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**DISSIPATION OF ACEPHATE AND BUPROFEZIN IN FOUR
DIFFERENT TROPICAL SOILS- CONFIRMATION OF RESIDUES
BY LC-MS / MS**

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ABSTRACT

A field study was carried to evaluate the fate of Acephate + Buprofezin in four different kind of soils i.e. sandy loam, loamy sand, sandy clay and clay soils, following a single application of combinational insecticide formulation of Acephate 50% + Buprofezin 20% WP, supplied by Rallis India Limited, Bangalore, India. The dosage was applied on the moist soil in the plots at @ Acephate + Buprofezin - 1000 g/ha formulation recommended dose and Acephate + Buprofezin - 2000 g/ha formulation doubles the recommended dose. Field experiment plots were laid in Randomized Block Design (RBD) with three replications for each treatment. Untreated control soil plots were maintained for comparison. The composite soil sample was collected from a depth of 0-15 cm 2 hours after the application (0th day), and the sampling was continued at pre-determined intervals (3rd, 5th, 7th, 10th, 20th, 30th and 50th day) after spraying. The collected soil samples were extracted and analyzed for the residues of Acephate by validated Gas chromatograph flame photometry detector (GC-FPD) and Buprofezin High Performance Liquid Chromatography with diode array detector (HPLC-DAD). The terminal residues were re-analyzed by LC-MS/MS in the AJS- ESI source and MRM with positive ion. The half-life for Acephate was DT 50 - 2.6 to 3.1 days and DT 90 - 8.8 to 10.5 days. The half-life for Buprofezin was 11.8 to 19.7 days and DT 90 39.4 to 63.3 days. This followed the first order Kinetics.

Keywords: Acephate, Buprofezin, dissipation, HPLC-DAD, GC-FPD, LC-MS/MS.

INTRODUCTION

Pesticides are used to control pests on crops and in soils. In the tropics, they are applied to vegetable crops throughout their growing seasons and also applied to soils after the vegetable crop have been harvested. This practice is common as tropical climate is very helpful / conducive for the proliferation of pests. The overuse of pesticides may result in contamination of soil and water which may be harmful to non-target organisms and to human health. Besides, these pesticides may also be taken up by new crops if they persist in soil. Therefore, there is an urgent need to study the fate of pesticides in tropical soils. Rallis India Limited (India) introduced a combinational insecticide (Ready mix) formulation containing Acephate (O, S- dimethyl acetylphos phoramidothioate; organo

phosphorus insecticides) and Buprofezin (2-tert-butylimino-3-isopropyl-5-phenyl-1, 3, 5-thiadiazinan-4-one; insect growth regulator) (20% WP formulation - containing Acephate-50% + Buprofezin-20%) for the control of a broad spectrum of sucking pests in agriculture. Figure-1 represents the basic structures of the pesticides.

Acephate is one of the highly effective, low poisonous organophosphorus insecticides also used as acaricides, which are widely used for protecting crops such as rice, wheat, cotton, tea, tobacco and vegetable [1]. Acephate residue in vegetables has been attracting much attention because the residue of its metabolite, methamidophos, may be hazardous to human health [2]. Acephate is readily metabolized in the soil under both aerobic and anaerobic conditions.

The metabolite formed is O, S-dimethyl phosphoramidothioate (methamidophos), which is a much more potent inhibitor of cholinesterase and O-methyl N acetylphosphoramidate [3, 4]. Methamidophos, a toxic metabolite of acephate, is a widely used organophosphorus insecticide with systemic properties and is effective against chewing and sucking insects. Methamidophos is much more toxic than Acephate. [5]. Acephate dissipates rapidly with half-lives of less than 3 and 6 days in aerobic and anaerobic soils, respectively. There are many studies concerning Acephate and methamidophos that have been realized in the past on fruit and vegetable samples. A few have been done successfully on water samples using high performance liquid chromatography (HPLC), gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) [6-16]. Although studies on Acephate contamination in ground water, soil and crop [17-22] have been conducted, only a few strains with the ability to degrade Acephate have been reported [23]. There are combinational chlorpyrifos and Acephate studies that were also conducted in tropical soil [24, 25].

Buprofezin is a contact and ingested insecticide also used as acaricides, active against Homoptera (whiteflies, leafhoppers, scale insects, etc.) in and on citrus, cotton, cucumber, tomatoes, sweet potatoes, rice, etc. Buprofezin is an insect growth regulator, which affects larval or nymphal development by the inhibition of chitin biosynthesis. The photo degradation of Buprofezin is reported [26]. There have been some reports on the detection of Buprofezin in soil and crops [27-29]. Cabras studied the dissipation behavior of Acephate and Buprofezin residues in olives and olive oil was using GC-NPD and GC-MS [30].

Until now, there are no reports on the dissipation behavior of this combinational insecticide in tropical soils. Thus, this paper aims at contributing to the knowledge of the fate of residues of Acephate and Buprofezin in four different tropical soils under field conditions.

EXPERIMENTAL

MATERIALS AND METHODS

The Acephate and Buprofezin purities were 98.4% and 99.5% were purchased from sigma Aldrich. All the chemicals were purchased from Merck Chemicals Ltd, Mumbai.

Dissipation studies in tropical soils

Dissipation of Acephate and Buprofezin was conducted in sandy loam, loamy sand, sandy clay and clay soils, at Padappai, Chennai, Kancheepuram District, Tamil Nadu 601 301 from June to August 2013 (Latitude:13°-28°; Longitude: 81°-03'). Based on the soil characteristics, four different types of soil were identified and selected for the study purpose. Each experimental block was of 20 m² in size, and nine plots were prepared with a minimum isolation distance of 3 m. All the plots were laid out

uniformly and labeled as RBD. Three replications were maintained for each treatment and control. Formulation used for the spray was Acephate + Buprofezin - 1000 g/ha formulation (1 x recommended dose) and Acephate + Buprofezin - 2000 g/ha (2 x recommended dose). A spray solution was prepared in water at 500 L/ha. A Hand operated Knap sack sprayer fitted with hollow cone nozzle was used for the application of spray solution. A mock trial was carried out to calculate the time required for spraying. All the treatment plots were irrigated separately to avoid cross contamination between the control and treated plots. The initial day soil sample was collected before irrigation, two hours after the application of the spray solution. Further samples were collected on the 3rd, 5th, 7th, 10th, 20th, 30th and 50th days. The physicochemical characteristics of the collected soil samples are presented in Table 1. The collected soil samples were extracted as follows,

For Acephate: An approximate 50 g of soil was transferred into an extraction bottle and about 200 mL of acetone was added and kept in an end over end mechanical shaker for 1 hour. The extract was filtered and the solvent was evaporated to a small volume under vacuum. To this, a 50 mL of Methylene chloride and 100 mL of 5 percent aqueous Sodium chloride solution were added and the residues were extracted by vigorous hand shaking for 5 minutes. The Methylene chloride layer was collected and the extraction was repeated using an additional 50mL volume of methylene chloride. Both the organic layers were combined and the volume was reduced to near dryness under vacuum. The residues were reconstituted to a smaller volume using ethyl ether for column cleanup. Simultaneously, a control sample was also analyzed without fortifying the test item.

A glass column was packed with 2g of silica gel slurry prepared with ethyl ether in between two layers (1 cm) of anhydrous sodium sulphate. The concentrated extract was poured on the top of the column and eluted with 20 mL of 5 percent methanol in ether. The collected eluate was concentrated and diluted with IPA and quantified with GC-FPD.

For Buprofezin: An approximate 50 g of soil was transferred into extraction bottle and about 200 mL of acetone was added and kept in an end over end mechanical shaker for 1 hour. The extract was filtered and the solvent was evaporated to small volume under vacuum.

To this, a 20 mL of n-Hexane and 30 mL of 1N Hydrochloric acid were added and the residues were extracted by vigorous hand shaking for 5 minutes. The n-Hexane layer was collected and the extraction was repeated using an additional 50mL volume of n-Hexane. Both the organic layers were combined and the volume was reduced to near dryness under vacuum. The residues were reconstituted to smaller volume in acetonitrile. Simultaneously, a control sample was also analyzed without fortifying the test item.

INSTRUMENTATION

Gas chromatograph flame photometry detector

Acephate was determined by a Shimadzu GC-FPD system (Shimadzu, Japan).

The DB-17 capillary column (30m x 0.52mm i.d with 1.0 µm film thickness) was used for separation. Injection was carried out in the split mode (5:1) at an injector temperature of 250°C. Nitrogen gas was used as a carrier gas with a flow rate of 9.74 mL/min. The column temperature was maintained at 60°C for 0.5 min and then programmed at 30°C min⁻¹ to 160°C for 0 min followed by a final ramp to 190°C at a rate of 5°C min⁻¹, and held for 5 min. The detector temperature was 260°C. Under these experimental conditions, the retention times were Acephate - 6.9 min and Metabolite, Methamidophos – 5.4 min.

High Performance Liquid Chromatography with Diode Array detector

Buprofezin was determined by an Agilent HPLC- DA.

The separation of and interference was performed on a reverse-phase, C₁₈ (15 cm length x 4.6 mm i.d x 5 µ particle size) obtained from Kromosil. The mixture of Acetonitrile: water (82:18, v/v) with a total flow rate of 1.0 mL/min. Detector was performed at 230, 4 nm. The injection volume was 10 µL. Under these experimental conditions, the retention time was Buprofezin was about 5.8 min.

Mass spectrometry (LC-MS/MS)

Mass spectra was recorded on an Agilent 6490 triple quadrupole (QQQ) mass spectrometer equipped with an ESI source. System control and data acquisition were controlled by Agilent Mass Hunter software. Aliquots of 5.0 µL were injected directly to the LC-

MS/MS system to test. Agilent 1290 series Infinity HPLC system with Agilent Zorbax SB c18 column (3.5µm particle size, 4.6 mm i.d., x 75 mm length), gradient elution of 0.5 ml per min having 0.1% formic acid in water as mobile phase A (20%) and Acetonitrile as mobile phase B (80%) were used. The column oven temperature was set at 40°C. Detailed MS conditions were: Capillary voltage – 3000 V; nozzle voltage-1500 V; gas temperature: 250°C; Gas flow-14 l/min, Nebilizer pressure: 45 psi; Sheat gas temperature: 325°C; Sheath gas flow-11 l/min. AJS-ESI was operated in the positive ion mode in the MRM (multiple reaction mode).

Recovery, Limit of quantification and repeatability

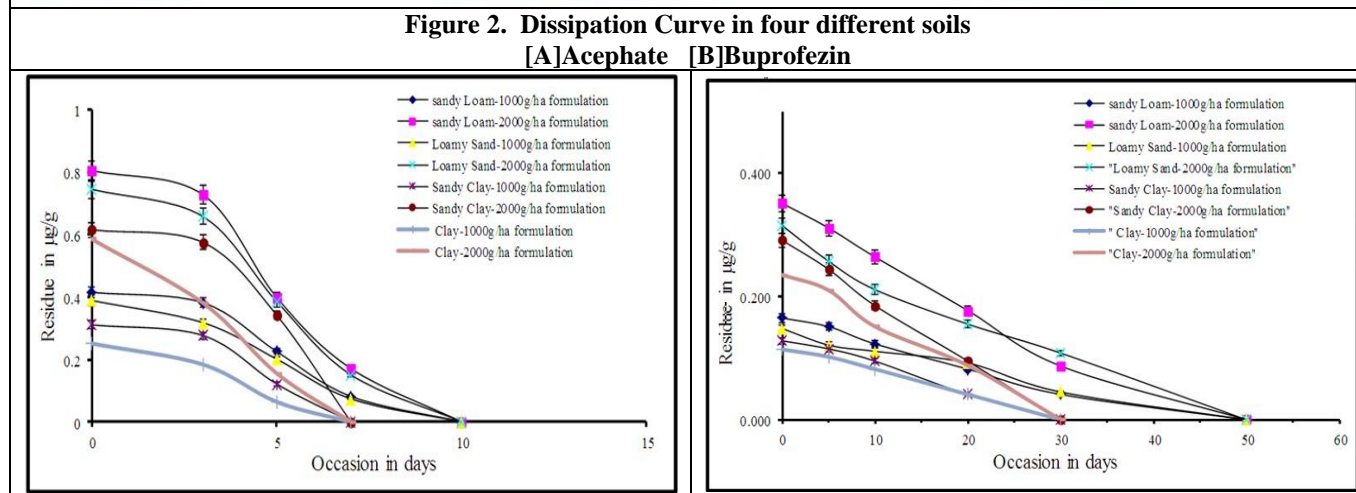
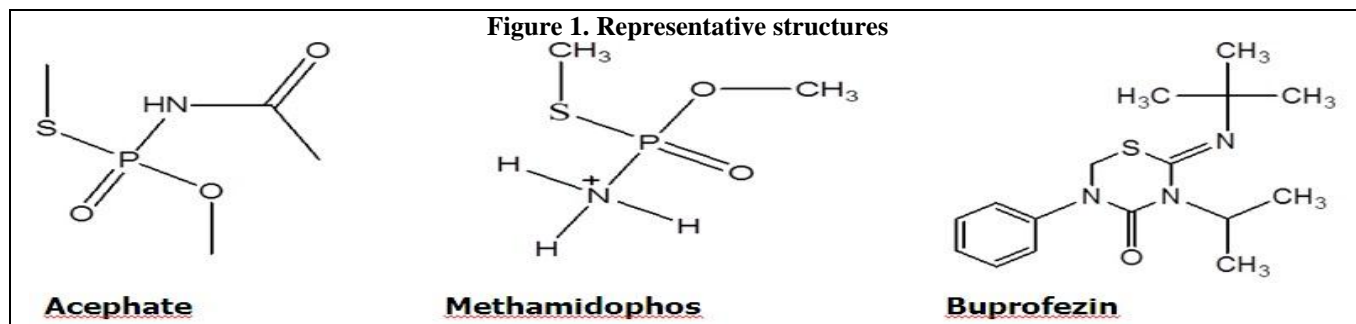
Recovery experiments were carried out in triplicate at three fortification levels (0.01, 0.1, 1.0 µg/g) by adding a known volume of the Acephate, methamidophos and Buprofezin standard solutions to different type of soil at the LOQ and at 10 x LOQ level and Higher level. Different known concentrations were prepared for Acephate its metabolite in IPA and Buprofezin in acetonitrile by diluting the stock solutions of reference analytical standards. Validation of the method was performed in terms of recovery studies before the analysis of unknown samples by spiking the three different known concentrations with five replicates in control soil samples. The percentage recovery ranges from Acephate 85 + 4% for metabolite Methamidophos 86 + 6% and Buprofezin 90 + 5%. No inference was observed at this quantification level as evidence by the control sample. From the analytical determinations, the limit of detection and limit of quantification were established as 0.01 µg/g for both Acephate and Buprofezin. The signal to noise ratio is 3:1, and 10:1 was considered for calculating the limit of detection and limit of quantification, respectively.

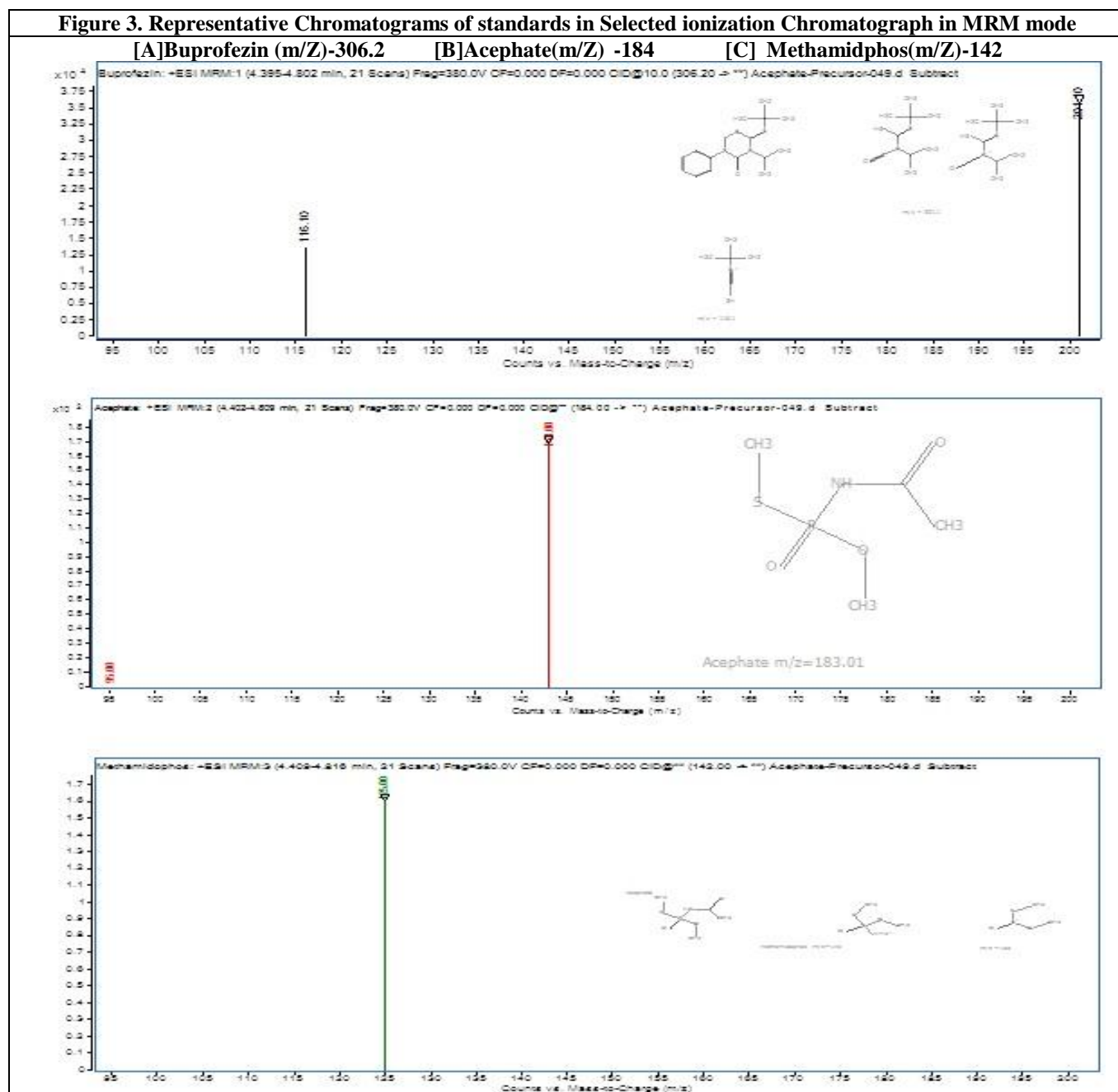
Table 1. Physicochemical Characteristics of soil samples

Parameters	Sandy Loam	Loamy Sand	Sandy Clay	Clay
Colour	Dark reddish brown	Brown to dark brown	Very dark greyish brown	Very dark greyish brown
pH	7.82	7.02	8.18	8.27
EC (m mhos/cm)	46.8	25.7	110.8	121.4
Sand (%)	57.8	65.8	30.6	16.4
Silt (%)	41.82	23.8	25.6	25.8
Clay (%)	6.8	19.4	48.9	66.4
Water holding capacity (%)	45	51.2	58	64
Organic matter (%)	0.58	0.47	0.65	0.58

Table 2. Residues of Acephate and Buprofezin in different soil types various sampling occasions

Days after treatment	Residues of Acephate in soil (µg/g)							
	Sandy Loam		Loamy Sand		Sandy Clay		Clay	
	1000 mL/ha	2000 mL/ha	1000 mL/ha	2000 mL/ha	1000 mL/ha	2000 mL/ha	1000 mL/ha	2000 mL/ha
0	0.415	0.805	0.389	0.745	0.311	0.615	0.251	0.585
3	0.382	0.728	0.318	0.658	0.277	0.575	0.184	0.381
5	0.225	0.398	0.201	0.385	0.081	0.165	0.064	0.155
7	0.082	0.171	0.074	0.151	BDL	BDL	BDL	BDL
10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
DT 50	3.10	3.17	3.06	3.13	2.76	2.84	2.67	2.71
DT-90	10.31	10.55	10.15	10.41	9.18	9.43	8.86	9.00
Regression equation	Y=-0.270-0.097X	Y= 0.006-0.095X	Y=-0.314-0.099X	Y=-0.026-0.096X	Y=-0.428-0.109X	Y=-0.129-0.106X	Y=-0.542-0.0113X	Y=-0.111-0.191X
Residues of Buprofezin in soil (µg/g)								
0	0.165	0.351	0.148	0.315	0.128	0.291	0.114	0.235
3	0.158	0.328	0.142	0.301	0.121	0.274	0.109	0.221
5	0.151	0.311	0.121	0.257	0.115	0.244	0.102	0.210
10	0.123	0.264	0.111	0.211	0.095	0.185	0.082	0.151
20	0.082	0.177	0.092	0.155	0.041	0.095	0.041	0.088
30	0.041	0.087	0.045	0.108	BDL	BDL	BDL	BDL
50	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
DT 50	14.90	15.07	19.06	19.77	11.87	12.13	13.19	13.56
DT-90	49.51	50.05	63.32	65.69	39.42	40.30	43.80	45.05
Regression equation	Y=-0.735-0.020X	Y=-0.411-0.020X	Y=-0.812-0.0016X	Y=-0.511-0.0015X	Y=-0.838-0.025X	Y=-0.509-0.025X	Y=-0.902-0.023X	Y=-0.602-0.022X





RESULT AND DISCUSSION

The dissipation of Acephate and Buprofezin in four different soils is presented in Table 1. The dissipation curve is presented in Figure 2A and 2B for Acephate and Buprofezin respectively. The half-life for Acephate was DT 50 - 2.6 to 3.1 days and DT 90 - 8.8 to 10.5 days. The half-life for Buprofezin was 11.8 to 19.7 days and DT 90 39.4 to 63.3 days.

Analysis of soil samples collected on 0 day and the 1st day showed that the residues of methamidphos metabolite were below the limit of determination in both the tested dosages. The metabolite methamidphos was

identified in the dose 2000 g/ha in the soil samples collected on the 3rd day at 0.035, 0.032, 0.027 and 0.024 µg/g, on the 5th day 0.027, 0.024, 0.021 and 0.018 µg/g in sandy loam, loamy sand, sandy clay and clay soils respectively. Only in the soil plots sprayed at 2000 g/ha dose, the residues of metabolite methamidphos were found to be below detectable level in the 7th day samples in sandy clay and clay soils, the residue levels were at 0.016, 0.018 µg/g in loamy sand and sandy loam soils at this sampling occasion. Complete dissipation of metabolite methamidphos was observed by the 10th day.

The terminal residues in the soil extract was confirmed by analyzing representative sample using LC-MS/MS. ESI was operated in the positive ion mode in the MRM.

Acephate and its metabolite methamidophos were eluted at 1.38, a molecular ion peak was observed at m/Z 184 and transitions ions at m/Z 143; 269.8. The metabolite methamidophos displayed a molecular ion peak at m/Z 142 and transitions ions at m/Z 125; 94. Buprofezin was eluted at 4.6 min and showed a molecular ion peak at m/Z 306.2 and the transitions ions at 201.1; 116.6. A representative confirmation spectrum is presented in Figure 3. There are no published data on field studies of four different soils of acephate + buprofezin. The dissipation of residues was faster in clay and sandy clay soil which has more organic carbon content and higher pH when compared to sandy loam and loamy sand soil. The degradation of the insecticide was faster in soil samples having higher clay content. This study demonstrates that

dissipation study of Acephate and Buprofezin under field tropical soils.

CONCLUSION

Dissipation of Acephate its metabolite and Buprofezin showed first order kinetics in soils. Calculated DT50 and DT90 - Acephate was DT 50 - 2.6 to 3.1 days and DT 90 - 8.8 to 10.5 days. The half-life for Buprofezin was 11.8 to 19.7 days and DT 90 - 39.4 to 63.3 days. The residues were confirmed by LC-MS/MS. The pesticides did not persist for longer times in the sub soils than in the top soils and after 10 and 50 days, acephate and Buprofezin were no longer detectable in the soil profile.

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