Xournals

Academic Journal of Forensic Sciences

ISSN: 2581-4273 | Volume 03 | Issue 02 | October-2020

Extraction and Identification of Pesticide Residue in Fruits and Vegetable Samples for Forensic Consideration

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Available online at: www.xournals.com

Received 01st October 2020 | Revised 10th October 2020 | Accepted 14th October 2020

Abstract:

Various pesticides and insecticides have been used in every region of most of the countries in the world to manage and mitigate different agricultural pests. Predicament of the presence of pesticide residues in fruits and vegetables is the major concern to the staid matter of individual health and wellbeing. The present study entitled "Extraction and Identification of Pesticide residue in Fruits and Vegetable samples for Forensic consideration" was carried out with an objective to analyze and identify pesticide residue in fruits and vegetables by using QuEChERS extraction procedure and TLC method. 4 Fruits (banana, apple, papaya, and guava) and 6 vegetable (eggplant, carrot, bell pepper, potato, okra, cauliflower) sample were collected from vegetable market of Allahabad. Extraction of pesticides was done by using QuEChERS extraction procedure and analyzed for qualitative determination of the presence of pesticides on samples by using thin-layer chromatography. Result revealed that 90% of samples were positively contaminated with as a minimum of one or more pesticide. The findings of the study might help in extending awareness to the farmers and local people about pesticides and their hazardous effects on humans. Whereas the methodology might be useful to the analyst working in the particular field as well as a forensic laboratory for rapid extraction of residues from biological and non-biological samples and to overcome the major issue of all the forensic laboratories i.e. pendency of cases.

Keywords: TLC, QuEChERS, Chlorpyrifos, Carbofuran, Cypermethrin.

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Introduction

The Environmental Protection Agency (EPA) defines the term pesticide as the use of any substance or combination of substances proposed for preventing, repelling, destroying, or extenuating any pest. The definition of pesticide according to the Food and Agriculture Organization (FAO) is very specific which ascertain that any substance or combination of substances which are purposefully used for controlling or preventing or even destroying any kind of pest including organism or pest which are capable of carrying any animal, plant or human disease; any plant or animal species which are capable of causing any kind of harm during manufacturing, processing, storage, transportation or even selling of any kind of food or farming products, any kind of coppice or even animal feedstuff; any substance or blend of substances which may be given to any animal for purpose of controlling or destroying any kind of pest, arachnids or insects living inside or even outside of the body of that animal **(Zacharia and Tano, 1-18)**. India ranks 12th in Asia and is being the largest producer in the world for using pesticides. Most of the population of India is majorly dependent on agriculture which requires the need for using pesticides to prevent the loss of agriculture products from any kind of pests. When we see the comparative use of pesticides in a global scenario, India is the country whose average consumption of pesticides in agricultural use is comparatively lower than any other developed country. Even then, the presence of pesticide residue in post harvested agricultural products is at great height **(Abhilash and Nandita, 1-12)**. The use of pesticides and other fertilizer has helped to a great extent to the farmers with an increase in yielding crop productivity but consequently, their bad effect on nature and human health is also unavoidable. Pesticides have been classified into 4 major categories with include *Organophosphate Pesticide:* These pesticides are basically affecting the activity of the enzyme which regulates the secretion of acetylcholine which is a neurotransmitter and in turn affects the nervous system. These pesticides show similar effects in both, insects and as well as humans. **(Cabello *et al.*, 471-479)**. *Carbamate Pesticides*: The effects of carbamate pesticides are quite similar to the effects of organophosphate pesticides as it also affects the activity of neurotransmitter enzyme acetylcholinesterase which in turn affects the nervous system. The effects of the enzyme are generally reversible **(Fernandez *et al.*, 43-56)**. *Organochlorine Insecticides*: these insecticides were generally being used in the ancient times, but a lot of them have been aloof from the commercial market because of their persistency in nature and hazards related to the human health as well as the environment the most common example of this group of pesticides are DDT and chlordane and many more **(Pandit *et al.*, 240-243)**. *Pyrethroid Pesticides*: These are generally synthesis from a natural pesticide “pyrethrin” found in the algae known as chrysanthemum. They have been adapted to augment their stability in the atmosphere. Some of these synthetically prepared pyrethroids are noxious to the nervous system **(Duara *et al.*, 43-46)**.

Effect of Pesticide on Human Health

Acute Effect: Acute effect involves immediate effects of the pesticides which are severe enough to seek urgent medical help and also cannot be ignored. These acute or Immediate affects human health due to the exposure of pesticide may inculcate the headache, hurtful eyes as well as skin, annoyance of nose and irritation on throat, skin burning, rashes, and blister, giddiness, diarrhea, severe pain on abdomen, vomiting, and sickness, distorted vision, sightlessness, and rarely death **(Mahmood *et al.*, 17-25)**

Chronic Effect: Chronic effects are often lethal and may appear even for a year. These are long-term effects that can damage body organs. Pesticide exposure for prolonged duration consequences in loss of coordination, and memory, reduced motor signaling, and reduced vision ability. Long-time pesticide exposure damages the immune system and can cause hypersensitivity, asthma, and allergies. Pesticide has been associated with leukemia, brain cancer, lymphoma, cancer of the breast, prostate, ovaries, and testes (pesticide and human health). Ingestion of organochlorine causes allergic reactions to light noise and contact, giddiness, tremors, seizures, queasiness, sickness, puzzlement, and anxiety. Pyrethroid pesticides may cause sensitivity to skin, ferociousness, overexcitation, reproductive, or growth deficiency **(Kumari and John, 162–167; Carbamate, 767-770)**.

Forensic Significance of Pesticide Analysis

In forensic science, there is ‘n’ number of cases related to the toxicological analysis of poison and pesticide in different suspected sample matrices including viscera to food commodities like grains, milk, fruits, vegetables, and even in a water sample. Forensic science laboratories work for justice and their responsibilities are very peculiar. The analysis proves that the suspected case is because of illness or toxicity, and if there is evidence of toxicity then whether it is the case of acute poisoning or chronic poisoning.

Whatever the reason for poisoning is, even if it is not intentional, still then the will be at least compensation posed by common regulatory or legal bodies for the hazardous effect which may be followed by intoxication. This characteristic is of great significance and shows an everlasting association between toxicology and forensic medicine, which requires an exceptional security method used in the laboratory **(Coulson, 47-56)**.

In view of various health effects associated with pesticide exposure, there is a need to gain an overview of the pesticides and their residue levels on crop produce such as fruits and vegetables which constitute a great part of the Indian diet. Particularly, *chlorpyrifos, carbofuran,* and *cypermethrin* are classified as hazardous pesticides yet are still in use in the country. Reliable residue analysis is of great value to indicate risks pesticide exposure poses on human health. This topic is significant in forensic science because monitoring all the fruits, vegetables, and other crops in different agro-climatic region of India for their contamination with such toxic constituent above MRL (Maximum Residue Level) value is the new merging field in forensic science which deals with public health and consumer safety. By keeping in mind the above significance objective of the present study is as follows.

Objective

To identify pesticide residue in fruits and vegetables using QuEChERS extraction and TLC.

Materials and Methods

The work entitled "Extraction and Identification of Pesticide residue in Fruits and Vegetables for Forensic consideration" was carried out in Department of Forensic Science with the objective, to Identify Pesticide residue in Fruits and Vegetables using QuEChERS extraction and TLC. Following materials was required for Experiment

Material Requirements: The instruments, apparatus, and the chemical reagents required for the study are given in table (1) and (2).

Table No. 1 – Required Instruments and necessary Apparatus

Instrument	Apparatus
Electronic Analytical Balance	Paper Bags, Metal Spatula, Dropper,
Hot Air Oven	Beakers (Borosil) Capillary Tubes (Borosil) Test Tubes (Borosil)
Water Bath	Centrifuge Tubes (Polypropylene) Measuring Cylinder Reagent Bottles Sprayer
U.V. Chamber	High-speed Electronic Mixture
Centrifuge Machine	TLC Plates (Precoated) TLC Chamber

Table No. 2 – Required Chemicals/Reagent

S. No.	Chemicals/ Reagents
1	Acetonitrile
2	Anhydrous Magnesium Sulphate
3	Anhydrous Sodium Sulphate
4	Sodium Hydroxide
5	Formic Acid
6	Primary Secondary Amine (PSA)
7	Hexane
8	Acetone
9	Distilled Water
10	Ethanol
11	Silver Nitrate
12	Fast Blue
13	Palladium Chloride
14	Hydrochloric Acid

Sample Collection

One kg of each 10 samples including 6 vegetables (okra, cauliflower, brinjal, bell pepper, potato, and carrot) and 4 fruits (banana, apple, papaya, and guava) were collected from Mundera Mandi Allahabad by random sampling. Samples were packed in paper bags and transferred to the laboratory. Samples of fruits and vegetables are given in table (3) and (4).

Table No. 3 – Selected Vegetable Samples

Common Name of Vegetable Sample	Scientific Name
Okra	*Hibiscus esculenta*
Egg Plant	*Solanum melongena*
Cauliflower	*Brassica oleracea*
Bell Pepper	*Capsicum annuum*
Potato	*Solanum tuberosum*
Carrot	*Daucus carota*

Table No. 4 – Selected Fruit Samples

Common Name of Fruit Samples	Scientific Name
Apple	*Malus domestica*
Banana	*Musa sapientum*
Guava	*Psidium guajava*
Papaya	*Carica papaya*

Pesticide Selection

Pesticide was selected after a short interview with farmers of the local reason of Allahabad about the maximum use of pesticide in general in fruits and vegetables, pesticide vendors, maximum purchased pesticides by farmers of local regions, and with a forensic expert about the maximum pesticide poisoning cases received in the laboratory. By analyzing their responses 3 pesticides are selected for the study which are Chlorpyrifos, Carbofuran, Cypermethrin stated in the table (5).

Table No. 5 – Selected Pesticides Standards

Pesticide Name	Molecular Formula	Classification
Chlorpyrifos	$CH_{11}C_{l3}NO_3PS$	Organophosphate
Carbofuran	$C_{12}H_{15}NO_3A$	Carbamate
Cypermethrin	$C_{22}H_{19}C_{l2}NO_3$	Pyrethroid

Sample Extraction and Cleanup

The extraction and cleanup method was based on QuEChERS (Quick Easy Cheap Effective Rugged and Safe) sample preparation method for pesticide **(Dasika, 19-28)**. First of all, the samples were well chopped into small pieces and homogenized with the help of an electrical blender. A 10g of homogenized subsample was taken in 15 ml polypropylene centrifuge tube with 10 ml of acetonitrile and vortexed for 2 minutes, followed by the addition of 4g anhydrous sodium sulphate and 1g NaCl. The sample was then centrifuged at 15000 rpm for about 5 minutes. For cleanup, the upper acetonitrile layer was transferred into another 15 ml centrifuge tube containing 150 mg anhydrous magnesium sulphate and 25mg PSA (Primary Secondary Amine) sorbent. Again vortexed for 2 minutes and centrifuged at 15000 rpm for about 2 minutes. The clean extract was then transferred into the test tube and concentrated on a water bath and then evaporated to dryness with help of a rotatory evaporator. The dried residue was reconstituted in 1 ml acetonitrile and spotted against precoated TLC plates **(Anastassiades *et al.*, 412–431)** as in figure (1) and (2).

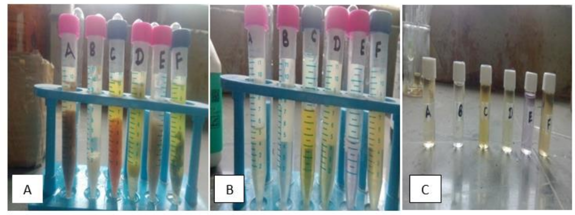

Figure No. 1 – Pesticide Residue Extraction from Vegetable Samples: A) Homogenized Vegetable Samples for Pesticide Residue Extraction, B) Pesticide Residue Extract of Vegetable Sample, C) Pesticide Residue Extract of Vegetable Sample after cleanup.

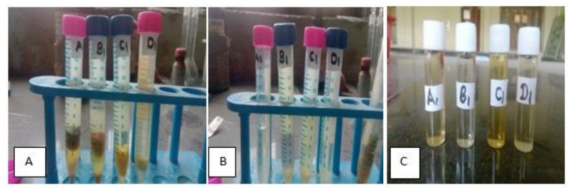

Figure No. 2 – Pesticide Residue Extraction from Fruit Samples: A) Homogenized Fruit Samples for Pesticide Residue Extraction, B) Pesticide Residue Extract of Fruit Sample, C) Pesticide Residue Extract of Fruit Sample after cleanup

Analysis by Thin layer chromatography

A pre-coated silica gel on an aluminum plate from Merck was used. A line of origin of spot was drawn. Another line was drawn 10 cm above from this line which will limit movement of solvent. On this line, the extracted clean sample was spotted along with standard with the help of microcapillary tubes (Spotting was done at 1 to 2 cm above from the bottom of plates and at sufficient distance from each other). Sample spotting was repeated at least 20 to 30 times. After drying of the spot, the loaded TLC plates were placed inside a saturated TLC chamber containing an eluting solvent mixture of hexane and acetone in a ratio of 8:2 (80 ml hexane and 20 ml acetone). Ensured that the level of solvent is above from the bottom of edge and below the line of origin of spot. Plates were allowed to run till 10 cm. After completion of movement of running solvent, plates were carefully removed from TLC chamber and allowed to dry on air.

Visualization of Spot

All the pesticides were not detected through the same detection technique and coloring reagent so different spry reagents were used according to the sensitivity of pesticide towards the spray reagent. *Palladium chloride* for *chlorpyrifos*, *silver nitrate* for *carbofuran*, and *fast blue* for *cypermethrin* were used. **(Shafi et al., 298-303).** For the detection of chlorpyrifos, plates were sprayed with palladium chloride spray reagent (prepared by dissolving 0.5 g palladium chloride in 100 ml water followed by 2 drops of HCl). For detection of Carbofuran, plates were sprayed with fast blue regent (prepared by dissolving fast blue B and sodium chloride in 100 ml distilled water). For detection of cypermethrin plated were sprayed with silver nitrate (prepared by dissolving 17 gm silver nitrate in 100 ml distilled water), followed by UV exposure for about 30 minutes. Color of spot and the Rf value was noted and compared with standard.

Result

Detection: Different spray reagents according to their sensitivity with specific pesticides produce different colors and some gave fluorescence with U.V. exposure as in Table (6).

Retention Factor: Retention factor of each pesticide was calculated by running the standard samples on TLC plate. Retention factor was calculated by measuring the distance travelled by the spots and solvent.

Rf = Distance travelled by the spot/ distance travelled by the solvent.

Retention factors of selected pesticides standard are given in Table (7).

Table No. 6 – Detection of Pesticides Standard on TLC Plates

Pesticide Standard	Detection on TLC with Spray Reagent	Color of Spot
Chlorpyrifos	Palladium Chloride	Yellow Color Spot
Carbofuran	Fast Blue	Yellowish Orange Spot
Cypermethrin	Silver nitrate followed by UV light at 254 nm for 30 minutes	Black Color Spot

Table No. 7 – Retention Factor of Pesticides Standard

Pesticide standard	Distance travelled by spot (in cm)	Distance travelled by solvent (in cm)	Retention Factor (Rf)
Chlorpyrifos	8.9 cm	10 cm	0.89
Carbofuran	2.5 cm	10cm	0.25
Cypermethrin	7.4 cm	10cm	0.74

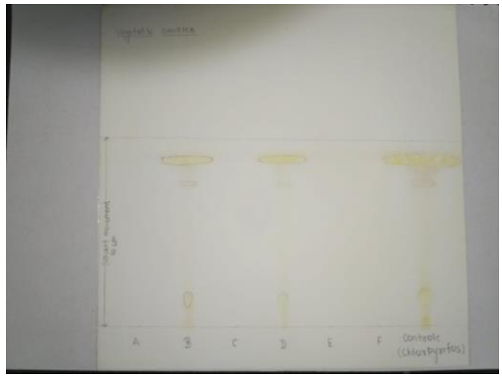

Figure No. 3 – TLC Plate of Vegetable Sample with standard Chlorpyrifos

In the above TLC plate, the extracted sample of A (Eggplant), B (Cauliflower), C (Carrot), D(Bell pepper), E(potato), F(Okra) were spotted and appearance of yellow spot was noticed. See figure (3)

Distance travelled by solvent = 10 cm

Distance travelled by sample B = 8.9 cm

Distance travelled by sample D = 8.9 cm

Distance travelled y standard chlorpyrifos = 8.9 cm

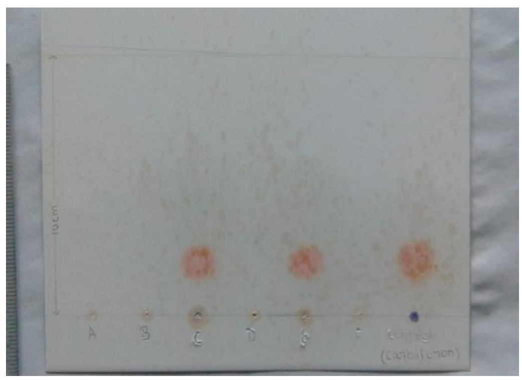

Figure No. 4 – TLC Plate of Vegetable Sample with standard Carbofuran

In the above TLC plate, the extracted sample of A (Eggplant), B (Cauliflower), C (Carrot), D(Bell pepper), E(potato), F(Okra) were spotted and appearance of orange color spot was noticed.

Distance travelled by solvent = 10 cm

Distance travelled by sample C = 2.5 cm

Distance travelled by sample E = 2.5 cm

Distance travelled y standard chlorpyrifos = 2.5 cm

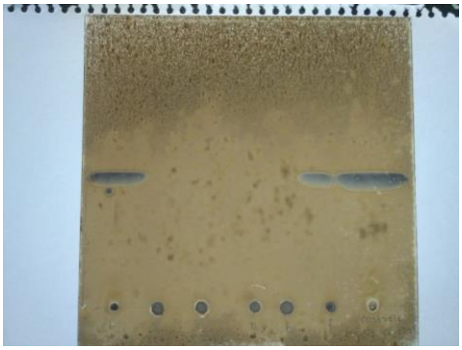

Figure No. 5 – TLC Plate of Vegetable Sample with standard Cypermethrin

In the above TLC plate, the extracted sample of A (Eggplant), B (Cauliflower), C (Carrot), D(Bell pepper), E(potato), F(Okra) were spotted and appearance of black colour spot was noticed. See figure (5)

Distance travelled by solvent = 10 cm

Distance travelled by sample A = 7.4 cm

Distance travelled by sample F = 7.4 cm

Distance travelled y standard Carbofuran = 7.4 cm

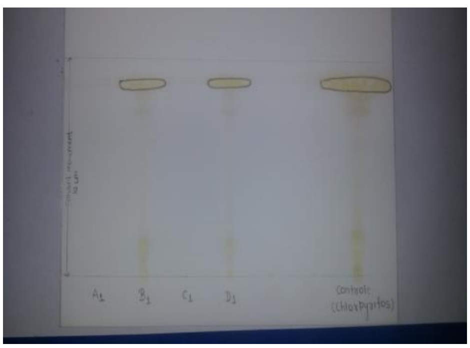

Figure No. 6 – TLC Plate of Fruits Sample with standard Chlorpyrifos

In the above TLC plate, the extracted sample of A1 (Banana), B1 (Apple), C1 (Papaya), D1 (Guava) were spotted and run with standard pesticide of chlorpyrifos and appearance of yellow spot was noticed. See figure (6)

Distance travelled by solvent = 10 cm

Distance travelled by sample B1 = 8.9cm

Distance travelled by sample D1 = 8.9 cm

Distance travelled y standard Chlorpyrifos = 8.9 cm

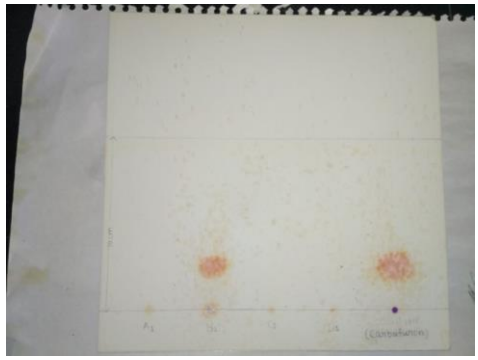

Figure No. 7 – TLC Plate of Fruits Sample with standard Carbofuran

In the above TLC plate, the extracted sample of A1 (Banana), B1 (Apple), C1 (Papaya), D1 (Guava) were spotted and run with standard pesticide of Carbofuran and appearance of orange colour spot was noticed. See figure (7)

Distance travelled by solvent = 10 cm

Distance travelled by sample B1 = 2.5 cm

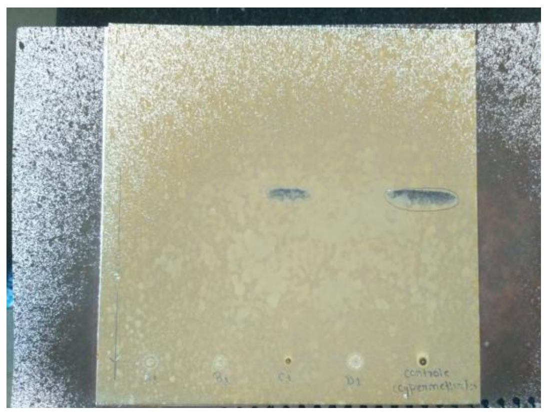

Figure No. 8 – TLC Plate of Fruits sample with standard Cypermethrin

In the above TLC plate, the extracted sample of A1 (Banana), B1 (Apple), C1 (Papaya), D1 (Guava) were spotted and run with standard pesticide of Cypermethrinand appearance of black color spot was noticed. See figure (8)

Distance travelled by solvent = 10 cm

Distance travelled by sample C1 = 7.4 cm

Distance travelled y standard Carbofuran = 7.4 cm

Table No. 8 – Detection of Presence of Pesticide in Selected Fruits and Vegetable Sample

S. No.	Samples	Color of Spot with Spray Reagent	Distance moved by Solute	Distance moved by Solvent	Retention Factor (Rf)	Observation
01	Eggplant (A)	Black Colour	7.4 cm	10 cm	0.74	Contaminated with cypermethrin
02	Cauliflower (B)	Yellow Colour	8.9 cm	10 cm	0.89	Contaminated with chlorpyrifos
03	Carrot (C)	Orange	2.5 cm	10 cm	0.25	Contaminated with Carbofuran
04	Bell pepper (D)	Yellow Colour	8.9 cm	10 cm	0.89	Contaminated with chlorpyrifos
05	Potato (E)	Yellowish Orange	2.5 cm	10 cm	0.25	Contaminated with Carbofuran
06	Okra (F)	Black	7.4 cm	10 cm	0.74	Contaminated with cypermethrin
07	Banana (A1)	No Color	-----	10 cm	-----	Not contaminated
08	Apple (B1)	Yellow with palladium chloride, orange with fast blue	8.9 cm, 2.5 cm	10 cm	0.89, 0.25	Contaminated with chlorpyrifos and Carbofuran
09	Papaya (C1)	Black	7.4 cm	10 cm	0.74	Contaminated with cypermethrin
10	Guava (D1)	Yellow	8.9 cm	10 cm	0.89	Contaminated with chlorpyrifos

A summarized result of this study is shown in table (8). In the above table, it is given that the samples of cauliflower, Bell pepper, Apple, and Guava shows a yellow color spot with palladium chloride reagent at Rf 0.89, which means that the sample of Cauliflower, Bell pepper, Apple, and Guava shows positive contamination of Chlorpyrifos.

Sample of Carrot, Potato, and Apple shows a yellowish orange color spot with Fast Blue reagent at Rf 0.25, which means that the sample of Carrot, Potato, and Apple shows positive contamination for Carbofuran. Whereas sample of Eggplant, Okra, and Papaya shows black color spot with silver nitrate reagent at Rf 0.74, which means that the sample of Eggplant, Okra, and Papaya shows positive contamination for Cypermethrin. Out of total samples, about 90% sample was found contaminated, in which Apple was found contaminated with both chlorpyrifos and carbofuran. Banana sample was found not contaminated with any of selected pesticides.

Discussion

Different kinds of pesticides are used to avoid pest infestation to the fruits. If the intended maximum residue limit of pesticides exceeds, then it causes different kinds of diseases **(Khan *et al.*, 816–821).** TLC method was used for the determination of pesticides in fruits and vegetables in connection with QuEChERS method **(Dasika 19-28)** and the outcome showed that the TLC technique was responsive and reproducible for the compounds tested and it could be used qualitative and preliminary evaluation of the presence of pesticide residues by monitoring laboratories in order to complement other instrumental analyses. Carbofuran is a systematic pesticide as when sprayed in the field, the plant absorbs it through the root and from there plant distribute it throughout organs such as roots, stem, leaves, and fruits, it becomes the part of fruit and vegetable then and no pests can destroy them **(Latif *et al.*, 46-52).** Chlorpyrifos belongs to the organophosphate class of pesticides which have a long persistent nature and can get accumulated inside body organs even when exposed to a small amount for a long time and can show nervous failure, birth defects reproductive cancer (**Montgomery *et al.*, 1235–1246).**

Conclusion

In the present study, it was observed that 90% of sample was contaminated with at least one of each selected pesticide. One sample was found contaminated with more than a single pesticide and one sample was found not contaminated with any of the selected pesticides. From the above interpretation and observation during the study, it was concluded that fruits and vegetables are more prone to the pesticide contamination and so as more hazardous to human health because more than half of the total population is depended on fruits and vegetable as their regular routine food and generally these commodities are consumed freshly.

To avoid these hazardous health effects this type of monitoring research should be done for each and every area so that data can be drawn that which area has the most pesticide contamination, which helps to monitor and control the excessive use of these hazardous pesticides. This research may help to spray awareness in general people and in among farmers about the hazardous effect of pesticides on humans as well as the environment. Since all the fruits which were analyzed are contaminated with pesticide residues. In forensic science laboratories, there are so many pending cases of pesticide poisoning due to a time-consuming laborious conventional process which used so large amount of toxic solvent and apply two to five steps long procedure for extraction and cleanup. In the present study, detection and identification of pesticides were done with TLC coupled with QuEChERS extraction procedure which used a small amount of environmentally safe solvent for extraction and fewer steps for both extractions as well as cleanup. If this method will be used in the forensic science laboratory, it will surely help to overcome the major problem of pendency of cases to some extent. Different methods can be used for the determination and quantification of pesticides, but by using TLC, it is convenient and easy.

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