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A Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) Determination of Pesticide Residues in Tender Coconut Water (elaneer/nariyal pani)

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Abstract

The occurrence of Monochrotophos, Copperoxychloride and Galaxy pesticides in 10 tender coconut water samples (Arasampattai, Sowcot pachai, kuttyaiadi (Kerala),Malayan orange, Malayan yellow, Alphanam, sowchot orange, Nettai, Kuttai and 1000 kaychi) in southern area of Tamilnadu, India was investigated. The HPLC results shows that the monochrotophos was found Malayan orange (5.847 mg kg^{-1}) and it was above the Maximum Residual Limit (MRL (0.5-1.0 mg kg^{-1}) and Malayan yellow (0.003 mg kg^{-1}) and Nettai (0.323 mg kg^{-1}) were below the Maximum Residual Limit. In Malayan yellow tender coconut samples copper oxychloride was found (0.003 mg kg^{-1}) below the MRL value (20.0 mg kg^{-1}). Analysis was carried out using HPLC-UV.

Keywords: Pesticide residue; Monochrotophos; Copperoxychloride; Tender coconut water; RP- HPLC -UV

Introduction

Coconut (Cocos nucifera L.) has been the focus of increased international attention as a functional ingredient in fruit juices, beverage blends, dietary supplements, and dairy [1]. Tender coconut water (elaneer/nariyal pani) is a rich source of nutrients. It is high in electrolytes, chlorides, potassium, and magnesium and has a moderate amount of sugar, sodium and protein. Coconut water is also a good source of dietary fibre, manganese, calcium, riboflavin and vitamin C. Coconut water is ideal in preventing dehydration, especially when children get diarrhoea. It replenishes the natural salts lost by the body. In the hot summer months, when your toddler is thirsty, coconut water is much more beneficial than packaged fruit juices and aerated drinks which only contain empty calories. The extraction and quantification of pesticide residues in food matrix mostly involved liquid-liquid extraction with a great variety of solvents and adsorbents for clean up. Analytical techniques such as Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) are widely used to monitor the presence of these compounds in water, soils, foods, fruits and vegetables. Several recent papers have reported advances in this field [2-5]. Therefore, pesticide residue in food has been strictly regulated by government in all countries in order to determine whether the concentrations of the pesticides used exceed their maximum residues limits (MRLs). The monitoring of pesticide residues in coconut water is of particular concern from the consumer safety perspective [6].

In the present study, a method employing HPLC with UV detection for the separation, identification and quantification of four widely used pesticides on cauliflower was developed and validated.

Materials and Methods

Sampling

Fresh samples of tender coconut water (elaneer/nariyal pani) were collected from pollachi (Coimbatore district), Tamilnadu. A total of 10 varieties (Arasampattai, Sowcot pachai, kuttyaiadi (Kerala), Malayan orange, Malayan yellow, Alphanam, sowchot orange, Nettai, Kuttai and 1000 kaychi) of tender coconut were collected for the analysis.

Chemicals and reagents

The organic solvents, acetonitrile, ethyl acetate used were HPLC grade and were purchased from E. Merck. with a purity of 95-99%. The standards were stored in a freezer at -5°C. Water and acetonitrile were degassed by vacuum suction. All samples and solvents were filtered through Millipore membrane filters (0.45 µm pore size) before injection on the column.

Standard preparation

A stock solution of monochrotophos, copper oxychloride and Galaxy (100 mg kg^{-1}) was prepared in acetonitrile. Standard calibration solutions (10 to 50 mg kg^{-1}) for the assessment of linearity were prepared from this stock solution using the mobile phase. The solutions were filtered through a 0.45-µm nylon filter. Working solutions were prepared daily by appropriate dilution with acetonitrile. The filtered solution was then injected into the HPLC system.

Extraction

The tender coconut water samples (50 ml) were homogenized and mixed with anhydrous sodium sulphate (50 g) and extracted with ethyl acetate [7] (200 mL) in a conical flask. The content was allowed to settle down for about half an hour and the ethyl acetate extract was then filtered through a filter paper covered by 20 g of anhydrous sodium sulphate. After filtration, the extract was evaporated to dryness and re-dissolved in 5 mL of acetonitrile and finally the sample was transferred in to the SPE cleanup using C18 SPE cartridge with acetonitrile (Strio™-E, 1CC, 30 mg, part#SSSE-1CC30, strio Chem Technologies, Inc, California, USA). The final extract was made up to 1 ml with acetonitrile. The clean samples were taken and were analyzed by a high performance liquid chromatography having UV/Visible detector.

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HPLC systems

A Shimadzu SCL-10AVP, Version 5.22 High performance liquid chromatography having UV/visible detector was used for identification and quantification of pesticides [7]. Separation was performed on reversed phase C-18 column (Nova pack). Samples were injected manually through a Rheodyne injector. Detector was connected to the computer for data processing. The working condition of HPLC was binary gradient, mobile phase was acetonitrile: water; (70:30), flow rate was 1 mL min^{-1}, injection volume was 20 µL and the wavelength of the UV/visible detector was fixed at 254 nm for the pesticides i.e., monochrotophos, copper oxychloride and Galaxy.

Results and Discussion

HPLC determination of pesticide standards

The pesticide residues was identified by comparing its retention time Retention time (Rt), Monochrotophos (Rt-2.258), Copperoxychloride (Rt-2.908) and Galaxy (Rt-3.608) and their standards have been shown in Figure 1 and Table 1. The quantitative determination was carried out with the help of a calibration curve (Figure 2). A good linearity was established by a correlation coefficient (R^2) value of 0.990 ± 0.0001 (Table 2). Correlation coefficient is a statistical tool used to measure the degree or strength of this type of relationship, and here, a high correlation coefficient value (a value very close to 1.0) indicates a high level of linear relationship between the concentration of monochrotophos and peak area. For quantification an external calibration curve with four different concentrations of each pesticide, with matrix matching were made.

HPLC determination of pesticide in Tender coconut water samples

The HPLC Result based on the Retention time (Rt), monochrotophos (Rt-2.25) content in tender coconut samples samples was given in Figure 3, monochrotophos was detected amount (5.847 ppm) more than MRL value in Malayan orange sample and the amount

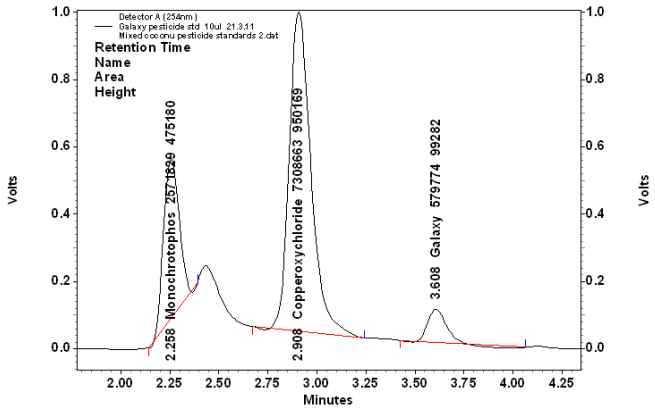

Figure 1: HPLC chromatogram of Pesticide standards.

Detector A (254nm)					
Pk #	**Retention Time**	**Area**	**Height**	**Name**	**Concentration (mg kg^{-1})**
1	2.258	2571829	475180	Monochrotophos	10.000
2	2.908	7308663	950169	Copperoxychloride	10.000
3	3.608	579774	99282	Galaxy	10.000

Table 1: Pesticide Standards.

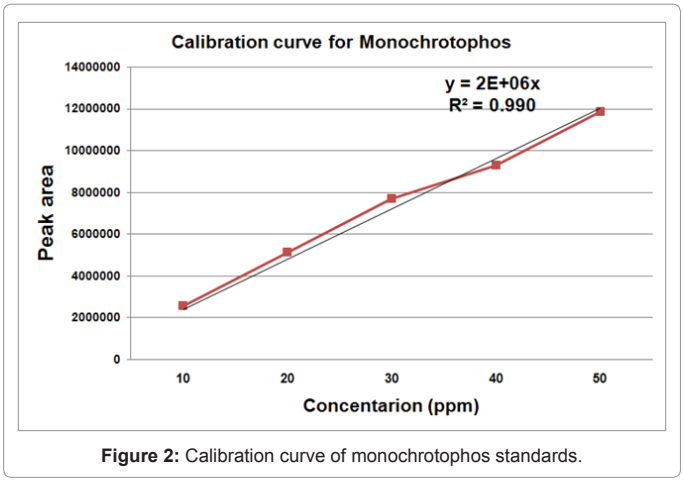

Figure 2: Calibration curve of monochrotophos standards.

S.No	Rf Value	Peak Hight	Peak Area	Monochrotophos Standard concentration (mg kg^{-1})
1	2.258	475180	2571829	10
2	2.243	762480	5133865	20
3	2.204	891930	7695748	30
4	2.256	1212580	9287764	40
5	2.255	1540910	11859321	50

Table 2: validation data of Monochrotophos standardsobtainedfrom HPLC chromatogram.

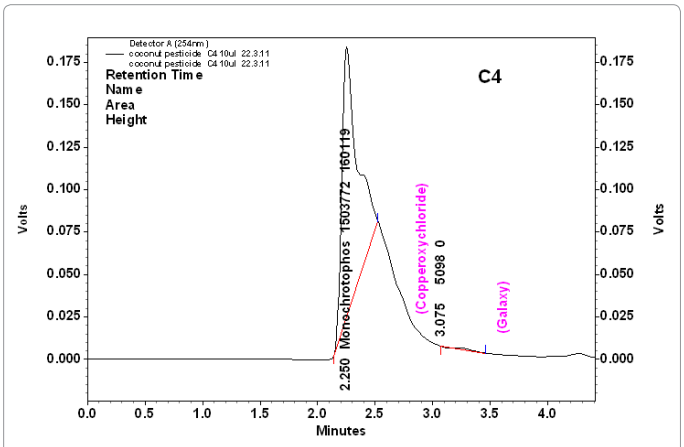

Figure 3: HPLC chromatogram of Pesticide in Malayan orange Tender coconut water.

was below the MRL value (0.5-1 mg kg^{-1}) as per the EU statndards in the 9 samples. Based on the the HPLC results Copperoxychloride was found in Malayan yellow (0.003 mg kg^{-1}) and the amount was below the MRL value (20 mg kg^{-1}) as per the EU statndards given in Table 3 and Table 4 and Figure 4. The galaxy pesticides was not detected in all the samples.

Conclusion

In this study the RP-HPLC method used to determine pesticides in tender coconut water samples. The results indicate that, among 10 samples of tender coconut water samples that were examined, only one two sample contained monochrotophos and copper oxychloride. The malayan orange sample that exceeded the FAO/WHO Codex Alimentarius Maximum Residue Limits (MRLs) (Codex Alimentarius Commission, 1996). Monocrotophos is a low cost and many possible

Tender coconut varieties	Retention time	Peak area	Peak height	Concentration (mg kg^{-1})	Maximum Residual Limit (MRL)
Arasampattai (C1)	BDL	BDL	BDL	BDL	(0.5-1.0 mg kg^{-1})
Sowcotpachai (C2)	BDL	BDL	BDL	BDL	
kuttyaiadi (Kerala) C3)	BDL	BDL	BDL	BDL	
Malayan orange (C4)	**2.250**	**1503772**	**160119**	**5.847**	
Malayan yellow (C5)	**2.267**	**1023**	**784**	**0.004**	
Alphanam (C6)	BDL	BDL	BDL	BDL	
sowchot orange (C7)	BDL	BDL	BDL	BDL	
Nettai (C8)	**2.275**	**83106**	**24363**	**0.323**	
Kuttai (C9)	BDL	BDL	BDL	BDL	
1000 kaychi (C10)	BDL	BDL	BDL	BDL	

*BDL-Below Detection Limit

Table 3: Monochrotophos residues content in tender coconut water samplesby HPLC.

Tender coconut varieties	Retention time	Peak area	Peak height	Concentration (mg kg^{-1})	Maximum Residual Limi (MRL)
Malayan yellow	2.900	2233	0	0.003	(20.0 mg kg^{-1})

Table 4: Copperoxychloride residues content in Tender coconut water samplesby HPLC.

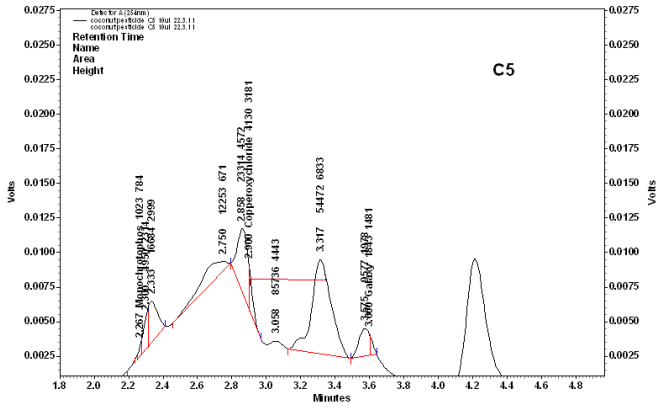

Figure 4: HPLC chromatogram of Pesticide in Malayan Yellow Tender coconut water.

applications have kept up demand in India despite growing evidence of its negative impact on human health. The results demonstrate that the accuracy of the method are used for multi residue analyses of pesticides. With regards to the 90% of the tender coconut water samples from a local market, no detectable residues of the pesticides were found.

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