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Determination of Pesticides in Fruits and Vegetables using Acetonitrile Extraction and GC/MS Technique

Zaib Hussain[a]*, Samia Siddique[b]
[a]Institute of Chemistry, University of the Punjab, Lahore, Pakistan
[b]Department of Chemistry, Government College University, Lahore

Abstract: A selection of oranges, guava and spinach were purchased from the local markets of Lahore, Pakistan. Extraction of the samples was carried out using an acetonitrile/toluene extraction procedure to determine the residual concentration of pesticides which may have been used during seasonal growth. Gas chromatography/mass spectrometry (GC/MS) was used for quantitative and confirmatory analysis of GC-amenable pesticides. The data obtained was compared with that of a referenced pesticide by matching molecular weight to a library of known pesticides. For comparison the same non-sprayed fruits/vegetable were extracted as a control reference. Factors studied were rentention time, elution time, relative abundance and molecular weight. The results showed that low molecular weight pesticides were eluted before the high molecular weight pesticides. Pesticides identified included crotoxyphos, fenoxycarb and methoxyfenozide.

Keywords: GC/MS, acetonitrile, extraction, pesticides, retention, elution.

Introduction:

Pesticides are very hazardous and lethal for organisms as well as for humans. They present danger to consumers, bystanders and workers during manufacture, transport or, during and after use. Pesticides are also toxic to plants and many food crops, including fruits and vegetables, contain pesticide residues after being washed or peeled. Pesticides decrease the biodiversity in soil and it has been found that the quality of soil is higher in the absence of pesticides with the additional effect of higher water retention[1].

A number of pesticides are highly toxic and even in very small quantities these pesticides can result in the death of humans and animals, while exposure to a sufficient amount of almost any pesticide can initiate long-term illness. Statistics show a 70 % increase in the risk of developing Parkinson's disease for individuals exposed to low levels of pesticides[2]. Long-term health problems such as respiratory, memory disorders, dermatological conditions[3,4], cancer[5], depression, neurological deficiencies[6,7], miscarriages and birth defects[8] have been known to be associated with pesticide exposure. Depending upon the duration of exposure of pesticides, short-term adverse, acute and chronic health effects can occur. Acute health effects include stinging eyes, rashes, blisters, blindness, nausea, dizziness, diarrhea and death, whereas chronic health effects include infertility, developmental toxicity, immunotoxicity and disruption of the endocrine system.

Children have been found to be especially susceptible to the harmful effects of pesticide exposure. The major source of pesticide exposure in children and infants is through diet. Early exposure results in brain cancer, leukemia and birth defects. However, children who have adopted an organic diet[9] are less exposed to the harmful effects of organophosphorus pesticides.

1-For Proof and Correspondence: **Zaib Hussaina**

Due to this the use of organophosphorus pesticides has been increased as they are less persistent and damaging to the environment when compared to organochlorine pesticides[10].

The most common pathway for pesticides to enter the body is orally, through the mouth and the digestive system, dermally through the skin, or by inhalation through the nose and respiratory system. Oral exposure may occur as a result of negligence, eating without proper hygiene after using pesticides, exposure to spills while mixing, and consumption of foods that have been sprayed with a pesticide. The extent of exposure depends upon the oral toxicity of the material and the amount consumed. Dermal exposure accounts for nearly 90 percent of the exposure pesticide users receive from non fumigant pesticides. This can occur whenever a pesticide is mixed, applied or handled, and it often goes undetected. Dry materials, dusts, wettable powders and granules, as well as liquid pesticides can be absorbed through the skin. Inhalation exposure results from breathing pesticide vapors dust or spray particles. It can also occur by breathing smoke from burning containers; breathing fumes from pesticides while applying without protective equipment, inhaling fumes during the mixing and pouring of pesticides and smoking tobacco products[11].

As pesticides are hazardous and toxic to human health, any pesticide residue remaining in fruits and vegetables can pose danger to humans and cause certain diseases. It is important to identify and quantify the pesticides which can be ingested by fruits and vegetables after pesticide spray.

Samples of fruits and vegetables with and without pesticide spray were examined for the determination of pesticide residue left after extraction. The solvent used for the extraction procedure was acetonitrile. After extraction, analysis was performed on GC / MS for the determination of concentration of each pesticide in the sample. The retention and elution time of each pesticide was determined alongwith the relative abundance which was calculated from the GC/MS spectra. The identity of each pesticide was then further confirmed by comparing molecular weights with those cited in the database library for GC/MS.

Materials and Method

The following analytical grade chemicals were used for each experiment:

Acetonitrile

Acetone

Buffer solution (phosphate)

NaCl

Na_2SO_4

Toluene

Method

For each fruit sample 20g was taken to which 20ml of distilled water was added. The mixture was left to stand for 15 minutes, after which 50ml of acetonitrile was added and the sample was homogenized by crushing in a pestal and mortar. The sample was then filtered by suction. To the remaining residue on the filter paper, 20ml of acetonitrile was added and again the sample was homogenized and filtered by suction. Both filtrates were combined together and the volume was increased to 100ml by the addition of acetonitrile. From this solution 20ml of sample was taken to which 10g of NaCl and 20ml of 0.5 mol/L of phosphate buffer (pH 7.0) was added and shaken. The solution was left to stand to allow for removal of the aqueous layer.

The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was dried at 40°C from which 2ml were added to a 3:1 mixture of toluene/acetonitrile.

Analytical Technique

For analysis, Gas Chromatography Mass Spectroscopy (GC/MS model QP 2010, Shimadzu) was used under the following conditions.

The solution obtained from the extraction step was applied on the column prepared from graphite carbon/aminopropylsilianized silica gel layered mini column (500mg/500mg) conditioned with 10ml of acetonitrile / toluene (3:1). The column was eluted with 20ml of acetonitrile/toluene (3:1) and the entire volume of effluent was collected. The effluent was concentrated to 1ml or less at 40^0 C or lower. The solution was concentrated by adding 10ml of acetone and again concentrated to 1ml at 40^0C or lower. By adding 5ml of acetone the solution was concentrated to dryness. A mixture of acetone / n-hexane (1:1) was added to the residue to make the volume up to 1ml.

The same procedure was performed for the fruit samples without pesticides and extracted residues from both experiments were analysed by GC/MS for analysis and determination of pesticides present.

Results and Discussion

From the spectra of experiments conducted it can be seen that a large concentration of pesticides is ingested and absorbed into the fruits which have been exposed to pesticides spray. The spectra of non sprayed fruits allowed for the elimination of any environmental factors which may have caused pesticide absorption in these fruits. Environmental factors do not contribute for the absorption of pesticides into those fruits which are not sprayed with the pesticides in the vicinity of those sprayed with pesticides. The amount of pesticides which have been left in the fruits after being sprayed causes harmful health effects. Therefore, it is required to determine the amount of pesticides left in fruit samples after being sprayed.

In the experiments the pesticide residues in fruit and vegetable samples were determined and confirmed from their molecular weights. The molecular weights of pesticides were compared with that given in the data bank of GC/MS for further confirmation of pesticides identified. From the elution time of pesticides on spectra it was found that elution time of the low molecular weight pesticides were lower than the high molecular weight pesticides.

The relative abundance of each pesticide was determined from the height of peaks in the spectra. Peaks were only shown in the spectra of fruits sprayed with pesticides. Glyophosphate was the common pesticide found in all sprayed fruit samples. Other pesticides found in orange, guava and spinach samples are presented in Tables I, II and III respectively alongwith their retention times, elution times, relative abundance and molecular weights.

Table I: Pesticides identified in orange by GC/MS

Pesticides Identified	Retention Time	Elution Time	Relative Abundance	Molecular Weight
Glyophosphate	3.425	2.806	1-2%	169
Methoxy fenozide	19.433	19.575	10%	368
Fenoxycarb	19.017	18.106	30%	300
Flumioxazin	19.675	19.400	2%	355

Table II: Pesticide identified in guava by GC/MS

Pesticides Identified	Retention Time	Elution Time	Relative Abundance	Molecular Weight
Glyophosphate	3.100	3.139	1-2%	169

Table III: Pesticides identified in spinach by GC/MS

Pesticides Identified	Retention Time	Elution Time	Relative Abundance	Molecular Weight
Piperonylbutoxide	16.275	17.661	2-3%	338
Crotoxyphos	17.283	17.901	3-4%	325
Glyophosphate	3.175	3.165	2-3%	169

The spectra of fruit and vegetable with and without pesticide spray are shown in Figures 1, 2 and 3. Figure 1 (a-e) shows the GC/MS spectra of orange sample with and without pesticide spray. Figure 2 (a-c) shows the GC/MS spectra of guava sample with and without pesticides spray. Figure 3 (a-d) shows the GC/MS spectra of spinach sample with and without pesticides spray. Each Figure also shows the pesticides identified in the respective sample.

GC/MS Spectra of Orange Sample

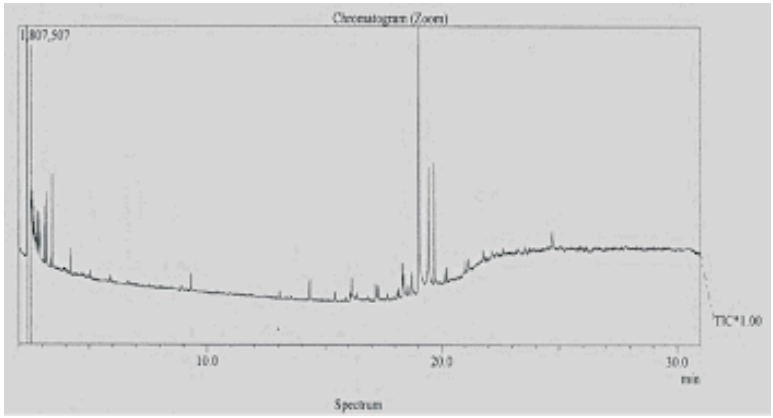

Figure 1(a): With Pesticides

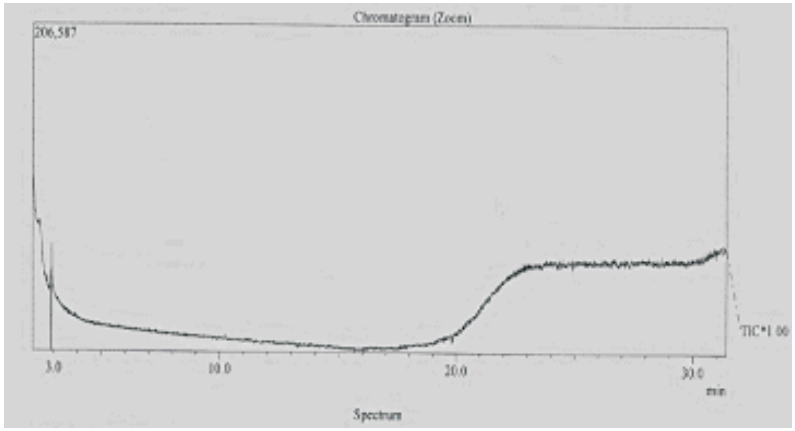

Figure 1(b): Without Pesticides

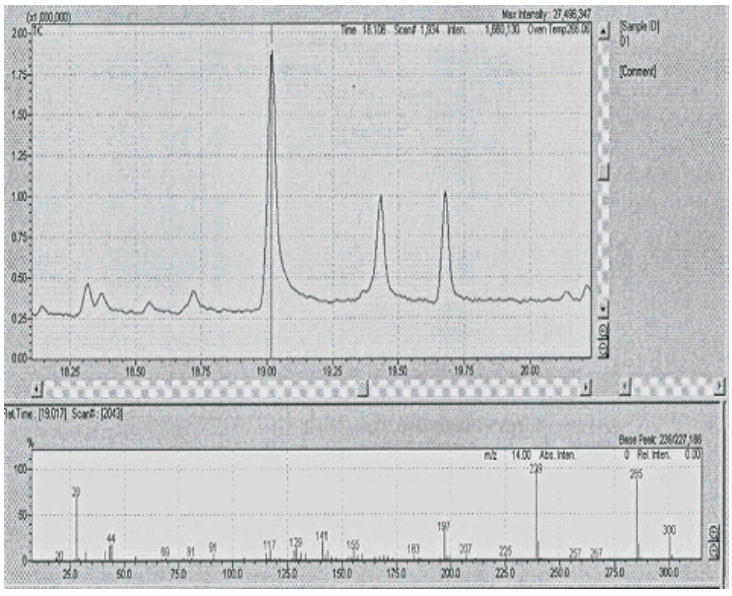

Figure 1(c): Identified Pesticide Fenoxycarb

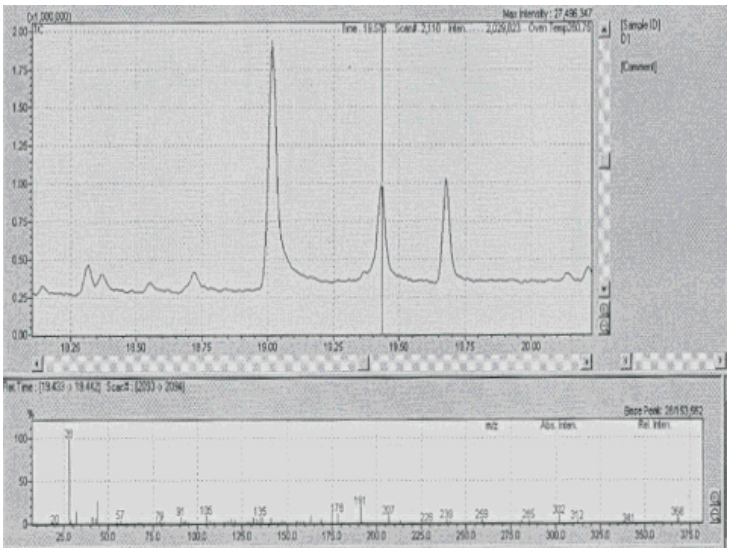

Figure 1(d): Identified Pesticide Methoxyfenozide

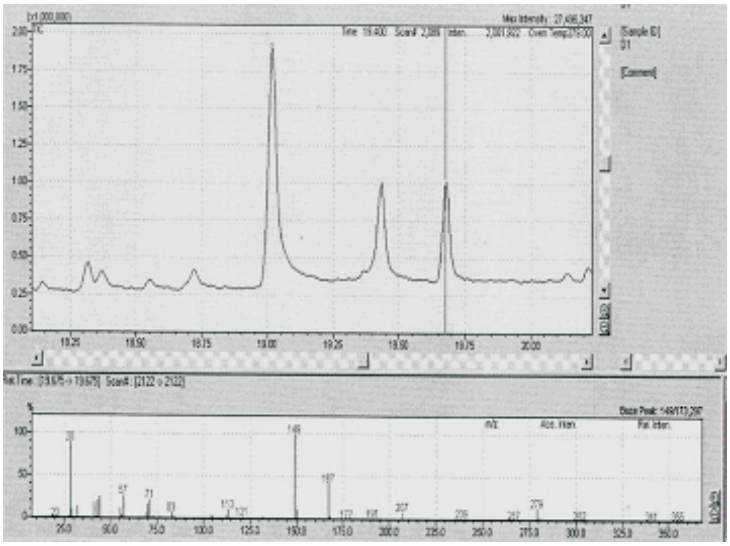

Figure 1(e): Identified Pesticide Flumioxazin

GC/MS Spectra of Guava Sample

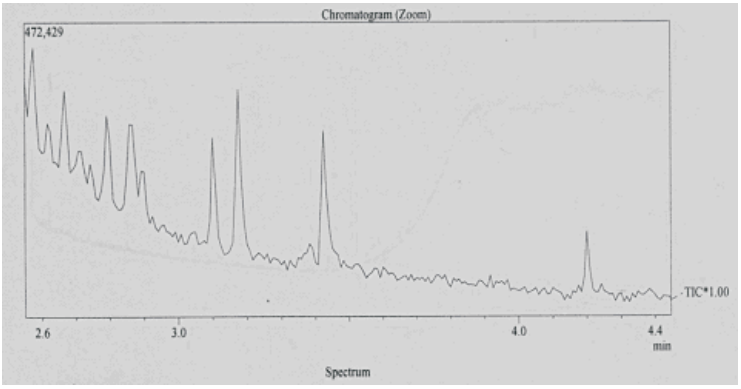

Figure 2(a): With Pesticide

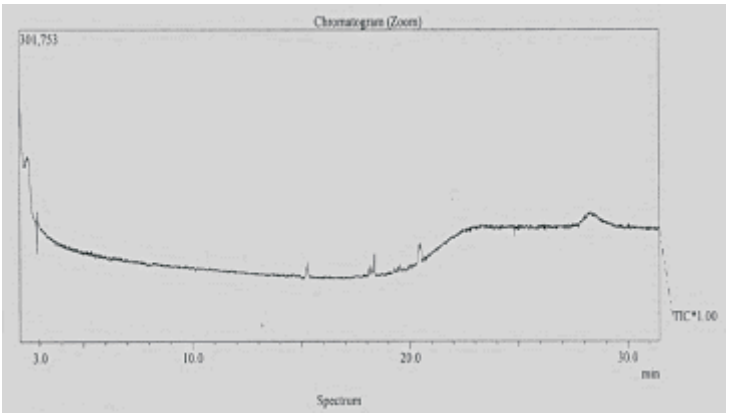

Figure 2(b): Without Pesticide

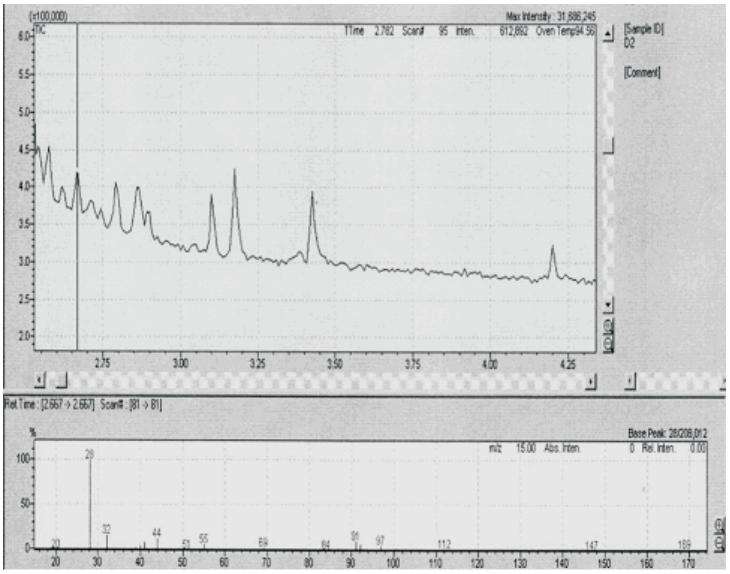

Figure 2(c): Identified Pesticide Glyophosphate

GC/MS Spectra of Spinach Sample

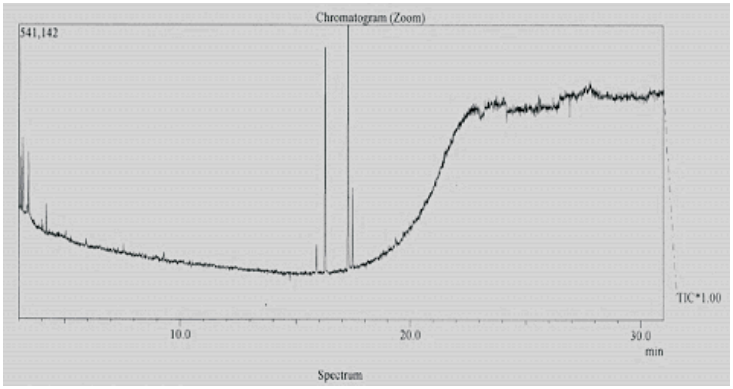

Figure 3(a): With Pesticides

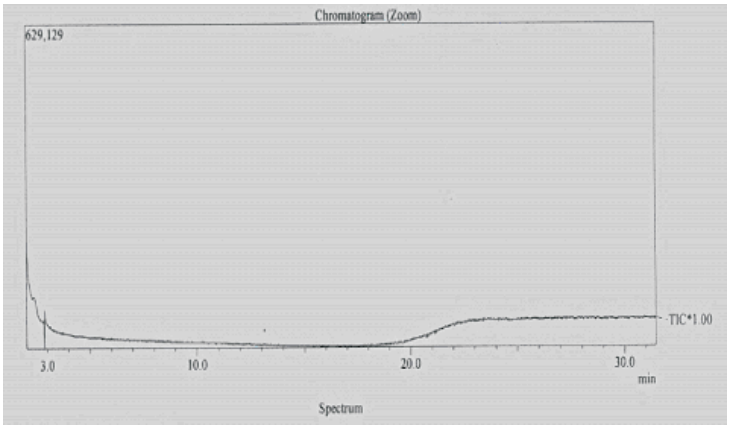

Figure 3(b): Without Pesticides

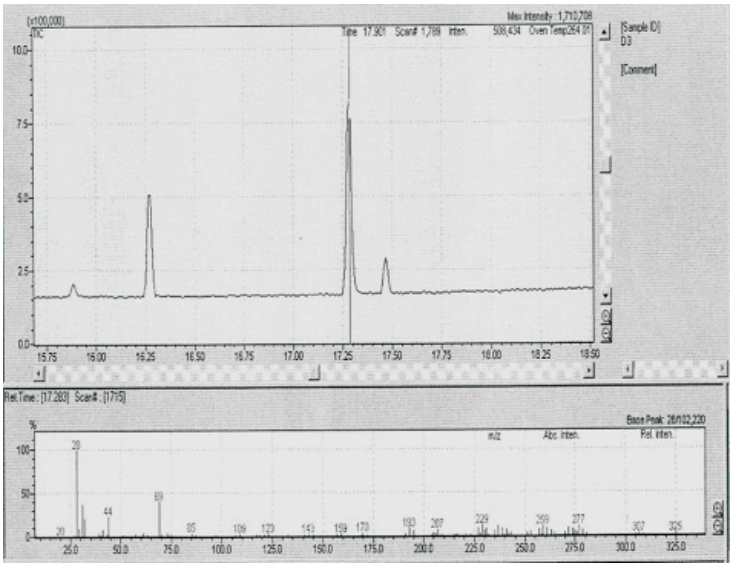

Figure 3(c): Identified Pesticide Crotoxyphose

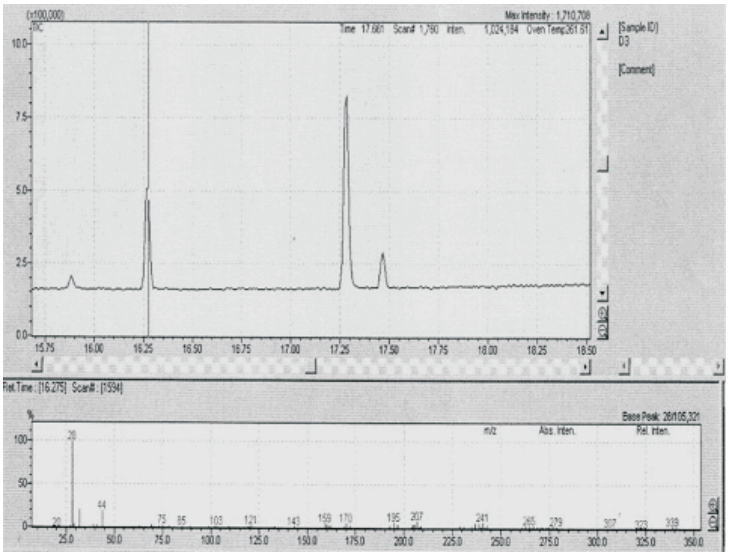

Figure 3(d): Identified pesticide Piperonyl butoxide

Piperonyl butoxide (PBO), a pesticide synergist, does not possess pesticidal properties. As PBO is a potent cytochrome P450 and a non-specific esterase inhibitor, it can act as a principal detoxification pathway for many pests. This mechanism allows for the higher unmetabolised systemic concentrations of the active insecticide to remain within the target species for a prolonged period of time. Crotoxyphos is an organophosphorus insecticide (OPs) and chiral compound which is mainly used for fly control. In excessive amounts it can lead to cholinesterase inhibition which may result in acetylcholinesterase inhibiton resulting from enantioselectivity of the chiral OPs in non-target organisms[12]. Glyophosphate was found to be common in all three samples. Its use as a broad spectrum systemic herbicide for killing weeds allows for its absorption to be enhanced through the leaves or the stumps of trees. Its mode of action is only effective on actively growing plants since it inhibits an enzyme involved in the synthesis of the aromatic amino acids; tyrosine, tryptophan and phenylalanine. As the samples were taken at random the recovery rates of Glyophosphate were much less than those reported for fortified samples[13]. The higher residual concentration of fenoxycarb can be attributed to the insecticidal activities of this early Juvenile Hormone Analog (JHA)[14]. Although it is a carbamate insecticide it is not considered to be an anti-cholinesterase inhibitor and so is thus rendered non-neurotoxic. Its low pressure and Henry's Law constant exempts it from being found in the air except possibly from drift which may be associated with spray applications. Its low water solubility and potential for leaching from the soil assigns a moderate to strong tendency for soil binding. The residual presence of Methoxyfenozide, a substituted dibenzoylhydrazine in the citrus fruit sample, is an insecticide which functions by accelerating the moulting process. It acts as an ecdysone agonist, substituting for the natural insect moulting hormone 20-hydroxyecdysone.

The metabolic pathway for degradation of methoxyfenozide in fruits suggests that the parent compound contributes to the large residual percentage and that minor amounts of B-ring carboxylic acid and B-ring mono-alcohols can be traced[15]. Trace residual amounts of

flumioxazin suggest its rapid degradation to 6-amino-7-fluoro-4-(2-propynyl)-1,4,-benzoxazin-3(2H)-one (APF) and 3,4,5,6-tetrahydrophthalic acid (THPA). The toxicity of these metabolites is found to be more soluble and persistent in water and the potential for the degradates to leach into ground water is very high.

Methods for the determination of pesticide residues are commonly being revised and improved with new conventional techniques. The broadening range of classes of pesticides and increasing diversity of structures and properties have made it difficult to determine all pesticides. Most of the methods for the determination of different pesticide residues in different types of food stuff consists of three major steps; extraction, clean-up and analysis. The method of extraction and the type of solvent used depends on the physical and chemical properties of the pesticides to be extracted, the type of substrate from which it will be quantitatively removed and the final method of analysis. As more polar pesticides, such as organophosphates, phenoxy acetic acids and triazines have come into use, more polar solvents, such as chloroform, acetone, acetonitrile and methanol have been found to be good extractants. Acetonitrile is well known for the extraction of multi-residues of pesticides from fruits and vegetables. The use of Mass spectrometry (MS), a universal selective detection approach, in Gas Chomatography analysis permits the detection and identification of a wide range of pesticides in complex extracts. In the present work, for samples which had been sprayed with pesticides, a number of peaks were obtained in the GC/MS spectrum while no peaks were found for the sample without pesticide spray.

GC/MS spectrum recorded elution time, retention time and molecular weight of each pesticide extracted. The relative abundance and the values of m/z ratio of each pesticide were also determined from the GC/MS spectrum.

Glyophosphate, Methoxy fenozide, Fenoxycarb and Flumioxazine pesticides were found from the GC/MS spectrum of orange with molecular weights of 169g/mol, 368g/mol, 300g/mol and 355g/mol respectively. Glyophosphate was the only pesticide obtained from the spectrum of guava with molecular weight 169g/mol while Glyophosphate, Piperonyl butoxide and Crotoxyphose pesticides were found in spinach with molecular weights 169g/mol, 339g/mol and 325g/mol respectively.

The present research shows that when fruits and vegetables are sprayed with pesticides, harmful health effects may be caused due to ingestion and absorption of these pesticides and that environmental factors do not necessarily contribute to the absorption of pesticides into those fruits which are not sprayed with pesticides in the vicinity of those sprayed with pesticides.

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