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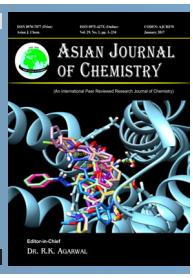

Dissipation of Monocrotophos Residues in Castor (*Ricinus communis*) and Harvest Time Residues in its Bi-products and Soil Using LC-MS/MS

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Field trials were conducted at two locations *viz*., Hyderabad and Palem to study the dissipation kinetics of monocrotophos in castor plant, its bi-products and soil. Monocrotophos 35 % EC at two test doses @ 437 mL/ha and 874 mL/ha was applied to castor crop at flowering stage followed by capsule formation stage. The leaf samples drawn periodically were analyzed by liquid chromatography and tandem mass detection (LC-MS/MS). The initial residues of recommended dose of monocrotophos in castor leaves were found to be 4.641 and 6.22 mg/kg at Hyderabad and Palem, respectively. More than 98 % of monocrotophos residues had dissipated after 10 days in both the test doses. Castor seeds, castor oil and castor cake and soil samples collected at harvest which happened to be 60 days after the last application did not show the presence of residues of monocrotophos at their determination limit of 0.05 mg/kg. These results would be helpful in setting maximum residual limit (MRL) of monocrotophos in castor in India.

Keywords: Residues, Dissipation, Monocrotophos, Castor, LC-MS/MS.

INTRODUCTION

Monocrotophos [(*E*)-dimethyl-1-methyl-3-(methylamino)-3-oxo-1-propenyl phosphate (9Cl); dimethyl phosphate ester with (*E*)-3-hydroxy-*N*-methylcrotonamide (8Cl is a broad-spectrum organophosphate insecticide, acaricide and termiticide and acts effectively against many insect pests *viz*., cutworms, corn rootworms, cockroaches, leaf folder and leaf hopper, *etc.* [1]. Approximately 30,000 tons of monocrotophos is used annually. As per data's Asia is the top user of monocrotophos as; India (43 %), South America (26 %), China (15 %) and South-east Asia (9 %) account for 90 % of the use, internationally. In India, Andhra Pradesh and Punjab are the chief consumers of monocrotophos [2]. Castor (*Ricinus communis*) is an important oil seed crop widely grown in rain fed conditions of southern Telangana regions. Even though castor is a non-edible oil seed crop, in India, castor oil can also be used as laxative to newborn babies and the castor cake will be applied as organic amendment to enrich the soil. A major constraint in the cultivation of the crop is the threat posed by an array of insects among which the lepidopteran pests like castor semilooper, tobacco cater pillar, hairy caterpillars and capsule borers take a heavy toll of the castor crop in the state. Quite often, insecticides are required to protect the crop from these noxious pests. Usage of monocrotophos during the capsule formation stage may lead to accumulation of residues in the seeds. Although monocrotophos residues have been reported in apple, cucumber, green vegetables [3,4], honey bees from the sunflowers (44 %), citrus groves (10 %) and cotton field (35 %), milk and potato [5,6]. But the literature pertaining to the persistence behaviour of monocrotophos residues on castor plant and its bi-products are scanty. As per the CIBRC, monocrotophos was registered for pest control on castor but the maximum residual limit was not fixed due to lack of residual data. Hence, the present studies were undertaken to investigate the dissipation of moncrotophos in castor plant, seed and soil. In this work, simple LC-MS/MS methods were established to detect the residues of monocrotophos in castor leaves, seed, oil, cake and soil, respectively.

EXPERIMENTAL

Field experiments were conducted at two sites: College of Agriculture, Rajendranagar, Hyderabad, Andhra Pradesh and Palem, Mahaboobnagar, India. Each experimental plot was 30 m^2 and each treatment was carried out in triplicate in randomized block design. To investigate the dissipation of monocrotophos in castor plant, seed and soil samples, the first application of monocrotophos 35 % EC at two test doses @ 437 mL/ha and 874 mL/ha at flowering stage followed by capsule formation stage. Castor leaf samples were collected at 0, 1, 3, 5, 7 and 10 days after last spray from all the plots separately, one leaf sample each from top, middle and bottom

parts of the plants was collected from randomly selected 8-10 plants and were made into small pieces, from which 500 g of sub sample was collected for analysis. At the time of harvesting, 2 kg seeds were collected randomly from each plot packed in poly bags to avoid contamination. The soil sample was collected at the time of harvest from 10 sites from each plot, weighing 0.5 kg each at 40 cm depth.

Analytical standard of monocrotophos (purity ≥ 99.8 %) was supplied from Sigma Aldrich, Germany. A standard stock solution (100 mg/L) was prepared with methanol and stored at -20 °C. The stock solution was diluted with methanol serially to obtain 0.01, 0.025, 0.05, 0.10, 0.25 and 0.5 mg/L solutions, respectively. 1 µL of each solution was injected into LC-MS and calibration curve was prepared by plotting concentration of monocrotophos on X axis against average peak area on Y axis.

Extraction and clean up of castor leaves, castor seed, oil and cake of castor: The castor leaf samples were analyzed for residues following the AOAC official method [7]. 500 g of the leaf samples collected from each plot and were homogenized with robot coupe blixer. 15 g sample was taken in 50 mL centrifuge tube and added with 30 mL acetonitrile and homogenized at 14000-15000 rpm for 2-3 min using Heidolph silent crusher. Then 3 ± 0.1 g sodium chloride was added to the sample and mixed by shaking gently followed by centrifugation for 3 min at 2500-3000 rpm. 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. From that 8 mL of extract was taken into 15 mL tube, containing 0.4 g PSA sorbent and 1.2 g anhydrous magnesium sulphate. The sample tube was vortexed for 30 sec then followed by centrifugation for 5 min at 2500-3000 rpm. 2 mL extract was transferred into test tubes and evaporated to dryness using turbovap with nitrogen gas and reconstituted with 1 mL methanol for LC-MS/MS analysis. The castor oil and castor cake were extracted from the castor seed and extraction and clean up procedures were carried out using QuEChERS method in a similar way as explained above.

Extraction and clean up of soil samples: The soil samples were analyzed using the QuEChERS method. 10 g soil sample was mixed with 20 mL acetonitrile, 1 g sodium chloride and 4g magnesium sulphate and taken into 50 mL centrifuge tube and centrifuged for 3 min at 3300 rpm. 10 mL of top organic layer was taken into the 15 mL centrifuge tube containing 1.5 g magnesium sulphate and 0.25 g PSA and sonicated for 1 min and centrifuged for 10 min at 3000 rpm. About 2 mL of supernatant was transferred into test tubes and evaporated to dryness using turbovap with nitrogen gas and reconstituted with 1 mL of methanol. The supernatant was transferred to LC/MS sample vial for instrumental analysis after filtered through 0.22 Um filter membrane for LC/MS analysis.

LC-MS/MS condition: SHIMADZU LC-MS/MS-8040 triple-quadruple mass spectrometry equipped with an electron spray ionization interface was operated in the positive ion mode. Shimpack XR-ODS-III column (75 × 2 mm, 1.6 µm) was used to separate the target compound from interferences at 40 °C. The mobile phase was methanol-water containing 10 mm ammonium formate at the flow rate of 0.4 mL/min. The injection volume was 1 µL.

Conditions for MS detection: Desolvation gas temperature: 250 °C; Heat block temperature about 300 °C; Drying gas flow – 15 L/min; Nebulizer gas (N_2) flow- 2 L/min; Dwell time – 50 msec; Interface voltage – 4.5-5.0 kv. Under these operating conditions the retention time of monocrotophos was found to be 1.01 min. The mutiple reaction monitoring (MRM) transitions used for the quantitative and qualitative estimation were *m/z* 224 -> 127 and 224 -> 193, respectively (Fig. 1). Linearity was assessed by the determination coefficient (r^2) of a calibration curve that was created based on six points of different concentrations of monocrotophos. Recovery was validated by fortifying the untreated castor plant samples with standard solutions of monocrotophos. The limit of detection (LOD) for monocrotophos was 0.01 mg/kg and the limit of quantification (LOQ) was 0.05 mg/kg. The degradation kinetics of monocrotophos in castor plant and soil were determined by plotting residue concentration over time. The rate of degradation (K) and half life ($t_{½}$) values were calculated using the following equation [8].

Rate of degradation (K) = 2.303 × Slope; Half-life ($t_{½}$) = 0.693/k.

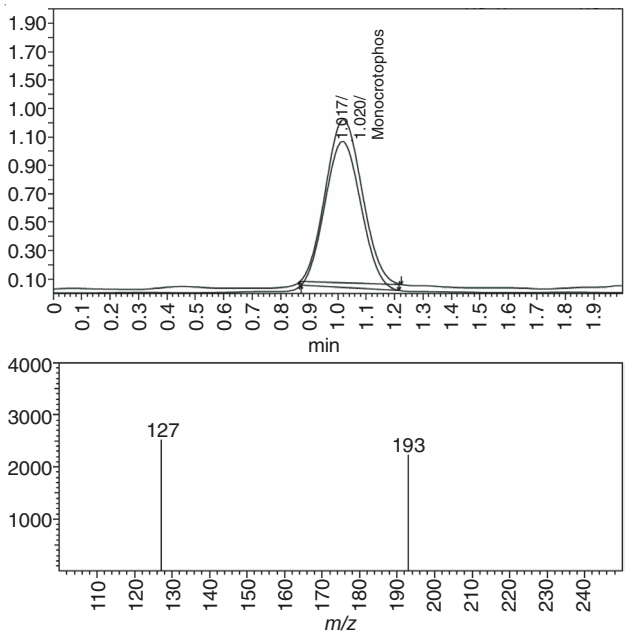

Fig. 1. LC-MS/MS multiple reaction monitoring chromatogram and mass spectra of monocrotophos standard

RESULTS AND DISCUSSION

Fortification and recovery studies: The analytical method was validated in terms of limit of quantitation, linearity and recovery. The fortification study was carried out by spiking the untreated castor plant samples and soil samples at 0.05, 0.25 and 0.5 mg kg^{-1} levels to determine the recovery levels and the average recoveries of the method (87.1-100.2 %) were satisfactory (Table-1 and Fig. 2). precision of the method in terms of relative standard deviations (RSD) ranged from 2.26 to 14.1 %. The limit of quantitation (LOQ) was found to be 0.05 mg kg^{-1} and limit of detection (LOD) being 0.01 mg kg^{-1}.

Dissipation of monocrotophos in castor plant and soil: The initial residues of monocrotophos in castor plants differed

TABLE-1
FORTIFIED RECOVERIES OF MONOCROTOPHOS ON CASTOR LEAVES, SEEDS, CASTOR CAKE, CASTOR OIL AND SOIL

Sample	Fortified level (mg/kg)	Mean (%) recovery	Relative standard deviation (%)
Castor leaves	0.05	92.6	5.27
	0.25	87.3	2.60
	0.50	94.1	2.87
Castor seed	0.05	101.0	14.01
	0.25	91.7	6.38
	0.50	98.5	6.72
Castor oil	0.05	87.8	4.84
	0.25	90.1	3.99
	0.50	88.5	6.27
Castor cake	0.05	93.0	3.44
	0.25	100.2	5.56
	0.50	101.3	2.56
Soil	0.05	87.1	5.56
	0.25	95.1	2.26
	0.50	92.6	3.25

between two experimental locations. The initial deposit of monocrotophos in castor plants grown in Hyderabad was 4.641 mg/kg (X dose) and 10.69 mg/kg (2X dose) with a half-life ($t_{½}$) of 3.78 days and 4.01 days, respectively. More than 98 % of this residue had dissipated after 10 days in both the test doses of monocrotophos. However, initial deposit of monocrotophos in castor plants grown in Palem, Mahaboobnagar was comparatively higher with a residue of 12.56 mg/kg (2X dose) and 6.217mg/kg with a half-life of 3.47 days and 4.71 days, respectively. More than 99 % of residues were dissipated within 10 days of spraying (Table-2). The differences in degradation pattern of monocrotophos at different locations suggest that the local soil characterestics and environmental conditions affect the dissipation of monocrotophos. However the factors that influence pesticide persistence are climate, physical and chemical properties of pesticide [6]. FAO/WHO, US [9] have not established maximum residue limits (MRLs) for monocrotophos in castor. Maximum residue limits for monocrotophos have been set in some crops in the range of 0.02-1 mg/kg and the acceptable daily take is 0.0006 mg/kg body weight. From the residue results in field at two locations, residues in leaves after 10 days of spray and residual monocrotophos levels in castor seed, castor oil, castor cake and soil collected at harvest time (60 days after second spray) are below detectable level (0.05 mg/kg). This result suggests that it is safe to harvest the castor seed after 10 days of application of monocrotophos at recommended dose. This work could be a reference for the maximum residual limit establishment and safe use of monocrotophos in castor.

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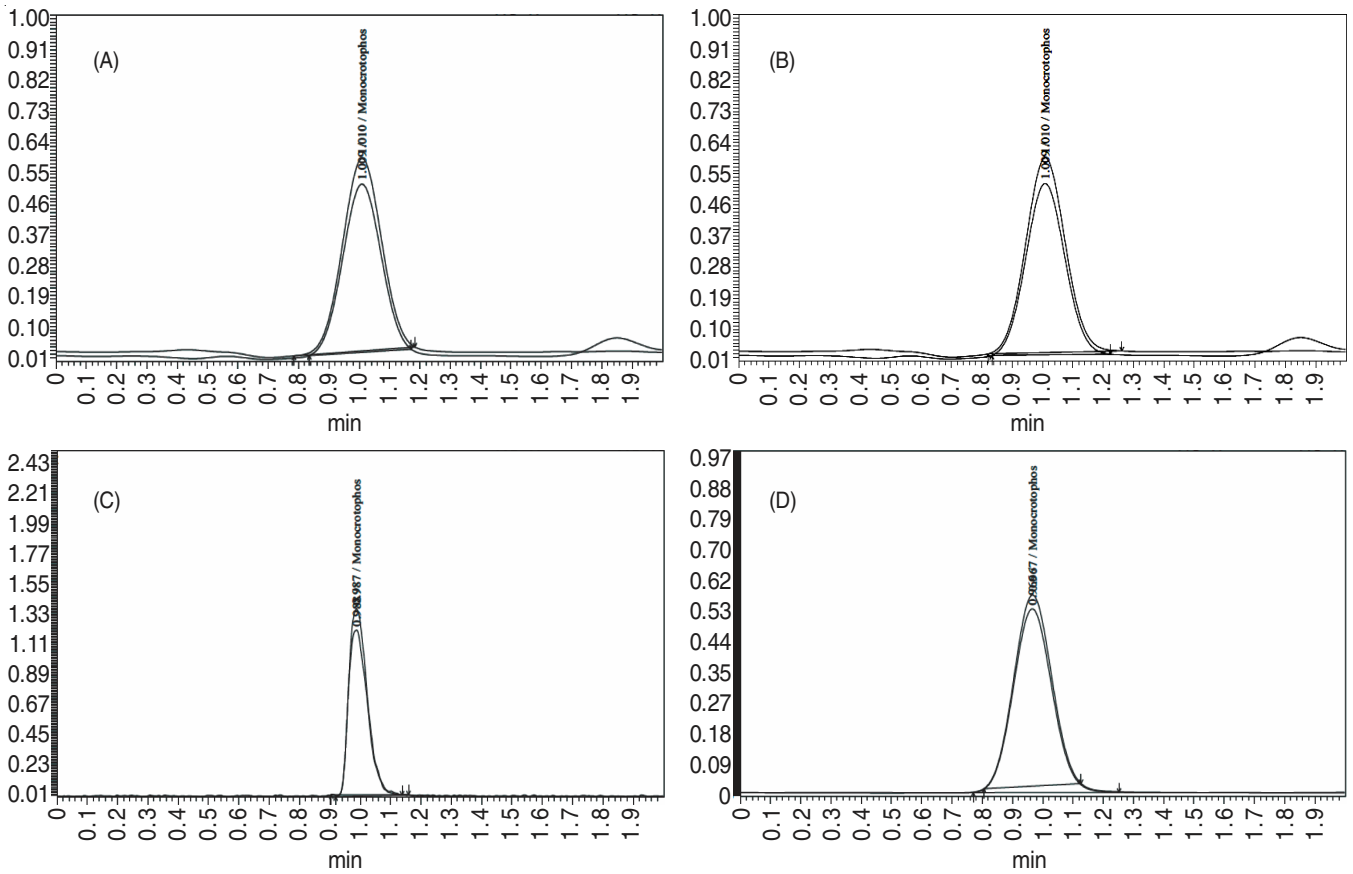

Fig. 2. Chromatogram of monocrotophos standard in LC-MS/MS; (A) Standard in castor leaf; (B) Standard in castor seed; (C) Standard in castor oil; (D) Standard in castor cake

TABLE-2
DISSIPATION OF MONOCROTOPHOS ON CASTOR LEAVES, SEEDS, OIL, CAKE AND SOIL

Sample	Days	Rajendranagar, Hyderabad				Palem, Mahaboobnagar			
		Monocrotophos @ 437 mL/ha		Monocrotophos @874 mL/ha		Monocrotophos @437 mL/ha		Monocrotophos @874 mL/ha	
		Residues (mg/kg) Mean±SD	Dissipation (%)	Residues (mg/kg) Mean±SD	Dissipation (%)	Residues (mg/kg) Mean±SD	Dissipation (%)	Residues (mg/kg) Mean±SD	Dissipation (%)
Castor leaves	0	4.641±0.11	0	10.687±0.091	0.00	6.217±0.27	0	12.559	0
	1	3.694±0.48	20.40	7.627±0.370	28.63	4.474±0.07	28.03	9.432	24.89
	3	2.865±0.16	38.26	6.302±0.149	41.03	2.731±0.25	56.07	5.941	52.69
	5	1.951±0.35	57.96	4.286±0.309	59.90	1.717±0.09	72.38	3.098	75.33
	7	0.498±0.02	89.26	1.329±0.353	87.56	0.488±0.03	92.15	0.952	92.41
	10	0.057±0.01	98.77	0.154±0.011	98.56	BDL	99.87	0.108	99.14
Seed at harvest		BDL		BDL		BDL		BDL	
Oil at harvest		BDL		BDL		BDL		BDL	
Cake at harvest		BDL		BDL		BDL		BDL	
Soil at harvest		BDL		BDL		BDL		BDL	
Regression equation		$y = -0.183^x \pm 3.865$		$y = -0.172^x \pm 4.190$		$y = -0.147^x \pm 3.83$		$y = -0.199^x \pm 4.257$	
R2 value		0.946		0.945		0.974		0.972	
Half life ($t_{½}$)		3.78 days		4.01 days		4.71 days		3.47 days	

BDL: Below determination level

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