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A STUDY ON ANALYSIS OF PESTICIDE RESIDUES IN SELECTED VEGETABLES OF PRAKASAM DISTRICT, ANDHRA PRADESH, INDIA

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ABSTRACT

The aim of this study was to investigate the pesticide residues in selcted vegetables (Green beans, Bitter gourd, Lady finger, Tomato, Brinal, and Cucumber) cultivated in Prakasam district of Andhra Pradesh, India. Samples collection and preparation were carried out using standard procedures. The concentrations of all the pesticides in the vegetables samples were determined using GC/MS techinque. A total of 187 samples of six types of fresh vegetables were analyzed for the presence of 15 pesticides using gas chromatography-mass spectrometry (GC-MS) technique. Pesticide residues above the maximum residue limits (MRL) were detected in 18.18% of the samples of the samples had no residues and 82.82% of the pesticides surveyed or contained residues above the MRL. The detected and most frequently found pesticide residues were Carbofuran (13 times) and Dichlorvos (20times) followed by Chlorpyrifos-Me (16 times). The findings of this study pointed to the following recommendations: the need for a monitoring program for pesticide residues in imported food crops. The differences in residual concentrations of pesticides could be due to different agricultural practices adopted by farmers and also accessibility of the pesticides. The occurrence of pesticides in the vegetables is a major threat to human that depends on these vegetables as food. Hence, the need for continuous monitoring is recommended so as to regulate the used of this pesticide in the study areas.

Keywords: Organophosphorus Pesticide, Vegetables, Agriculture, Prakasam district, India

Introduction

Agriculture is the mainstay of Indian economy. Agriculture and agriculture allied sectors contribute nearly 22 percent of Gross Domestic Product (GDP) of India, while about 65 -70 per cent of population depends on agriculture for livelihood. The rural population in India constitutes 72.22 % of the total population and majority of this population is engaged in agriculture related activities. Agriculture in South India is primarily a subsistence production system that involves 127 million cultivators and 107 million agricultural laborers. Pesticides are largely applied to protect commercial crops. Farmers are often exposed to increased health risks through the mixing, application, and disposal of pesticides. This exposure can lead to pesticide poisoning causing short- and/or long-term health effects. India is the land of spices and over 50 million tons (8-/10%) is exported to more than 150 countries. The Indian share of the world trade in spices is 45-/50% by volume. However, of late there has been a 30% decline in exports of spices due to the presence of agricultural contaminants and pesticides. Of particular interest are residues of the chlorinated hydrocarbons. Several consignments of spices such as turmeric, sweet peppers and Chillies sent from India to different countries such as Germany, Spain and Finland have been rejected due to the presence of pesticide residues (Pesticide Safety

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Directorate, 2001). Andhra Pradesh is one of the agriculturally rich and food grains surplus states of the country.

There has been a significant increase in India's crop production since the introduction of 'New Technology'. Technological innovations (referred as 'New Technology') such as improved short duration and high yielding crop varieties, modified cultural practices like cropping patterns, optimum use of fertilizers, irrigation management, insecticides and pesticides were introduced in India during 1965-66. However, it has been observed that the crop production under HYV technology has become more sensitive to weather fluctuations than it was under the traditional methods of cultivation[1]. Pesticide is any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. They are human-made and naturally occurring chemicals that control insects, weeds, fungi, and other pests that destroy crops. Although there are benefits to the use of pesticides, some also have drawbacks, such as potential toxicity to humans and other animals. Pesticides may be chemical or biological in their origin. The chemical pesticides fall into 4 categories: Organophosphates, Carbamates, Organochlorines, and Pyrethroid pesticides. Pesticide residues refer to the pesticides that remain in diet after spraying in food crops. The concentration of pesticide residues in commodity are specified by statutory bodies in most of the countries. Most of the pesticides are chemical derivatives of chlorinated pesticides whose appearance and bioaccumulation are unsafe in the humans and environment.

In India, about 13 to 14% of the total pesticides used in agriculture are used on fruits and vegetables covering only 3% of the cropped area. Repeated application of pesticides on vegetables often results in the buildup of their residues. Surveys carried out in the country showed that 50 to 70 percent of vegetables were polluted with pesticide residues. Studies on the vegetable monitoring farm gate samples in different places revealed that they were contaminated more with OP and SP insecticides which indicate of the changes in the usage pattern from OCs to other groups of pesticides. Organophosphate insecticides have been used widely in agriculture and in household applications as pesticides due to their high insecticidal activity and relatively low persistence[2]. Organophosphorus pesticides (OPs) are one of the most common classes of chemicals used for the control of insects on vegetables because of their high efficacy and broad spectrum of activity. As a result, OP residues are likely to occur in vegetables. The inappropriate and illegal usage of OPs further increases the risk of human exposure. Therefore, it is important to determine the levels of OPs in vegetables to protect human health[3].

Research has often emphasized the need to increase the awareness of farmers about the consequences of unsafe pesticide use and the importance of communication and education programs aiming to reduction of risk. Plant root uptake of persistent residues is a common form of plant contamination. The quantity of pesticides absorbed by a given plant generally depends upon the water solubility of the pesticide, the quantity of pesticide within the soil and the organic matter content of the soil. The total amount absorbed by a single plant increases with time if the residue is persistent. For non-polar pesticides, soil organic matter is the most important soil factor influencing the sorption of residues. Government and private laboratories around the world have monitor the levels of pesticide residues in imported and locally grown agricultural produce and varying levels of pesticide residues have been reported in the produce. Significantly high quantities of pesticide residues have been reported in vegetables and fruits[4]. A study was conducted on the levels of various pesticide deposits in samples (vegetables) collected in surrounding areas of Hyderabad, Guntur, and Srikakulam from farmer's fields in harvest time during 1992-1993[5]. The HCH residues from Hyderabad samples were 0.588, 1.513 and 0.250 ppm in brinjal, chilies and spinach respectively which were beyond maximum residue limits of 0.25 mg/kg. The vegetable samples viz., tomatoes, brinjal, bhendi, cabbage, and palak samples collected in vegetable markets of Hyderabad were analyzed for pesticide residues[6]. Traces

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of HCH, DDT, aldrin, endosulfan, cypermethrin, fenvalerate and quinalphos residues were found. The main objective of this research Studies on pesticide residues in major consuming vegetables of different places of Prakasam district of Andhra Pradesh, Estimation of pesticide residues by multi residues method in vegetables and fruits by Gas chromatography mass spectroscopy and Assessment of permissibility of market samples of vegetables for the safe consumption.

Methods and Materials

All the materials used in the study and the methods of analysis have been discussed. This section has been divided into three sub-sections, which have been discussed in following text.

Methodology &Location of Vegetable samples : Sampling was carried out, from July 2014 to the last week of october 2014 and from July 2015 to the last week of December 2015, the farmer's fields of ten prominent places of Prakasam district of Andhra Pradesh., i.e., Ongole, Kandukur, Chirala, Vetapalem, Markapur, Parchur, Chinaganjam, Ardhaveedu, Inkollu and Markapur. Some parts of the study area, were suffering from waterlogging and salinity, fluoride, hardness problems. Different depths of water tables were found in the study area. Normally, in the month of October, the temperature is low and the evaporation is slow. Surveys were conducted in the above locations from 2014-2015 and the survey revealed that farmers mostly used methyl parathion and dichlorovos pesticides for the vegetables pests. Two seasons' winter and summer were utilized to carry out the residual monitoring program of seasonal vegetables .Samples were taken randomly from the field before placed for the market. Three replicates of each vegetable were collected directly from the fields in Zip lock polythene bags and stored at 4^{o}C. Sampling techniques, sample preservations and preparations were conducted following Sharma (2007)[7]. Survey revealed that mostly used pesticides in vegetables are of organophosphate group. Hence, the analysis was carried out for organophosphate group following the methodology given in APHA 1989[8].

Collection of Vegetable Samples: At each sampling sites, 20g each of the five vegetables namely, (1) **Green beans,** (2) **Bitter gourd, (3) Lady finger, (4) tomato, (5) Brinal, and (6) Cucumber** were also collected from three different locations in each area to provide replicate samples of each crop. The Vegetables samples were collected in clean polyethylene bag; labeled and transported to the laboratory and preserved in a refrigerator at 4^{O}C, pending extraction.

Extraction of Vegetable Samples: The method of extraction used for the vegetables was the USEPA method 3510 for extracting pesticide residues in non-fatty crops, using ethyl acetate as the solvent. Sodium hydrogen carbonate ($NaHCO_3$) was used to neutralize any acid that may be present and the vegetable samples were washed thoroughly with distilled water. Twenty grams (20g) of each of the samples was placed in a mortar and anhydrous sodium sulphate (Na_2SO_4) was used to remove water from the sample matrix. After weighing, the samples were washed thoroughly with distilled water and placed in a mortar and ground to a paste using a pestle. The paste was transferred into a conical flask with the help of a spatula and 40ml of Ethyl acetate was added and shaken thoroughly. A 5g portion of sodium hydrogen carbonate (NaHCO3) was added to the mixture followed by 20g of anhydrous sodium sulphate (Na2SO4) and the entire mixture was shaken vigorously for one hour. This process was to ensure that enough of the pesticide residue dissolved in the ethyl acetate. The procedure was repeated for the samples from each area and the mixture was filtered into a labeled container before being centrifuged at a speed of 1800 rpm for 5 mins. The organic layer was decanted into a container and a 1:1 mixture of 5 ml ethyl acetate and cyclohexane was added.

Cleaning up of Vegetable Extracts: The vegetable extracts were cleaned up as follows; A 10mm chromatographic column was filled with 3g activated silica gel and topped up with 2 to 3g of anhydrous sodium sulphate, and 5 ml of n-hexane was added to the column. The residue in 2 ml n-hexane was transferred onto the column and the extract was rinsed thrice with 2 ml hexane. The procedure was repeated

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for all the samples. The sample was collected in a 2 ml vial, sealed and placed in the refrigerator in the laboratory with temperature below normal room temperature, to prevent evaporation of the ethyl acetate.

Determination of Pesticide Residues : The SHIMADZU GC/MS (GC – 17A), equipped with fluorescence detector was used for the chromatographic separation and was achieved by using a 35% diphenyl, 65% dimethyl polysiloxane column. The oven was programmed as follows: initial temperature 40°C, 1.5 min, to 150°C, 15.0 min, 5°C/min to 200°C, 7.5 min, 25°C/min to 290°C with a final hold time of 12 min and a constant columnflow rate of 1 ml/min. The detection of pesticides was performed using the GC-ion trap MS with optional MSn mode. The scanning mode offer enhances selectivity over either full scan or selected ion monitoring (SIM). In SIM at the elution time of each pesticide, the ration of the intensity of matrix ions increase exponentially versus that of the pesticide ions as the concentration of the pesticide approach the detection limit, decrease the accuracy at lower levels. The GC-ion trap MS was operated in MSn mode and perform tandem MS function by injecting ions into the ion trap and destabilizing matrix ions, isolating only the pesticide ions. The retention time, peak area and peak height of the sample was compared with those of the standards for quantization.

Validation of themethod: In order to check the feasibility of the GC-MS method for the analysis of pesticide residues in fresh vegetable sample extracts, it was validated using (1) Green beans, (2) Bitter gourd, (3) Lady finger, (4) tomato, (5) Brinal, and (6) Cucumber extracts.

Identification and confirmation of target analytes: The identification of the pesticides was based on the retention timewindows (RTW) that are defined as the retention time average 63 S.Ds of the retention time when 10 blank samples spiked at the second calibration level of each compoundwere analysed. The confirmationofapreviouslyidentified compoundwasdone by comparing the GC-MS spectra obtained in the sample with another stored as reference spectrum in the same experimental conditions.The reference spectrawere obtained daily by injecting a blank selected vegitable sample spiked at the concentration of the second calibration point.

Identification and quantification: The compound was identified by comparing its retention time with respect to technical grade reference standard. The quantitative determination was carried out with the help of a calibration curve drawn from chromatographic experiments with standard solution. For quantificationan external calibration curve with four different concentrations of each pesticide, with matrix matching were made. The standard solutions for the calibration curves were prepared in controlmatrix because
samples may possess co-extractants in the matrix which may affect the peak area of the unknown samples.

Minimum Detection Limit (MDL)

To determine the maximum residue limits (MRL), retention time (Rt), limit of quantification (LOQ), ion target, qualifier ions m/z by scan mode, and perform the GC-MS quantification (Table 1) by using a four-point calibration curve plotting peak area versus mg/L concentration of 86 pesticides using the dilution levels ranged from 0.0001 to 5.00 mg/L

Table 1: Parameter of retention time, LOQ, target, and qualifier ions m/z by scan mode.

S.No	Pesticide Compounds	Retention time min	LOQ	Target ion m/z	Qualifier ions	
					Q1	Q2
1	Dichlorvos	7.211	0.02	109	185	79
2	Hexachlorobenzene	19.949	0.05	284	249	142
3	Dichloran	20.412	0.03	176	206	124
4	Dimethoate	20.694	0.02	87	93	125
5	Carbofuran	21.517	0.01	164	149	123

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6	Lindane	21.953	0.01	219	181	111
7	Fonofos	22.837	0.06	109	137	246
8	Diazinon	24.181	0.03	179	137	152
9	Dichlofenthion	26.869	0.01	279	223	162
10	Allethrin	54.515	0.04	123	181	81
11	Malathion	31.31	0.03	127	173	99
12	Chlorpyrifos-Me	27.53	0.03	286	125	288
13	Carbaryl	27.846	0.03	144	115	116
14	Chlordane-trans	35.99	0.04	373	375	237
15	Beta-endosulfan	41.82	0.03	207	239	195

RESULTS AND DISCUSSION

A multiresidue procedure was carried out to monitor the pesticide residues in a wide range of the most common consumed six types selected vegetables samples collected during two years, that is, 2014-2015. The analyzed samples composed of 6 species of vegetables, that is, Green beans, Bitter gourd, Lady finger, Tomato, Brinal, and Cucumber. A wide range of pesticide residues were detected and quantified in the analyzed samples during the two years of this study. In 2014, it was detected the residues of 15 pesticides while in 2015 it was detected the residues of 15 pesticides.

Pesticide Residues in Analyzed Samples

The level of pesticide residues of 6 vegetables and 187 samples was determined. Pesticide residues were not detected in 34 samples (18.18%), while 153 samples (87.82%) contained detectable amount of pesticide residues (Table 2).

Table 2: Number of fruit and vegetable samples without pesticide residue, and with residue below the maximum residue limits (MRL) and above the MRL

Vegitable	**Number of Samples**	Without Pesticided Residue	< MRL (%)	With Residue	> MRL (%)
Tomato	42	8	19.05	34	80.95
Green beans	26	7	26.92	19	73.08
Bitter gourd	29	4	13.79	25	86.21
Lady finger	28	5	17.86	23	82.14
Brinal	30	6	20.00	24	80.00
Cucumber	32	4	12.50	28	87.50
Total	187	34	18.18	153	81.82

Data in Table 2 shows the amounts of the detected pesticide residues in vegetable samples from different locations collected from agricultural fields of prakasam district during the year of 2014-2015. According to the detected pesticides, it is clear that there are a wide range of compounds which included insecticides, herbicides, and fungicides. The detected insecticide residues which represent the majority of the detected compounds, it was found that such insecticides could be classified chemically into their major four chemical groups, that is, organochlorines, organophosphorus, pyrethroids, and carbamates, while the organophosphorus insecticides included that is, chlorpyrifos, dimethoate, malathion and disulfoton. The corresponging GC MS spectra of various pesisied given in Figure 1 (2014) and Figure 2 (2015)

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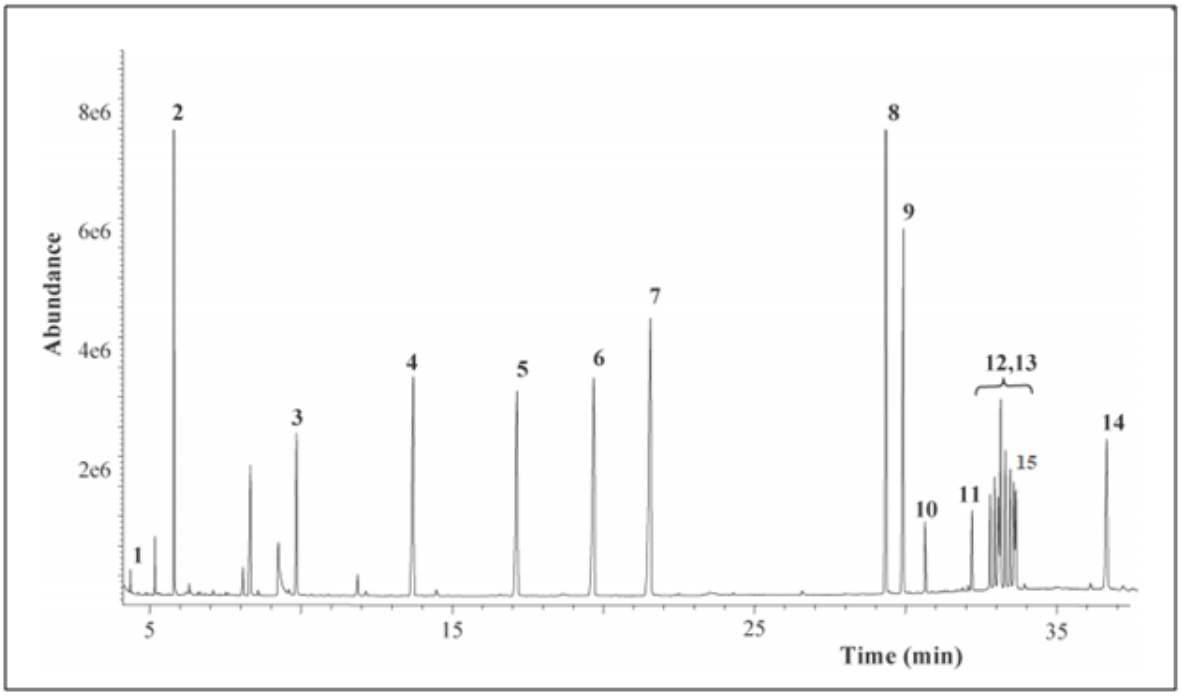

Figure 1:GC MS Scan spectra of Pesicides identified in 6 vegetable analysed in the rea 2014

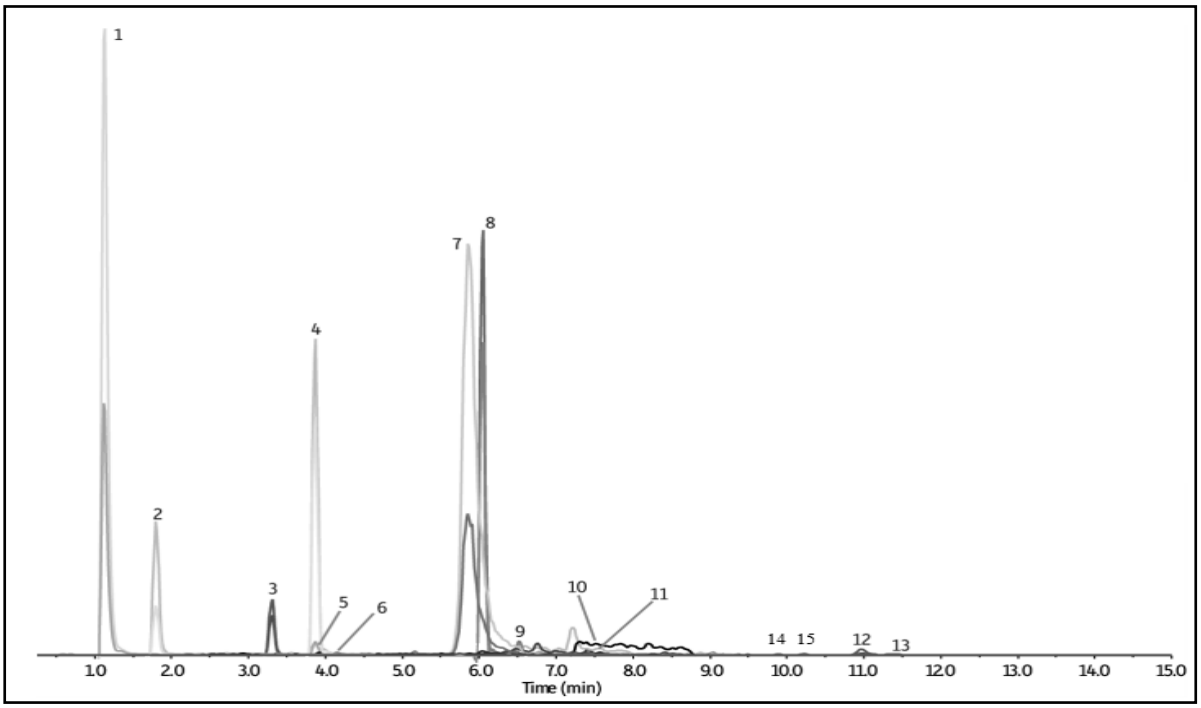

Figure 2 GC MS Scan spectra of Pesicides identified in 6 vegetable analysed in the rea 2015

Multiple Residues in Analyzed Samples

Fruits and vegetable samples containing no residue, one, and multiple residues are shown in Figure 3. Some samples contained only one pesticide residue, but 14.97 % (28 out of 187) of the samples had multiple residues of more than one pesticide found in the same sample. A single residue was detected in 3.20 % of the samples, and two, three, and four residues in 20.32%, 27.27%, and 19% of the samples, respectively. The most

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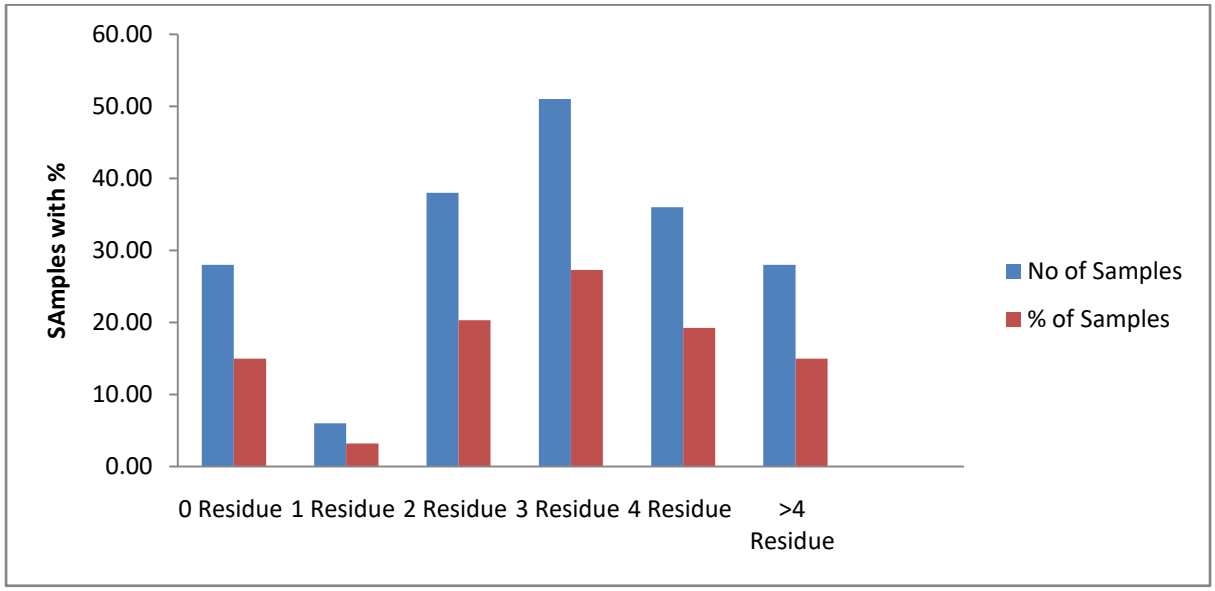
frequent combinations of two pesticides detected in the same sample were Dichlorvos and Hexachlorobenzene (14 samples), and Allethrin and Malathion (08 samples).



Conclusions

This study investigated the levels of pesticide residues in commonly used vegetables (Green beans, Bitter gourd, Lady finger, Tomato, Brinal, and Cucumber) in prakasam district . The results indicated that majority of the vegetable samples were contaminated with pesticide residues, with concentrations above the MRL. The samples no residues and contained pesticide residues at or below MRL were detected in 18.12%. Meanwhile, the detected pesticides concentration had exceeded the MRL in 69.89% of the total tested samples. From a public health perspective, the observed levels of pesticide residues pose a potential health risk to consumers. Therefore, to reduce this risk, sensitization of farmers to better pesticide safety practices and the need for continuous pesticide residue monitoring is highly recommended. However, the detected amounts of the mentioned insecticides in these important vegetables make the necessary to continue the pesticide residue monitoring programs which must be implemented to assure the minimum allowable residue levels in plant foods.

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