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DETERMINATION OF PESTICIDES IN THE TOMATOES (*LYCOPERSICON ESCULENTUM*) FROM GUNTUR DISTRICT OF ANDHRA PRADESH USING A MULTI RESIDUE GC-MS METHOD

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ABSTRACT

A reliable, rapid and accurate method based on spiked calibration curves and modified QuEChERS sample preparation was developed for determination of 10 pesticide residues in Tomatoes by gas chromatography-mass spectrometry (GC/MS). The use of spiked calibration standards for constructing the calibration curve substantially reduced adverse matrix-related effects. The recovery of pesticides at 5 concentration levels (n = 3) was in the range of 80.6-112.3. The method was proved to be repeatable with RSD lower than 20%. The limits of detection and quantification for all pesticides were <10 ng/g and <25 ng/g, respectively. The developed method was used for simultaneous determination of the selected pesticides in 60 Tomatoes samples. Among the 60 analyzed samples, 41.7% of them were contaminated with pesticide residues which 31.7% of samples had pesticide residues lower than maximum residue limit and 10% of samples had residue higher than maximum residue limit.

Keywords: Pesticide; Spiked calibration curve; GC/MS; Tomatoes; Multi residue

Introduction

Tomato (*Lycopersicon esculentum Mill*) is worldwide one of the most important vegetable components of the human diet and it is consumed in nature, cooked or processed. Besides the basic nutritional properties, tomatoes contain bioactive compounds with antioxidant properties such as ascorbic acid, vitamin E, carotenoids (lycopene), flavonoids and phenolic acids that benefit human health. Nevertheless, tomato plants are susceptible to several pests and diseases that have been controlled with pesticides in different plant stadium in order to avoid significant yield losses.[1]

Tomato production has enhanced the livelihood standard of rural farmers due to its good production yield, commercial use and high rate of consumption . This vegetable is affected by many pests due to favorable weather conditions during winter season. Farmers applied excessive amounts of different types of pesticides to protect their crops. Cypermethrin, is one of the most common pesticides, which is used on tomato and other vegetables frequently. Although it is a profitable and delicious vegetable, it is highly susceptible to pests and farmers have no other option except application of pesticides to protect this crop[2]. The use of pesticides in agriculture is necessary to or processed as a canned product, Juice or paste and combat a variety of pests that could destroy crops and to pesticides are widely used in tomato because its improve the quality of the food produced. The diversity of conditions under use, the presence of residues in food that was critical which beans are grown, coupled with highly- specific elements of overall population health is unavoidable and local preferences for particular seed types or colors have pesticide residues in food is of great importance in the complicated attempts at bean improvement[3].

A very limited work has been done about pesticides residues in tomatoes and related studies about the decontamination of pesticides residues in tomatoes are scarce in Andhra Pradesh selectively in Guntur

District. It is necessary to have cheap and effective decontamination techniques and methods to reduce pesticides´ contamination in vegetable samples for developing countries like India. It is necessary to know about the present scenario of pesticides´ residues in tomatoes, which will help to improve the environment quality and to minimize the potential health risk. This study is an attempt to provide information about pesticides residues in tomatoes collected from local Markets of Guntur District, Andhra Pradesh during the period of January 2016 to April 2016. This paper presents a rapid multi-residue method of analysis based on QuEChERS extraction procedure using spiked calibration curve to simultaneously determine and confirm 10 pesticide residues in Tomatoes. The selected pesticides included GC-amenable pesticides, those for which MRL is issued by Indian Institute of Standards.

Material and Method

Chemicals

Reagents: Standard pesticides which were >98% pure were procured from RFCL, Delhi, India All organic solvents, intended for extraction, were at least AR grade and purchased from Merck India.

Standard materials: Standard pesticides which were >98% pure were procured from RFCL, Delhi, India. The standard stock solutions (100 ppm) were prepared in ethyl acetate and stored at -4°C.

Apparatus

Estimation of selected Pesticide residues were carried out on a GC (Shimadzu 2010) coupled with mass detector (Fisons MD-800, quadrupole mass detector) equipped with capillary column (Rtx-5 Sil MS, 30 m × 0.25 mm i.d. × 0.25 µm film thickness). Rotary vacuum film evaporator (Heidolph Labrota 4002) was supplied by Heidolph, Germany was used for concentration of sample. A high speed homogenizer (Heidolph Silent Crusher) was used for homogenization of Tomato sample.

Preparation of Tomato Samples

Tomato samples were collected from local markets in Guntur, During January 2016 to April 2016. Four groups (Randomly selected from four different markets) of tomato samples, 250g each, were taken for the experiment and served as the blank or spiked sample. All the samples were extracted fresh. The unit was generally more than 250 g[4]. For the analysis, only the edible portions were included, whereas bruised or rotten parts were removed. Samples of tomato, were washed, sliced into a suitable size and cooked. Vegetable samples (raw) were dry, cleaned to remove soil contamination with a disposable paper towel and blended to mace a homogeneous sample for pesticide analysis. Vegetables were washed by placing in a plastic colander and rinsed under normal tap water (25-30°c) for 30 second[5] with gentle rotation by hands and blotted dry with a paper towel. These samples were divided into two portions, of which one was analyzed as such after homogenizing in blender and other was further boiled and cooked. Boling Sliced vegetables were boiled by placing 75 ml of water in saucepan. Vegetable (50g) was added immediately to boil for 5-10 min / boiled still softness was subjected to pesticide analysis. Sliced vegetables were cooked by placing 15 ml of water in saucepan. Vegetable (50g) was added immediately to cook for 10-12 min was subjected to pesticide analysis. Washed, boiled and cooked samples were processed in a similar manner as of unprocessed samples. Commercially purchased tomato served as the blank or spiked sample. All the samples were extracted fresh. Each sample was chopped into small pieces and after quartering, a representative sample (50g) was macerated with 5-10g anhydrous sodium sulphate in Warring blender to make a fine paste. The macerated sample was extracted with 100ml acetone on mechanical shaker for 1 h by using the method of Kumari et al[6]. Extract was filtered, concentrated up to 40ml and subjected to liquid-liquid partitioning with ethyl acetate (50, 30, 20 ml) after diluting 4-5 times with 10% aqueous NaCl solution. Concentrated the organic phase up to 10ml on rotary evaporator and divide it into two equal parts. One part was kept for OC and second for OP. For OC, clean-up was carried out by using column chromatography. Column (60cm × 22mm) was packed with, Florisil and activated charcoal (5:1 w/w) in between the two layers of anhydrous sodium sulphate. Extract was eluted with 125ml mixture of ethyl acetate: hexane (3:7 v/v). Eluate was concentrated to 2ml for residue analysis. Residues of OP were also cleaned by adopting column chromatographic technique. Column was packed with silica gel and activated charcoal (5:1 w/w) in between the layers of anhydrous sodium sulphate. Extract was eluted with 125ml mixture of acetone: hexane (3:7 v/v). After concentrating the eluate on rotary evaporator, final volume was made to 2ml for analysis by gas liquid chromatography (GC). An external method was employed in the determination of the quantities of residues in the sample extracts. A standard mixture of known concentration of pesticide was run and the response of the detector for each compound ascertained. The area of the corresponding peak in the sample was compared with that of the standard. All analyses were carried out in triplicates and the mean concentrations computed accordingly.

GC/MS analysis

The GC/MS was employed with helium as the carrier gas at a constant flow of 1 mL/min. The oven temperature started at 75 °C and remained at this temperature for 3 min increasing to 120 °C at 25°C/min ramp rate and then increased to 300 °C at 5 °C/min ramp, holding at 300 °C for 11 min. Injection port was adjusted at 250 °C and splitless injection mode was used. After acquisition of the total ion chromatogram for the mixed stock standard solutions in scan mode, peaks were identified by their retention time and mass spectra. The most abundant ion that showed no evidence of chromatographic interference and had the highest signal-to-noise ratio was selected for quantification purposes.

Results and Discussion

Chlorpyrifos, diazinon, phosalone, endosulfan carbyl, were detected in 18.5, 44.4, 25.9, 11.1, 18.5, 37, and 51.8 µg/g of the total samples of tomatoes with the mean levels of 0.22, 0.059, 0.177, 0.11, 0.37, 0.28, and 0.99 µg/g, respectively (Table 1). Different amounts of the pesticide residues were observed in the whole samples of tomatoes (Tables 1). The highest level of the above pesticide residues (55.5%) was concerning the diazinon. The MRLs were established on pesticide residue by Codex Committee. The Food and Drug Administration (FDA)/World Health Organization (WHO) in 1992, which is the highest level of a pesticide residue legally tolerated in or on food or feed when pesticides are applied correctly in accordance with Good Agricultural Practice. The detected pesticide MRLs in the vegetables were generally ranged from 0.5 to 5.0 ppm. In the current study, the detected pesticide MRLs were violated and analyzed in five tomato samples (Table 1). The highest residue levels were found in the collected tomato samples in January and April. Results of the current study demonstrated that some pesticide residues such as chlopyrifos, diazinon, and permethrin in most samples were violated more than Codex MRL alimentarius. Since the current study was conducted just on pesticide residues in the fruit samples, then it is suggested that the fruit producers should regard the preharvest periods (e.g. five days for diazinon; four days for permethrin, and 13 days for chlorpyrifos) before distributing the crops into the markets. As the previous studies showed, considering preharvest period on the fruits and vegetables decreased the level of different pesticide residues[7].

Table 1: Pesticide Residue in Tomato Samples Collected from Guntur Markets

Pesticide	Residue, µg/gc	Maximum, µg/g	Minimum, µg/g	Number of Samples	Percentage of Samples	Codex MRL, Maximum Residue Limit, µg/g (ppm)
Carbaryl	0.22 ± 0.44	1.9	0.04	5	18.5	1
Diazinon	0.059 ± 0.03	0.109	0.01	12	44.4	0.1
Cypermethrin	0.177 ± 0.06	0.27	0.04	7	25.9	2
Chlorpyrifos	0.11 ± 0.07	29	0.24	3	11.1	2

a-Endosulfan	0.37 ± 0.33	0.61	0.016	5	18.5	3
b-Endosulfan	0.28 ± 0.34	0.64	0.01	10	37	0.5
Propargite	0.99 ± 1.1	1.51	0.01	14	51.8	0.05
phosalone	0.177 ± 0.06	0.27	0.04	7	25.9	2
Permethrin 2	0.11 ± 0.07	29	0.24	3	11.1	2
Quinalphos	0.177 ± 0.06	0.27	0.04	7	25.9	2

Gas chromatographic determination: Analysis was performed in the SIM mode based on the use of one target and two or three qualifier ions. Pesticides were identified according to their retention times and target and qualifier ions. The quantization was based on the peak area ratio of the targets to that of internal standard. Table 2a summarizes pesticides studied with their diagnostic and quantification ions used in SIM mode in this study.

Table 2. The retention time, diagnostic ions and selected quantification ion for the target pesticides and internal standard.

No.	Compound	Diagnostic ions (m/z)	Quantification ions (m/z)	Retention time (min)
1.	Carbaryl	144, 115.1, 145.1, 116.1	139.777	7.9476
2.	Diazinon	304, 276.1, 179,1	294.977	10.4332
3.	Cypermethrin	238.2, 166.1, 138	161.117	10.8864
4.	Chlorpyrifos	314, 257.8, 316	304.58	19.32928
5.	a-Endosulfan	236.9, 264.9, 338.9	229.793	21.3536
6.	b-Endosulfan	339.1, 264.9,236.9	229.793	22.76672
7.	Propargite	350.2, 173.1,201.1	339.694	23.9296
8.	phosalone	183.1,163.1,184.1	177.607	27.02912
9.	Permethrin 2	183.1,163.1,184.1	177.607	33.15
10.	Quinalphos	419.2, 225.1,167.1	162.087	36.85968

Method validation:

Linearity of the calibration curves: The twelve pesticides showed linearity in SIM mode. Linear spiked calibration curves for all the interest pesticides were obtained with correlation factors >0.996. The Calibration data (equation and regression coefficient) of 12 pesticides in spiked Tomatoes calibration curves is showed in Table 3.

Table3. Calibration data (equation and regression coefficient) of 10 pesticides in spiked Tomatoes calibration curves.

Compound	Equation	Regression Coefficient
Carbaryl	$y = 0.1899x – 0.016$	0.999
Diazinon	$y = 0.1482x + 0.0003$	0.999
Cypermethrin	$y = 0.6757x - 0.0012$	0.999
Chlorpyrifos	$y = 0.2803x + 0.0056$	0.998
a-Endosulfan	$y = 0.3587x + 0002$	0.998
b-Endosulfan	$y = 0.1978x - 0.0001$	0.999
Propargite	$y = 0.11797x - 0.0023$	0.999
Phosalone	$y = 0.0568x + 0.0003$	0.996
Permethrin 2	$y = 0.0965x - 0.0003$	0.999
Quinalphos	$y = 0.1351x+ 0.0006$	0.999

LOD and LOQ: Limits of detection (LOD) and limits of quantification (LOQ) of the proposed method were measured in spiked samples and calculated by considering a value 3 and 10 times that of background noise, respectively. The LODs and LOQ for all the pesticides were ≤10 ng/g and ≤25 ng/g respectively.

Accuracy and Precision: Table 4 presents the recovery and repeatability for five concentration levels of pesticides. The recovery of pesticides at 5 concentration levels triplicates was in the range of 80.6-112.3%. In terms of repeatability, the majority of the pesticides gave RSD<20%. The recoveries and repeatabilities are in accordance with the criteria set by SANCO Guideline[8].

Table 4. Average recoveries (%) and relative standard deviations (%) of pesticides obtained by GC-MS analysis of Tomatoes samples at 5 spiking levels (n=3).

Compound	Average recovery (%) (n=3)					Total Average recovery (%)	Range of RDS (%)
	30ng/g	60ng/g	150ng/g	250ng/g	350ng/g	(n=3)	
Carbaryl	102.52	104.44	113.75	79.04	88.35	97.66	5.4-16.8
Diazinon	91.79	109.80	113.95	99.28	103.63	103.73	1.8-11.2
Cypermethrin	113.75	119.21	115.67	103.93	110.00	112.53	10.1-19.9
Chlorpyrifos	76.71	81.16	91.59	83.49	85.11	83.59	0.6-24.1
a-Endosulfan	89.46	109.50	119.62	93.31	98.77	102.11	7.1-23.3
b-Endosulfan	98.37	104.03	105.35	97.35	97.76	97.76	2.9-5.6
Propargite	117.90	89.46	68.92	75.60	81.67	86.73	0.9-9.9
phosalone	75.50	85.21	86.93	78.43	81.57	81.57	3.3-9.3
Permethrin 2	106.77	120.73	122.76	107.37	109.19	113.45	1.7-22.8
Quinalphos	75.50	94.62	102.31	91.28	97.05	92.19	2.7-19.1

The major source of inaccuracy in pesticide residue analysis by GC-MS, especially in food, is related to the presence of interfering components in the sample, the so-called ‘‘matrix effect’’. In other words, in conventional gas chromatographic analysis, such as the analysis of pesticide residues in foods, co-extracted matrix components may be problematic in obtaining the true data[9].Theoretically, elimination of matrix components or active sites in the injection port would surmount the matrix-induced enhancement effect; but, complete and permanent GC system deactivation or comprehensive sample clean up is practically impossible (Schenck FJ et al 2000). There are a number of approaches for preventing, reducing, or compensating for the occurrence of matrix effects including the application of alternative calibration methods including the use of (A) matrix-matched calibration method, (B) standard addition method, (C) isotopically labeled internal standards (not feasible in multiresidue pesticide analysis due to their unavailability or high price) and (D) usage of analyte protectants[10]. In the present study, we used spiked calibration curves approach to overcome the problems caused by the matrix. In this approach, calibration curves are constructed by the addition of standard solution to blank Tomatoes samples and these samples are subjected to the same sample preparation procedure which is intended to be used for unknown samples. This way, the standard sample matrices will have the same composition as the unknown samples and therefore the effect of matrix is reflected in both standards and unknown samples. The calibration curve is constructed using these spiked calibration standards and it is easily used to calculate the concentration of analytes in unknown sample without being concerned about the matrix effects. Figure 1 represents an overlaid GC-MS-SIM chromatogram of a Tomatoes sample spiked at 100 ng/g of various pesticides contaminated Tomatoes sample including chlorpyrifos. It appears that the proposed spiked calibration curve method is a proper approach for elimination of matrix effects in pesticide residues analysis. The method could be considered as an alternative method along with matrix-matched standards, standard addition method, isotopically labeled internal standards and usage of analyte protectants, for determination of various classes of pesticide residues. It is rapid, simple, sensitive, selective and rugged. In greenhouse Tomatoes samples, Alpha-Endosulfan, Beta-Endosulfan, and Chlorpryfos were detected while in market samples.

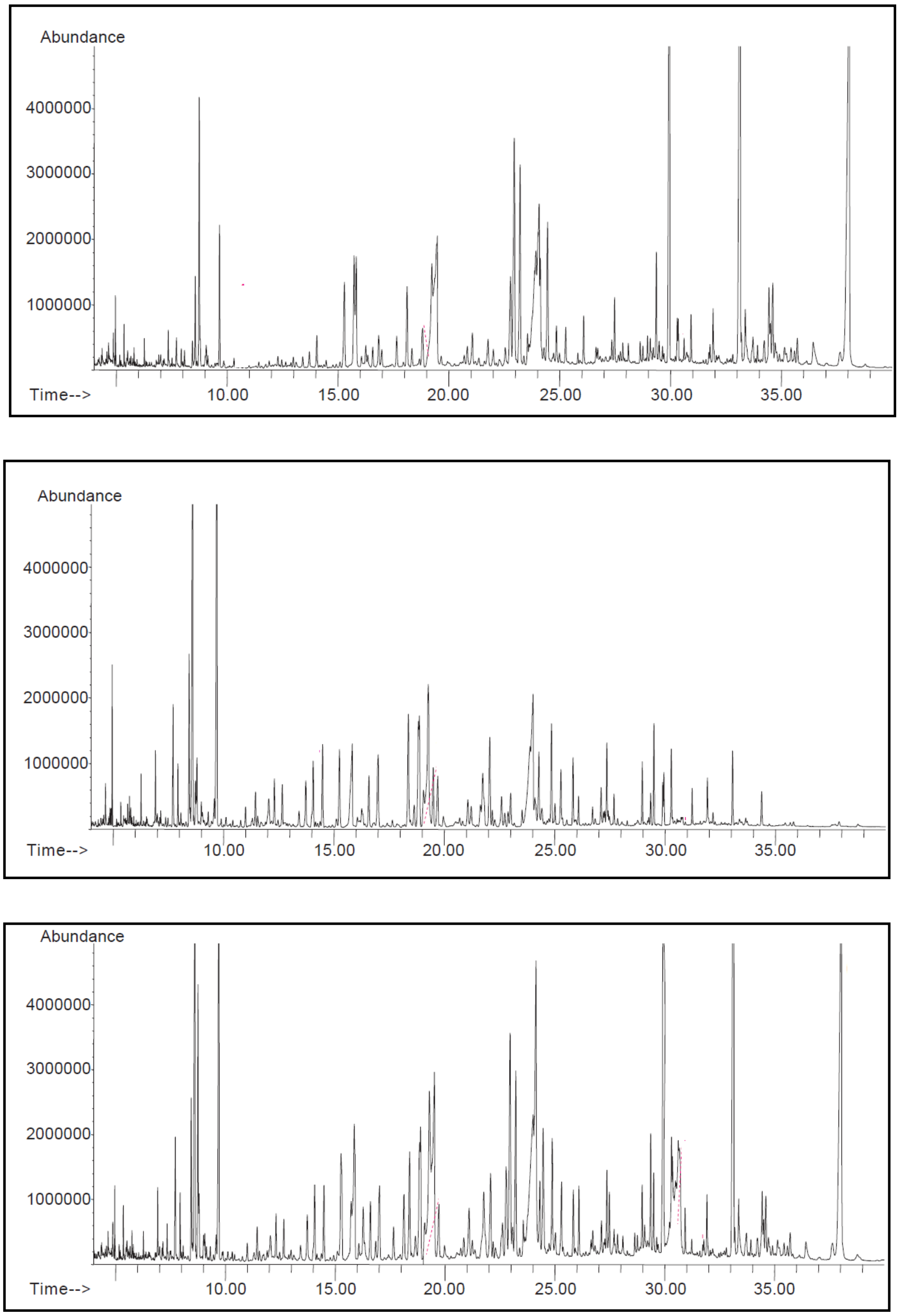

Figure 1. An overlaid GC-MS-SIM chromatograms of contaminated pesticides in Tomatoes sample (Three different types of Locations)

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