Research Article

# Analysis of Pesticide Residue in Coconut Water and Tomatoes around Tirupati Region by Using Reverse Phase Ultra High Performance Liquid Chromatography

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**Abstract**

The presence of pesticide residue in primary and derived agricultural product raises serious health concerns for consumers. The present study was done to assess the level of pesticides residues by employing ultra high performance liquid chromatography with photo diode array (PDA) for identification and determined the concentration of metalaxyl in tomatoes, monochrotophos and carbofuran in coconut water around tirupati region. The results of the study showed that sample obtained were far below the maximum recommended safety limits. However presence of pesticides residues is worrisome because of possibility synergic tendencies.

**Keywords:** Tomatoes, Coconut water, Pesticides, UHPLC

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## Introduction

During the last decades, the increasing demand of food safety gave rise to research regarding the risk associated with consumption of food stuffs contaminated by pesticides. Pesticides are widely used during the growth process of vegetables and fruits even after harvest. Therefore pesticide residue analysis is of overriding importance not only for the protection of human health but also for trade and regulatory control [1]. Tomato is a basic ingredient of the Mediterranean diet and is used almost daily in several countries for home- cooked or processed as canned products. But a significant oncogenic risk has been associated to this crop considers the possibility of contamination by fungicides. Metalaxyl [n-(2,6-dimethylphenyl)-n-(methoxy-acetyl)-dl-alanine ester] is an acylalanine compound used as systemic fungicides it can be used to control diseases caused by air and soil borne peronosporales [2]. Coconut water is a clear isotonic, refreshing drink and rich source of nutrients, electrolytes, chlorides, potassium and moderate amount of sugar. Coconut water is ideal in preventing dehydration especially in children an increasing international demand for this product could be a highly positive issue. Current research on coconut water is specific uses, biochemical composition and preservative techniques. Coconut palms are very frequently attacked by various insects/pests and the total productivity is reduced hence to improve production of coconuts, the use of organophosphorus pesticides mainly monochrotophos (acts as cholinesterase inhibitor) is recommended. The injection of pesticides into trunk of the coconut trees cause contamination of coconut water and kernel by monocrotophos [3]. And coconut fruit is attacked by a number of phytophagous insects among different products used for the pest control, carbofuran belong to the chemical class of n-methyl carbamates is commonly employed in the coconut culture because it is an acetyl cholinesterase inhibitors [4] these studies indicate that the coconut water can be contaminated with pesticide residues by either way of treatment. The presence of pesticide residues in coconut water may constitute a health risk to the consumers due to their potency to cause toxicity. Hence, the pesticide residues in coconut water need to be monitored periodically from the consumer's safety point of view. UHPLC with PDA is employed for identification and determination of metalaxyl, monochrotophos and carbofuran.

## Experimental

### *Reagents*

All solvents employed throughout the study were of hplc -grade and were purchased from merk - water, acetonitrile, sodium sulfate anhydrous, sodium chloride, PSA, magnesium sulphate, ethyl acetate.

Reference standards of all pesticides were purchased from sigma aldrich (labor chemikalien). All standards were at least 98% pure. Three stock solutions containing different pesticides were prepared. 12.7 mg of carbofuran and metalaxyl was dissolved in acetonitrile and monochrotophos was dissolved in methanol. The working standard solutions were prepared by diluting the stock solution as required.

***Equipment***

HPLC instrument was a shimadzu UHPLC NEXERA X2 attached with photo diode array detector and software used was lab solutions.

## Methodology

***Method of extraction for coconut water***

The coconut water sample (50 ml) is homogenized and mixed with anhydrous sodium sulphate (50g) and extracted with ethyl acetate (200ml) in a conical flask. The contents was allowed to settle down for about half an hour and filtered through a filter paper covered by 20g of anhydrous sodium sulfate. After filtration, the extract will evaporated and re-dissolved in 5ml of acetonitrile and finally the sample was transferred into the C18 spe cartridge with acetonitrile. The final extract was made to 1 ml with acetonitrile. The cleaned samples are taken and are analyzed by a high performance liquid chromatography using pda detector [5].

***Method of extraction for tomato fruits***

One kg of unwashed tomatoes was chopped and homogenized for 5min in a laboratory homogenizer. 15g of the homogenized sample is weighed into a 50-ml centrifuge tube. Added 30ml of acetonitrile and homogenized the sample at 14000-15000 rpm for 3 min. 3g of sodium chloride was added and shaken gently, centrifuged for 3mins at 2500-3000 rpm to separate the organic layer. 16 ml of organic layer was transferred into 50ml centrifuged tube having 9g of sodium sulfate and mix thoroughly. Then transfer 8ml of extract into 15ml centrifuge tube with 1.2g of magnesium sulfate and 0.4g of psa and centrifuge for 2min at 2500 rpm. Finally 2ml of supernatant layer was taken into 15ml tube and filter through ptfe filter (0.22µm) and take 1ml of filtered solution was taken into hplc vial for analysis directly (Association of Analytical Communities [6].

***Liquid chromatographic analysis for Metalaxyl***

UHPLC analysis was performed with SHIMADZU with a model NEXERA X2 with auto injector and attached with PDA detector. The chromatographic column was Ascentis ® $c_{18}$ (250mm x4.6 i.d, 5µm film thickness) the column oven temperature was kept at $35^0$c. Flow rate of mobile phase (acetonitrile: water =50:50) was 0.5ml/min and injection volume was 10µl. Detection wavelength for detection of metalaxyl was set at 270nm. The residues were estimated by comparing retention of standard with that of the unknown or spiked samples under identical conditions.

***Liquid chromatographic analysis for Monochrotophos***

UHPLC analysis was performed with SHIMADZU with a model NEXERA X2 with auto injector and attached with PDA detector. The chromatographic column was Ascentis ® $c_{18}$ (250mm x4.6 i.d, 5µm film thickness) the column oven temperature was kept at $40^0$c. Flow rate of mobile phase (water: methanol=60:40) was 0.4ml/min and injection volume was 10µl. Detection wavelength for detection of monochrotophos was set at 280nm. The residues were estimated by comparing retention of standard with that of the unknown or spiked samples under identical conditions.

***Liquid chromatographic analysis for Carbofuran***

UHPLC analysis was performed with SHIMADZU of a model NEXERA X2 with auto injector and attached with PDA detector. The chromatographic column was Ascentis ® $c_{18}$ (250mm x4.6 i.d, 5µm film thickness) the column oven temperature was kept at $35^0$c. Flow rate of mobile phase (water: acetonitrile=40:60) was 1.00ml/min and injection volume was 10µl. Detection wavelength for detection of carbofuran was set at 272nm. The residues were estimated by comparing retention of standard with that of the unknown or spiked samples under identical conditions.

## Results and Discussion

The pesticide residues were calculated by using the following formula.

$$\text{Residue } (\mu g/g) = \frac{\text{Height or area of sample}}{\text{height or area of the sample}} \times \frac{\mu l \text{ of sample injected}}{\mu l \text{ of the standard injected}} \times \text{conc. of Standard / weight of sample x final volume} \quad (1)$$

***Results for Monochrotophos in Coconut water Sample***

The linearity of the method was determined at five concentration levels ranging from 0.1-1 ppm for monochrotophos. The regression line equation for monochrotophos was y=12325x +2661 and the regression coefficient value were 0.997 respectively. The regression data for the calibration curve showed good linear relationship over a concentration range 0.1-1ppm for monochrotophos with respect to peak area at 280nm.

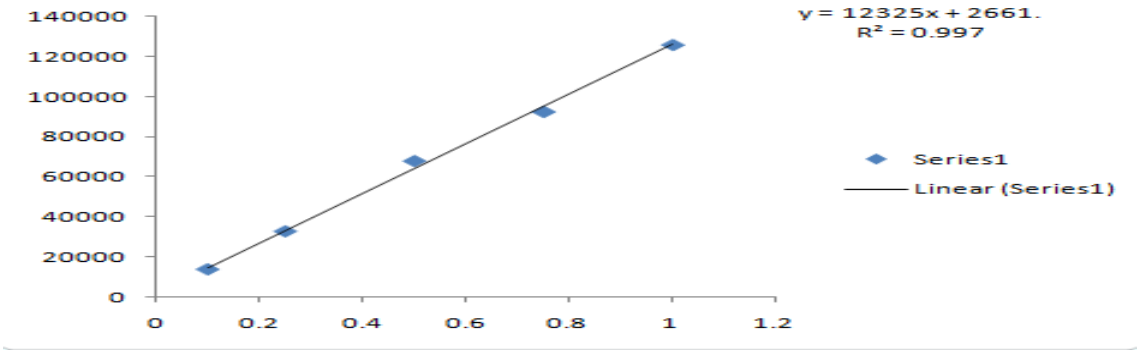


**Figure 1** Standard calibration curve for monochrotophos

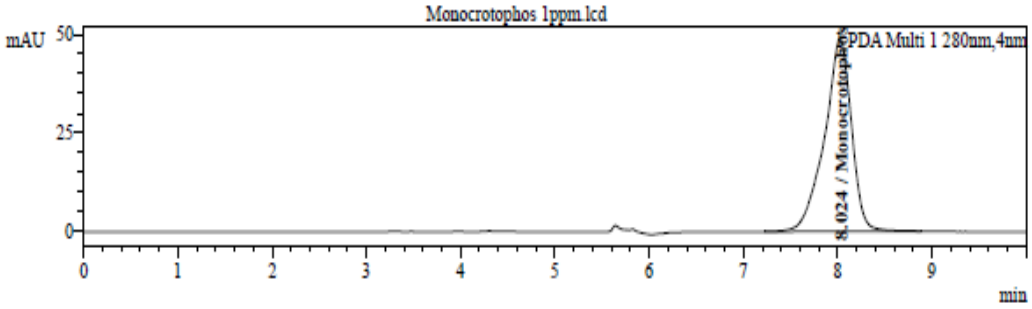


**Figure 2** Optimized concentration (1ppm) of monochrotophos

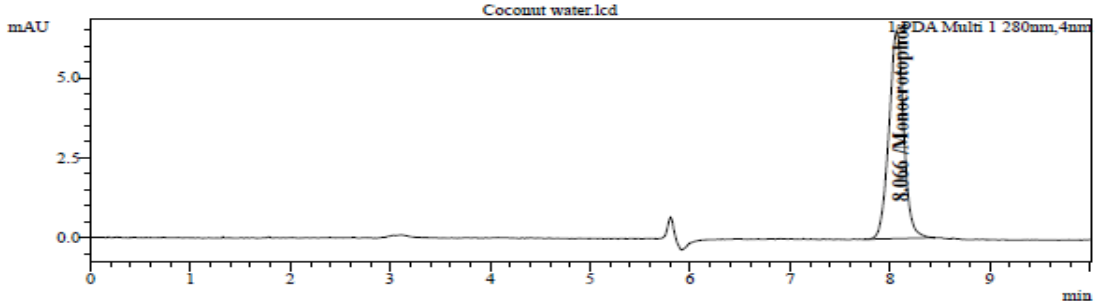


**Figure 3** Chromatogram for coconut water sample

The results showed that no interfering endogenous peaks are appeared in the chromatogram of monochrotophos hence the identification of the monochrotophos pesticide residue was performed by comparing peak retention times in the sample chromatogram to those peaks in the standard chromatogram. An analytical chromatogram of monochrotophos and its retention time is (RT=8.024) and sample chromatogram of monochrotophos and its retention time (RT=8.066). An amount of (0.00195ppm) monochrotophos was detected in the coconut sample and it was found to be below detectable levels corresponding to the MRLs. MRLs for monochrotophos was (0.02mg/kg) established by European Union pesticide data base (2010).

***Results for Carbofuran in Coconut water Sample***

The linearity of the method was determined at five concentration levels ranging from 0.1-1 ppm for carbofuran. The regression line equation for carboburan was y=47685x +600.3and the regression coefficient value was 0.996 respectively. The regression data for the calibration curve showed good linear relationship over a concentration range 0.1-1ppm for metalaxyl with respect to peak area at 272nm. Results of the present study revealed that carbofuran was

not detected in coconut water sample. Because carbamates (carbofuran) are easily decomposed.

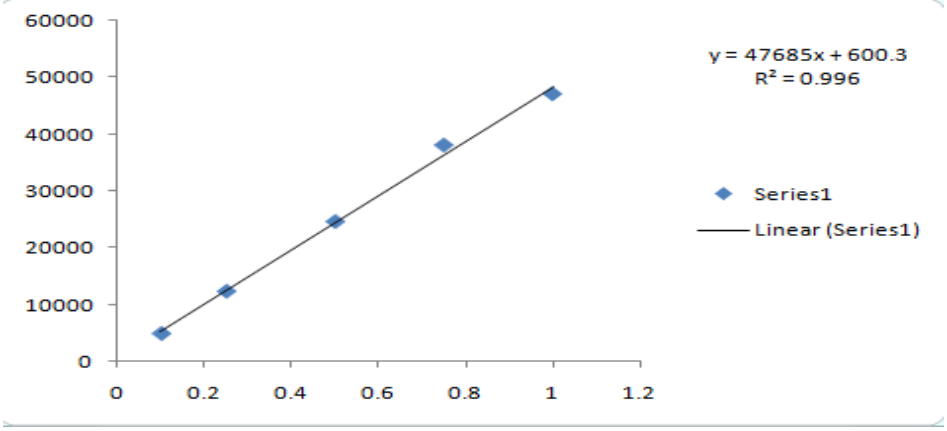


**Figure 4** Standard calibration curve for carbofuran

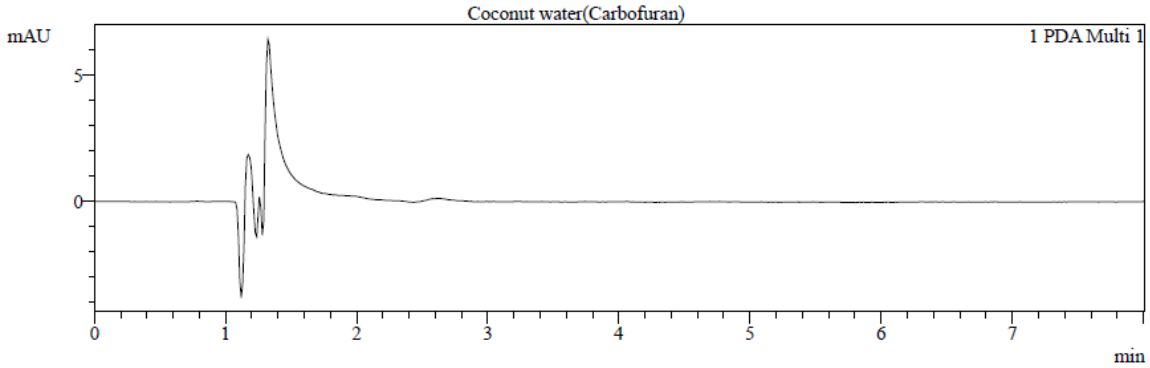


**Figure 5** chromatogram for coconut sample

***Results for Metalaxyl in Tomato Sample***

The linearity of the method was determined at five concentration levels ranging from 0.1-1 ppm for metalaxyl. The regression line equation for metalaxyl was y=88920x +11669 and the regression coefficient value were 0.999 respectively. The regression data for the calibration curve showed good linear relationship over a concentration range 0.1-1ppm for metalaxyl with respect to peak area at 270nm.

The results of the present study showed that, an analytical chromatogram of standard metalaxyl and its retention time was found to be (RT=5.750) and sample chromatogram of tomato sample and its retention time was RT=5.738). Hence the identification of the metalaxyl pesticide was performed by comparing the peak retention times in the sample chromatogram was compared with standard chromatogram. The pesticide residue level of (0.0038) metalaxyl was found and it was found to be below detectable levels corresponding to the MRLs. MRLs for metalaxyl was (0.5mg/kg) established by WHO/FAO codex Alimentarius International Food Standards.

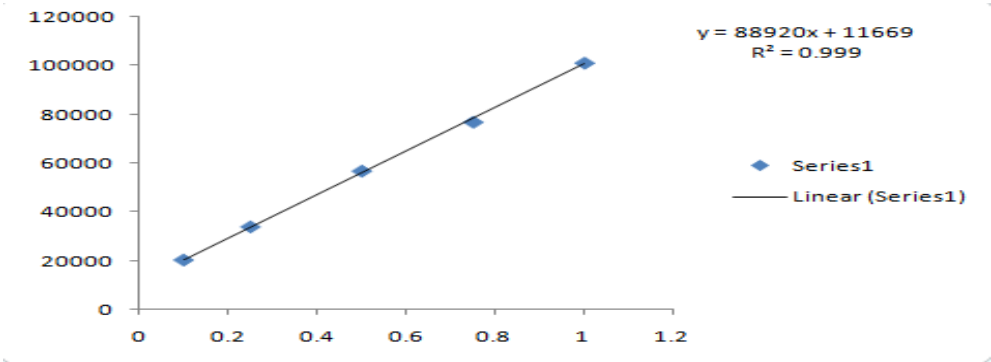


**Figure 6** Standard calibration curve for metalaxyl

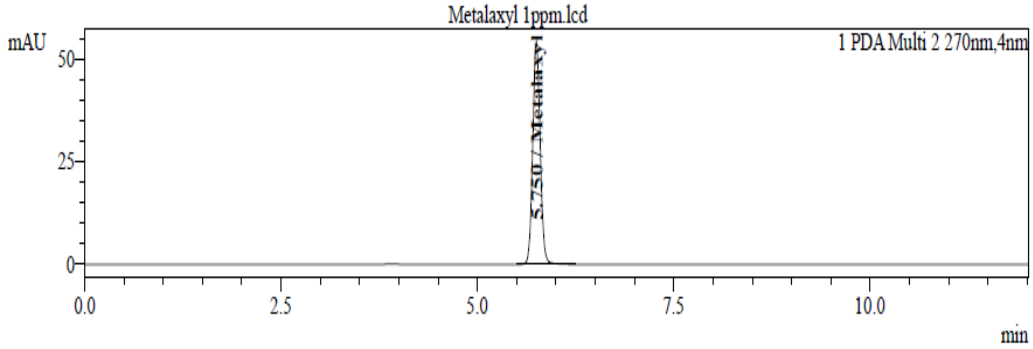


**Figure 7** Optimized concentration (1ppm) of metalaxyl

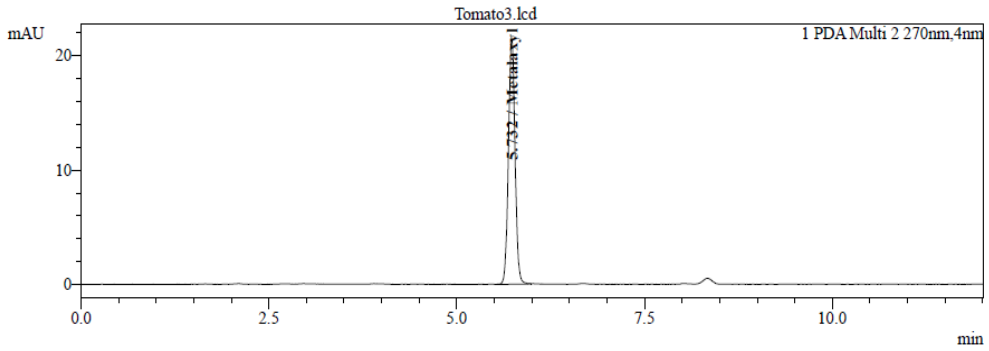


**Figure 8** Chromatogram for tomato sample

## Discussion

The use of RP-HPLC with conventional detection methods makes possible the direct, reliable, efficient and economical determination of pesticides in coconut water samples. The pesticide studies include compounds of organophosphorous and they are used frequently in coconut culture. The result indicated that detected monochrotophos(0.00195mg/kg) was found in below detectable levels corresponding to the MRLs. Carbamates (carbofuran) are easily decomposed, but due to usage in control of various pests in vegetables, fruits and in coconut culture which can translocate in coconut fruit and contaminate the coconut water. Among the most common fungal diseases in tomatoes are late blight, de berry is the most destructive disease which are controlled by usage of suitable pesticides like metalaxyl, mancozeb, captan etc., Metalaxyl is widely used around the world and it is registered for use in many countries worldwide [7].

## Conclusion

The presence of pesticide in primary and derived agriculture product raises serious health concerns for consumer. The most alarming concern is that pesticides use is very indiscriminate, vegetables and fruits growers been using the pesticides frequently to have higher yield. The wide spread use of pesticides may contaminate the environment as well as food stuffs which may pose health problems like carcinogenic, mutagenic and teratogenic effects. The analysis of the pesticide residue remains a challenge and analysis is not only for the protection of human health but also for official control purpose. Routine monitoring programs for pesticides in vegetables, food stuffs and fruits are needed to prevent, control and reduce the pollution and to minimize health risks. Many researchers have estimated pesticide residues in water, soil, foods stuffs, fruits and vegetables by using RP-HPLC although new extractions are published time to time.

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