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SCREENING OF ORGANOPHOSPHORUS PESTICIDE RESIDUES IN VEGETABLES OF SELECTED AREAS IN GUNTUR DISTRICT

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ABSTRACT

The Concentrations of selected organophosphorus pesticide residues were determined in 15 different vegetables from ten agricultural zones in Guntur district, Andhra Pradesh, India. The concentrations of all the pesticides in the vegetables samples were determined using GC/MS technique and the highest concentrations of diclorvos, diazinon, chlorpiryfos and fenithrothion in the selected agricultural areas were observed in tomatos samples, while the lowest concentrations were observed in the bitter guard samples. The results of organophosphorus pesticides in the vegetables from the five agricultural areas were observed to be at alarming levels, much higher than the maximum residue limits (MRLs). 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, Guntur district, India.

INTRODUCTION

Agriculture is the mainstay of Indian economy. Agriculture and agriculture allied sectors contribute nearly 22 per cent 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.

The term pesticide is a general term that includes compounds used for a wide variety of purposes to control a range of living organisms. Basically they may be subdivided into two main groups' agricultural pesticides: used in agriculture, horticulture, forestry and weed killers around water courses and nonagricultural pesticides: used for wood preservation, treatment of masonry, antifouling agents and for control of insects for public hygiene [1]. 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 (OPPs) 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, OPPs residues are likely to occur in vegetables. The inappropriate and illegal usage of OPPs further increases the risk

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of human exposure. Therefore, it is important to determine the levels of OPPs in vegetables to protect human health [3]. General population is mainly exposed to organophosphorus pesticide residues through the ingestion of contaminated foods (such as cereals, vegetables, and fruits), which are directly treated with OPPs pesticides or are grown in contaminated fields. Compared with organochlorine pesticides, OPPs demonstrate relatively low environmental persistence but a higher toxicity acute. Therefore, the OPPs 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 residue limits (MRLs) [4].

Origin of the research problem

Organophosphosphate pesticide self-poisoning is an important clinical problem in rural regions of the developing world, and kills an estimated 200 000 people every year. Unintentional poisoning kills far fewer people but is a problem in places where highly toxic organophosphates pesticides are available. According to the World Health Organization (WHO), there are about 1 million people a year admitted to hospital with accidental poisoning and 2 million with suicidal intent. It is estimated that there could be as many as 25 million agricultural workers in the developing world suffering an episode of poisoning each year. The WHO figures appear to be an underestimate and a recent call was made for more reliable data to be collected

In Guntur district known agricultural zones (Figure1) are Tenali, Narakodur, Chebrolu, Mangalagiri, Pedavadlapudi, chiluvur, Bapatla, Narasaraopet, Repalle, Macharla mandalams (revenue sub divisions) and Tallayapalem, Venkatayapalem and Uddandarayapalem villages are known for their vegetable cultivation, including leafy vegetables. These villages cultivate donda, ladyfinger, onion, cauliflower, cabbage, beetroot, kanda, carrot, banana and other varieties of vegetables. Likewise, Linayapalem, Rayapudi, Borupalem and Abbirajupalem villages are known for their lime, which are exported to several districts of the state.

Figure 1 : Map showing study area (selected agri-zones in Guntur district)

The main objectives of the study were to: Determine the levels of OPPs residues in the selected vegetables grown in selected agri-zones of Guntur district. To estimate the potential health risk associated with the daily intake of the selected vegetables.

Methods and Materials

In this section, 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.

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Methodology & Location of Vegetable samples : Sampling was carried out, from July 2013 to the last week of October 2013 and from July 2014 to the last week of December 2014, the farmer's fields of ten prominent places of Guntur district of Andhra Pradesh., i.e., surroundings fields of Tenali, Narakodur, Chebrolu, Mangalagiri, Pedavadlapudi, chiluvur, Bapatla, Narasaraopet, Repalle, Macharla . Some parts of the study area, were suffering from waterlogging and salinity, 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 2013-2014 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) [5]. 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 [6].

Collection of Vegetable Samples: At each sampling sites, 100g each of vegetables namely, Tomotos (S1), Lady Finger (S2), Cauliflower (S3), Bitter guard (S4), Snake guard (S5), Brinjal (S6), Spinach (S7), Pumpkin (S8), Cucumber (S9), Gherkin (S10), Green Chilli (S11), Cluster Beans (S12), Ridgegourd (S13), Broad Beans (S14) and Sorrel Leaves (S15) 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 ($NaHCO_3$) was added to the mixture followed by 20g of anhydrous sodium sulphate (Na_2SO_4) 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 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.

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Pesticides

Six different pesticdes Chlorpyrifos, Diazinon,Fenitrothion, Monocrotophos, Dimethoate, Dichlorvos of common OPI's to be were investigated standard of these compounds were obtained from AGRIPEC COMPANY. Stock solutions were prepared containing 100 mg·L−1 of each compound investigated. Working standards were prepared by serial dilution. Final concentrations (in acetonitrile) ranged to 0.1, 0.25, 0.50, 1.0, 3.0, 7.0, and 10.0 mg/L for each analyte.

Determination of Pesticide Residues [9]

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^{O}C, 1.5 min, to 150 ^{O}C, 15.0 min, 5 ^{O}C/min to 200 ^{O}C, 7.5 min, 25^{O}C/min to 290 ^{O}C with a final hold time of 12 min and a constant column flow 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.

Results and Discussion

Method Validation

Method validation (for the orange juice) was carried out using the following parameters: limit of detection (LOD), limit of quantification (LOQ), linearity, precision and recovery. For observing the LOQ calculated as 3·LOD, see Tables 1 and 2.

Table 1: Parameters of validation of the method.

Insecticides	Linear range (µg/L)	r^2	LOD (µg/L)	LOQ (µg/L)
diazinon,	0.3–2.5	0.9994	0.23	0.69
fenitrothion	0.3–2.5	0.999	0.26	0.78
Monocrotophos	0.3–2.6	0.9973	0.31	0.93
Chlorpyrifos	0.3–2.5	0.9998	0.25	0.75
Dimethoate	0.3–2.5	0.99875	0.29	0.87
dichlorvos	0.3–2.5	0.9987	0.301	0.903

Table 2: Precision and recovery tests to selected vegitable (Tamota/Brinjal)

Insecticides	Precision (%)	Recovery (%)
diazinon,	10	108.5 ± 9.30
fenitrothion	9.5	52.1 ± 8.86
Monocrotophos	9.8.0	57.0 ± 11.8
Chlorpyrifos	8	49.5 ± 2.48

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Dimethoate	9	108.5 ± 9.31
dichlorvos	8.5	52.1 ± 8.87

Regression analysis was studied to approximate the linearity of the calibration curves. Good determination correlation coefficients were obtained for all of the compounds ranging from 0.9994 to 0.9999 (Table 1)

The limits of detection (LOD) for four insecticides were based on the lowest detectable peak that had signal/noise = 3. The results are also listed in Table1. The obtained values were satisfactory and allow the determination of these compounds at the levels required to method validation Selectivity or the existence of potential interferences in the chromatographic determination of the pesticides in the vegitable samples was monitored by running control of blank samples in each calibration. The absence of any chromatographic components at the same retention times in the target insecticides suggested that no chemical interferences occurred. To evaluate the precision of the measurement, the reproducibility of the method was determined by performing the measurement 5 times for each spiked orange fruit samples for a 3 day period. The RSD values were found to be typically below 10%. The solutions of orange fruit spiked with different amounts of target analyte were also analyzed to evaluate the recovery. The results are listed in Table 2. The recoveries and reproducibility (RSD) varied from of 49.5%–108.5% and 8.0%–10.0%, respectively, and were considered acceptable, indicating satisfactory accuracy and precision of the proposed method for determination of 2,4-dichlorophenol, chlorpyrifos, parathion-methyl, and malathion in orange fruit. These values are similar to the recoveries reported by other authors for the MSPD extraction of several pesticides from vegitable samples[10]

Chromatography Analysis

The most frequently used detectors for pesticide residues analysis include ECD, NPD, FPD, and MSD. The most frequently used detectors for pesticide residues analysis include ECD, NPD, FPD, and MSD. However, it is well known that ECD has been the most used detector in pesticide residues analysis due to its high sensitivity, in particular to halogenated pesticides although all kinds of electron-attracting functional groups such as nitro groups and aromatic structures also give a response on this detector. Figure 2 shows a chromatogram of insecticide standards analyzed by our GC-ECD system. One can observe four peaks of the compounds studied in this work, appearing at distinct retention times. Figure 2 shows a typical gas chromatogram of an Tomota extract with a 1 $mg{\cdot}L^{-1}$ of the pesticide. This chromatogram also exhibits other peaks (not identified), and one can notice the complexity of the tomoto components.

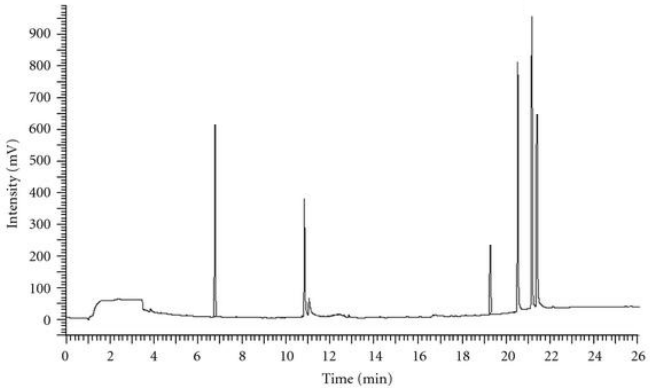

Figure 2: Chromatogram of insecticide standards (1.0 $mg{\cdot}L^{-1}$) in acetonitrile. Peaks from left to right Chlorpyrifos, Diazinon,Fenitrothion, Monocrotophos, Dimethoate, Dichlorvos

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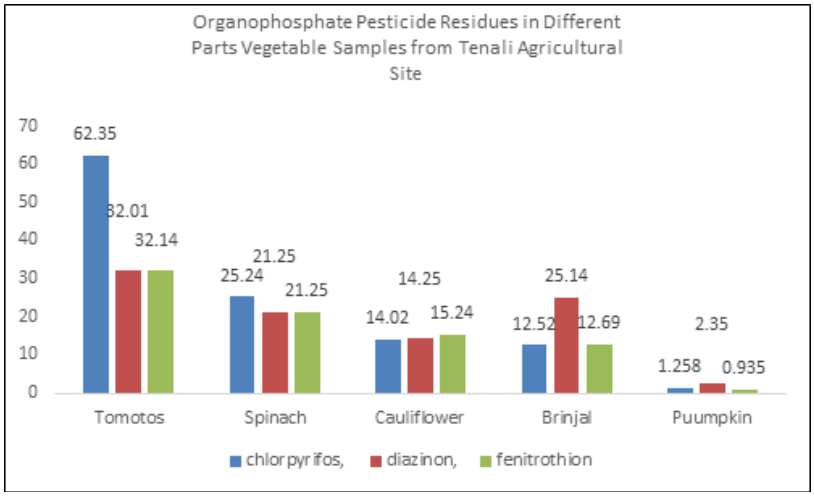
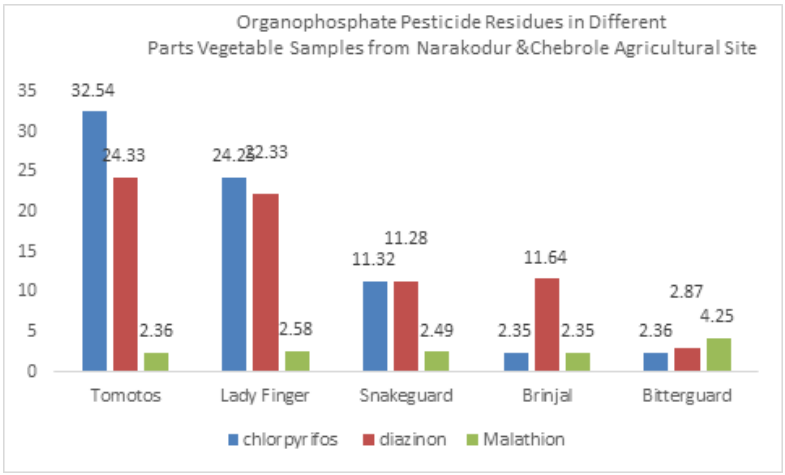
Determination of Real Samples

The optimized experimental conditions were applied to real samples to evaluate the efficiency of the method in the determination of each compounds investigated. The results are shown in Table 4. Average concentrations of the chlorpyrifos is observed in higher concentrationsin tomotos of all areas in the study area. Next in concentration diazinon in spinach, Lady, monochrotophos in cauliflower. The levels of pesticides residues are controlled by Maximum Residue Levels (MRLs), which are established by each country. In India the MRLs are established by ANVISA through the Program for Analysis of Pesticide Residues in Food (PARA), started in 2001, which monitors the levels of pesticides in fruits, vegetables, and grains consumed by Indian. Since not all the ANVISA's data for these insecticide residues were available during the fruit studies, it was compared with the MRLs established by the European Union (EU) and US. Different MRLs specific values are given in Table 4 for each fruit/vegetable, and the reason for it is perhaps the quantity of daily intake in average.





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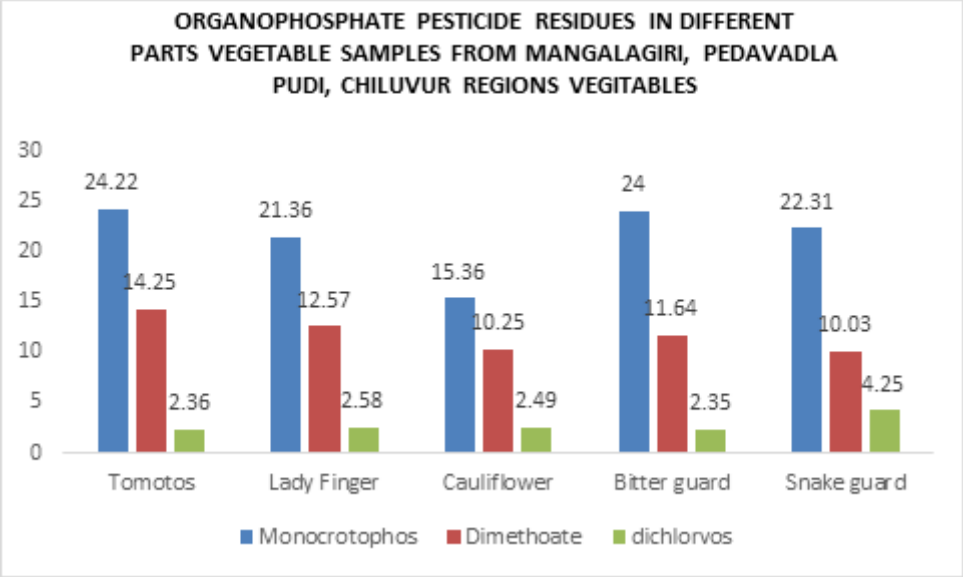

Table 4: Average distribution of the pesticide residues in vegitanle samples.

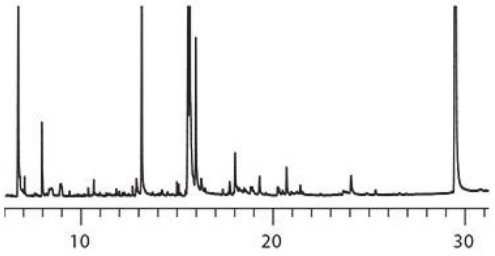

Figure 3: Chromatogram of pesticides present Bitter guard samples collected from various locations.

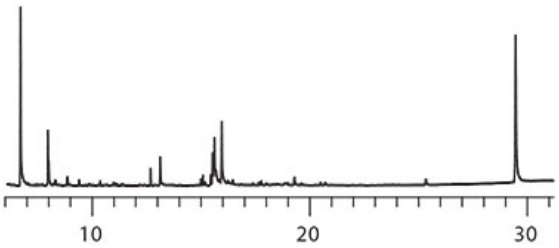

Figure 4: Chromatogram of pesticides present Brinjal samples collected from various locations.

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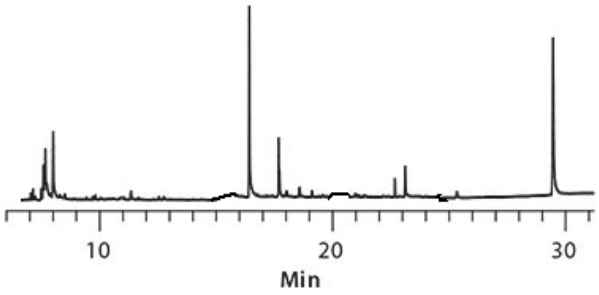

Figure 5: Chromatogram of pesticides present Cauliflower samples collected from various locations

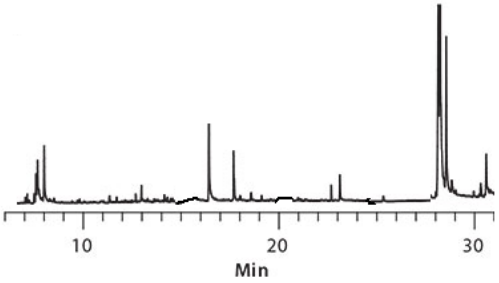

Figure 6: Chromatogram of pesticides present Lady Finger samples collected from various locations

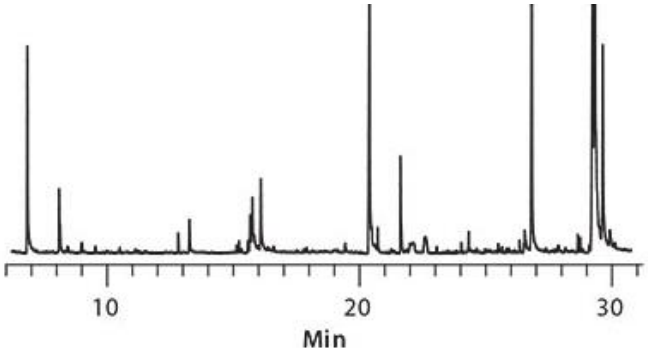

Figure 7: Chromatogram of pesticides present Pumpkin samples collected from various locations

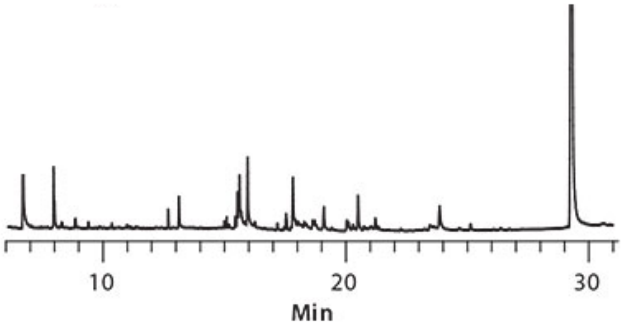

Figure 7: Chromatogram of pesticides present Snakeguard samples collected from various locations

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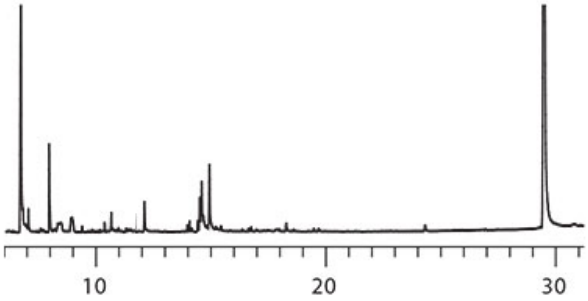

Figure 7: Chromatogram of pesticides present Spinach samples collected from various locations

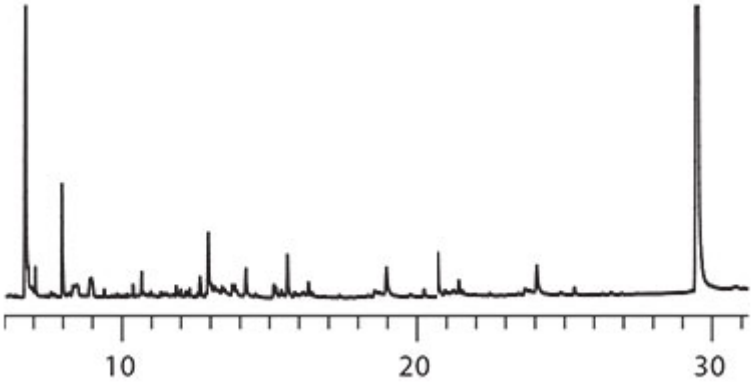

Figure 7: Chromatogram of pesticides present Tomotos samples collected from various locations

Conclusions

Organophosphorus pesticide residues were detected in all the vegetable and soil samples collected from Guntur agrizones. The concentrations of all the pesticides were observed to be higher in the tamoto and leaf of all the vegetable samples studied, while the root had the lowest concentrations in the study areas. The concentrations of all the pesticides in the vegetables from the three agricultural zones were much higher than the European Union (EU) set maximum residue limits (MRLs) and acceptable daily intake values (ADIs) set for vegetables by ISI. Based on the results of this study, Government should enforced routine monitoring of pesticide residues in these study areas so as to prevent, control and reduces environmental pollution. The farmers and the inhabitants of the study areas should be educated on the dangers of pesticides for pest control.

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