-
Regression equation	$y = -0.0335x + 0.4009$				
MRL value	0.05 mg kg-1				
Waiting period	13.16 days				

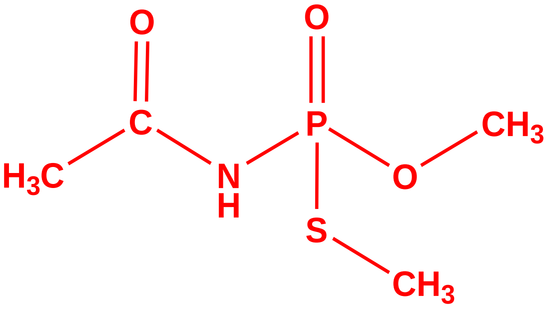

Fig. 1:

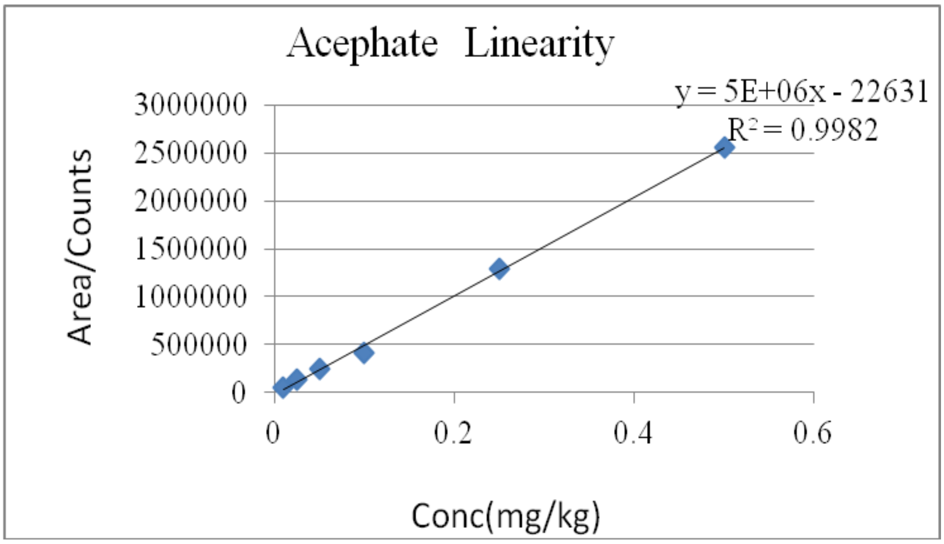

Fig. 2: Linearity curve for Acephate

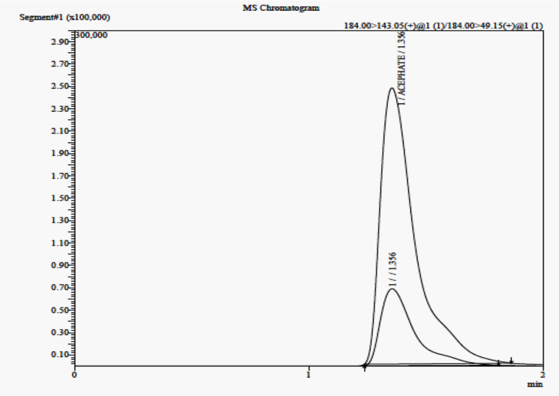

Fig. 3: Chromatogram of Acephate standard 0.5 mg kg^{-1}

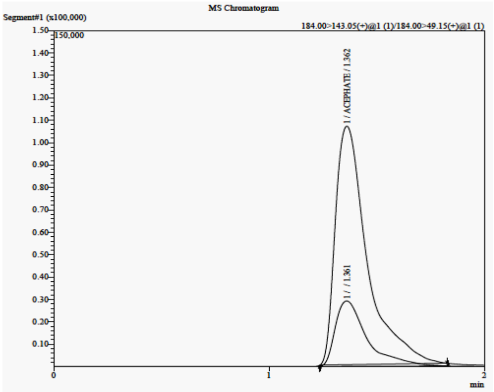

Fig. 4: Chromatogram of Acephate standard 0.25 mg kg^{-1}

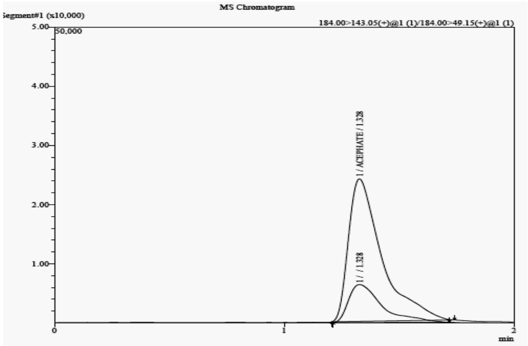

Fig. 5: Chromatogram of Acephate standard 0.05 mg kg^{-1}

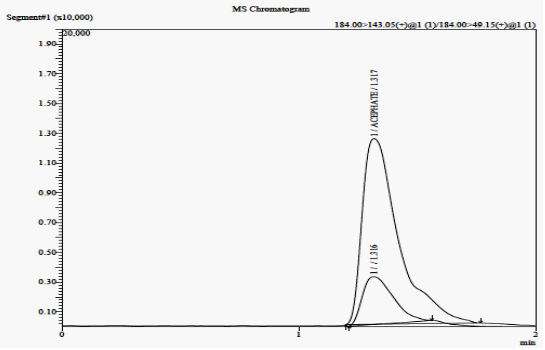

Fig. 6: Chromatogram of Acephate standard 0.025 mg kg^{-1}

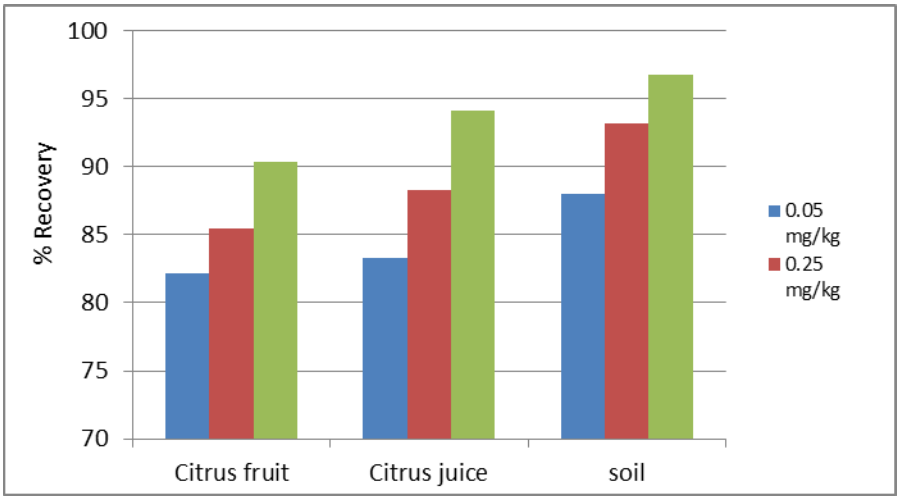

Fig. 7: Recoveries bar graph of Acephate in citrus fruit, citrus juice and soil

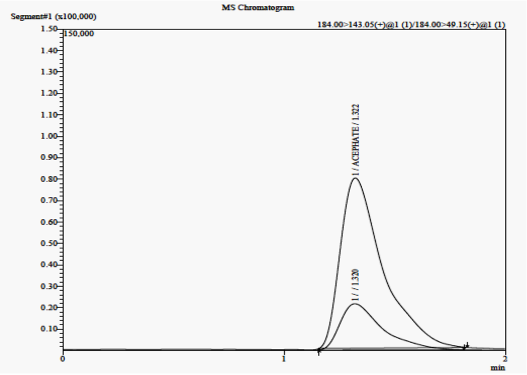

Fig. 8: Chromatogram Zero days citrus fruit sample

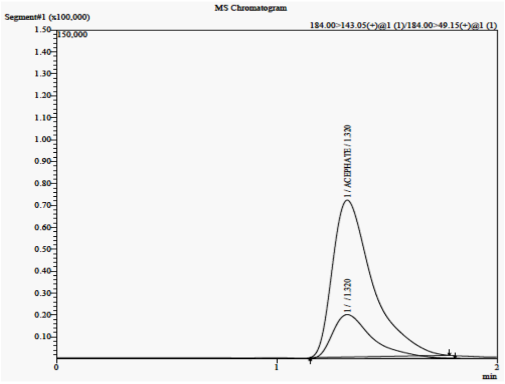

Fig. 9: Chromatogram of 1 day citrus fruit sample

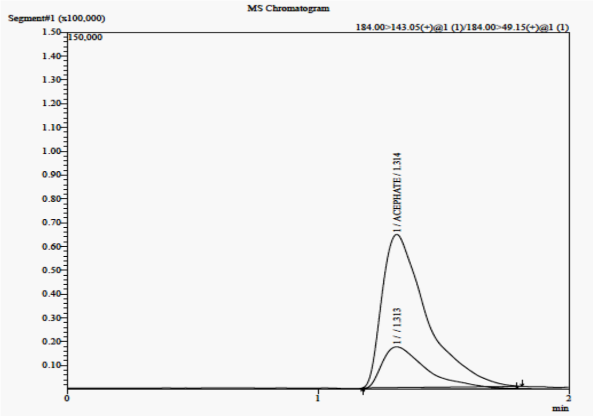

Fig. 10: Chromatogram of 3 day citrus fruit sample

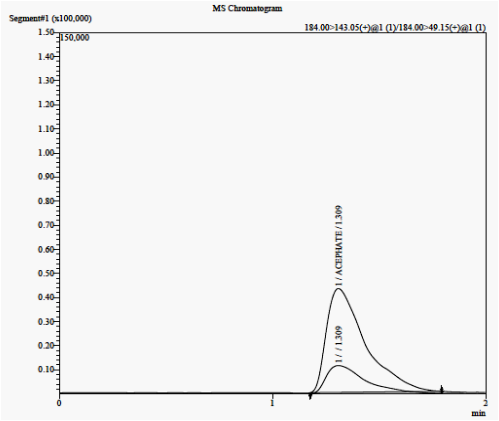

Fig. 11: Chromatogram of 5 day citrus fruit sample

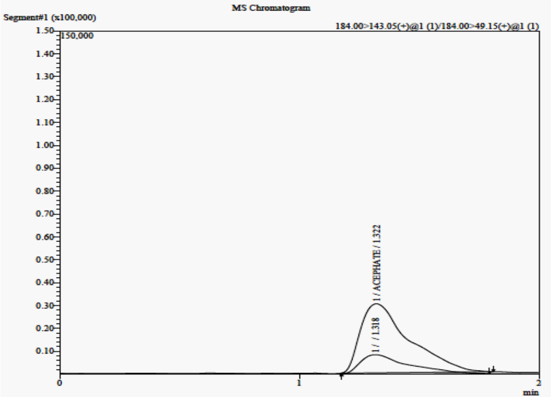

Fig. 12: Chromatogram of 7 day citrus fruit Sample

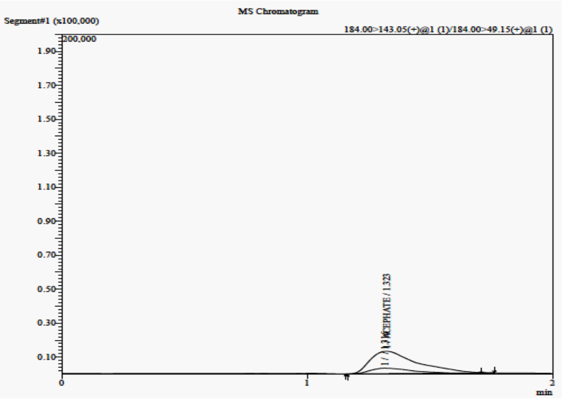

Fig. 13: Chromatogram 10 day citrus fruit Sample

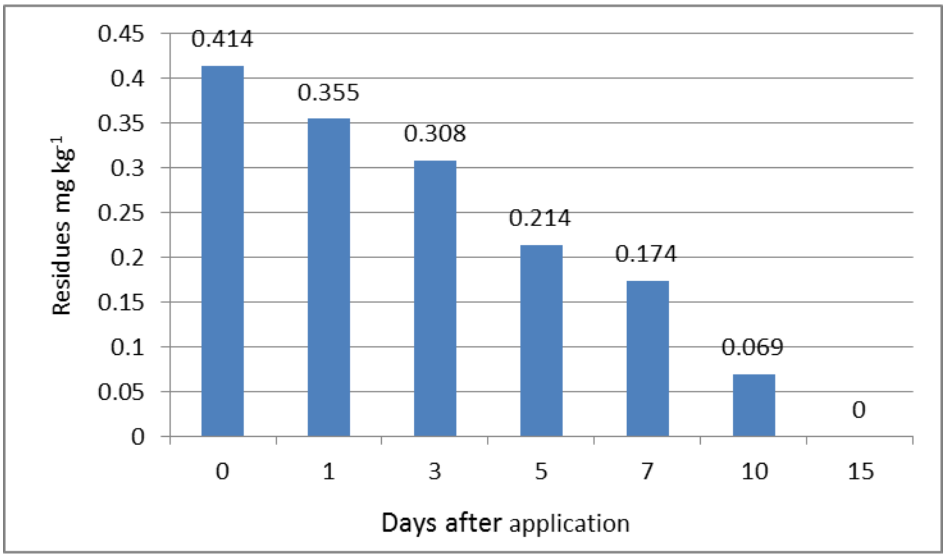

Fig. 14: Dissipation Bar graph of Acephate in citrus fruit

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