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Determination of Pesticide Residues in Strawberry (*Fragaria ananassa*) from Upper Punjab Districts of Pakistan

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ABSTRACT

Strawberry (*Fragaria ananassa*) is grown from mid-October to the end of April in Lahore, Sheikhupura, Sialkot and Gujranwala Districts of the Punjab. A huge amounts of pesticides especially fungicides are sprayed on it indiscriminately to protect it from different diseases and fungal attacks. Present experiment was planned for determination of pesticide residues in strawberry. For this purpose, 40 samples of strawberry were collected from Sheikhupura, Lahore, Sialkot, and Gujranwala districts of Punjab, Pakistan for determination of residues of Malathion, Chlorpyrifos, Profenofos, Atrazine, Carbofuron, Tricyclazole, Difenoconazole, Azoxystrobin, Tebuconazole and Cypermethrin in strawberry. The accuracy, precision, specificity, linearity, limit of detection and quantification were measured. The recovery ranged from 89.3 % to 108% with RSD ranging from 1.66 % to 14.2 %. The linearity showed reliable range (0.995–0.999). The limit of detection ranged from 0.006 to 0.04 μg g^{-1}. Data showed that all the samples contain multi-residues of different pesticides. Among these 40 samples pesticide residues were most often found. All the samples contained multiresidues of different pesticides. The percentage of samples having pesticides residues are , Carbofuron (50 %), Malathion (37.50 %), Profenofos (32.50 %), Chlorpyrifos(25 %), Cypermethrin (20 %), Difenoconazole in (47.50 %), Tricyclazol in (52.50 %), Tebuconazole in (50.00 %), and Azoxystrobin in (20 %) of the samples analyzed.

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INTRODUCTION

Strawberry (*Fragaria xananassa*) is a fruit of family Rosacea grown throughout the world including Pakistan.

It is famous for its appealing look and aroma. Its cultivation is getting popularity since last 5 years in Sheikhupura, Sialkot, Gujranwala, and Lahore districts of Punjab (Mehmood *et al*., 2018) It is rich in vitamins C, D, B9 organic acids, anthocyanin, phosphorus, iron, Potassium, Manganese and other minerals. It is a rich source of antioxidants and have benefits in controlling heart beat and blood sugar (Benchikh *et al*., 2020). Despite all these merits strawberry and other fruits are potential sources of toxic chemicals like pesticide residues (Song *et al*., 2020). Various pesticides formulations are widely used in agricultural around the world for controlling pest and maximize crop yield, but their un judicious use results in environmental contamination and residues accumulation in food, especially in vegetables and fruits (Sun *et al*., 2018). The pesticides pollution is a major environmental concern and is a cause of contamination of agricultural land and commodities (Sidhu *et al*., 2019). Heavy pesticide sprays are a common practice on fruit crops to save them from different insect pests and diseases. Since pesticides are toxic and can cause environmental contamination, they must be used carefully to protect the environment and human health (Boudh *et al*., 2019). Same is the case with strawberry. Heavy pesticides are sprayed on strawberry during its cultivation. Grey mould (*Botrytis cineria*) is a serious disease of strawberry that causes significant yield losses due to fruit rotting (Weber and Hahn, 2019). Powdery mildew (*Sphaerotheca macularis* f. sp. *Frgariae*) readily infects strawberry leaves and fruits (Bajpai *et al*., 2019). Strawberry shelf life is very low. Huge amounts of fungicides are sprayed on during harvesting and to maintain its freshness until it reaches end user. Use of pesticide on fruits is also evident from the literature review. The agricultural yield is greatly enhanced by exogenous application of pesticides whether applied on a standing crop or as post-harvest treatment (Adisa *et al*., 2019). Accumulation of pesticide residues in food has always been a great concern, particularly in fruits which are consumed fresh. Similar is the case with strawberry. Strawberry is on the top of Dirty Dozen products published by US Environmental Working Group. Exposure to toxic pesticide residues through diet is estimated to be 5 times the magnitude of exposure through other sources such as air and water (Mathews, 2019). In Belgium, pesticides residues were found in 72 % of fresh fruits while in south asia, pesticides residues were detected in 51% of food commodities (Choudhary *et al*., 2018). David 2019, revealed that 35.62 % of total contaminated samples exceed the maximum residue limit MRL values recommended by the Food and Agricultural Organization (FAO) and World Health Organization (WHO). Another study conducted by Ramadan *et al* (2020) revealed the presence of Monocrotophos, Chlorpyrifos, Cypermethrin and endosulfon etc. in the vegetables. A study on fresh vegetables and fruits in Croatia found 246 (28.5%) of samples show pesticide residues at or below the established MRL while 46 (5.3%) show pesticides above the MRL (Reichert *et al*. 2020). In Saudi Arabia it was found 34 % of samples had toxic residues above the MRL (Osman *et al*., 2011). Taking into account the above stated facts, present study was conducted to determine pesticide residues in strawberry collected from different districts of Punjab, Pakistan. Our results provide useful information to the scientist working on pesticide residues and also contributed in risk assessment of consumers exposure to the expected pesticide residues.

METHODS AND MATERIAL

Sampling

Keeping in view the above facts a study was planned at the, Pesticide Residue Laboratory (ISO 17205-2005 accredited) Kala Shah Kaku (Annual maximum and minimum temperature 35°C and 22°C, Relative humidity 58%) in collaboration with, College of Earth and Environmental Sciences, University of Punjab, Pakistan, to assess the pesticide residues in strawberries collected from different locations of Districts Lahore, Sheikhupura, Sialkot and Gujranwala. Total of 40 samples were collected during the months of March and April from these four districts. After collection of samples in the field, they were properly stored in ice boxes and brought to laboratory. Samples were kept at -20 ˚C for further processing and analysis.

Reagents and Chemicals

Certified reference materials of selected pesticides, including; Acephate, Chlorpyrifos, Cypermethrin, Fipronil, Carbofuron, Malathion, Profenofos, Azoxystribin, Difenoconazole, Tricyclazole,Tebuconazole and Atrazine were purchased from (Augsburg, Germany), purity ranging from 97.00 - 99.50 %. Other solvents and chemicals, including; Acetonitrile, Acetic acid, Triphenylphosphate (TPP), sodium chloride (NaCl), anhydrous magnesium Sulfate ($MgSO_4$) and PSA sorbents

40 um. All the organic solvents used were of HPLC grade (Figure 1) with certificates of analysis.

Extraction and Cleanup

The QuEChERS sample extraction and cleanup method for pesticide residue analysis (AOAC Official Method 2007.01. (10)) was used to prepare all the samples. After homogenization with a high speed varying blender, 15 g portion of completely homogenized and comminuted sample was taken in a 50 mL Teflon tube. After adding 15 mL of 1% acetic acid in acetonitrile, 1.5 g of Sodium acetate, 6 g of anhydrous Magnesium sulfate, and 75 u L^{-1} Internal standard the sample was vigorously shaken for I minute. Then the sample was centrifuged at 4000 rpm for 1 minute. Upper 1-8 mL of supernatant layer was transferred to tube with 150 mg of anhydrous Magnesium sulfate and 50 mg of PSA per m L^{-1} extract and then it was shaken for 30 second. After centrifuging it for 1 minute 0.5-1.0 mL extract was added to GC vial for analysis. Instrumental conditions are presented below.

Instrument Conditions GC Conditions	
GC.	Agilent 7890B series (G3440A)
Auto sampler	Agilent 7693A Automatic Liquid Sampler injector and sample tray
Column	Agilent J&W DB-5MS, 30 m length × 0.2 mm diameter, 0.25 µm film thickness.
Inlet	Split/splitless
Inlet liner	Splitless, single taper, Ultra Inert liner with glass
Wool	(Agilent p/n 5190-3167)
Carrier	Helium (99.999 % pure)
Inlet pressure	36 psi (constant pressure mode) during run, 1 psi during back flush.
Inlet temp	280 °C
Inj.vol.	1 µ L^{-1}
Purge flow to split vent	30 m L-1/min at 0.75 min
Gas saver	On (20 m L^{-1}/min at 2.0 min)
Oven temp.	50 °C (1 min), 20 °C/min to 180 °C (0 min), 10 °C/min to 190 °C (0 min), 3 °C/min to 240 °C (0 min) and 10 °C/min to 300 °C (5min)
Retention time locking	Chlorpyrifos locked at 13.77 min
MS conditions	
Spectrometer	Agilent 7890B Single Quadrupole GC/MS System
Mode	Electron Impact
Transfer line temp	280 °C
Solvent delay	2.3 min
Ion Source temp	300 °C

RESULTS

Accredited AOAC (Association of Official Analytical Chemists) method of analysis was used during the study. Method performance parameters like Accuracy, Precision, (Loss on drying) LOD/Limit for quantification (LOQ), and linearity were performed and found satisfactory. Method validation results are summarized in Table 2. Matrix matched calibration standards were used for plotting calibration curve. For identification and quantification of 10 target pesticides in this study, both SIM and SCAN modes were used. First identification of different pesticide were confirmed by running the matrix matched calibration standards, recording retention times and scanning the m/z values ranged from 50-500. After identification and calibration real samples were analyzed by using same procedure. Information about target ion, retention times and LOQs for all the pesticides is listed in the Table 1.

Table.1. List of Pesticides with RT, MRL, LOQ, and Target Ion (m/z).

Name of Pesticide	Retention Time (RT) (min)	MRLs	Target Ion (m/z)	LOQ(ug/g)
Carbofuron	10.24	0.01	164.20	0.02
Atrazine	10.43	0.1	200.20	0.01

Malathion	13.34	0.05	173.20	0.02
Chlorpyrifos	13.77	0.2	197.00	0.006
Tricyclazole	16.11	0.01	189.20	0.04
Profenofos	16.97	0.07	399.20	0.02
Cypermethrin	29.52	2.0	163.10	0.03
Difenoconazole	31.14	0.4	323.20	0.01
Azoxystrobin	31.76	10	344.30	0.01

Table 2. Types of pesticide, residue range and mean residual level.

Pesticide	Type of pesticide	No of samples with residues	Residue range ug/g	Samples with residues above MRL	Mean residue level ug/g
Atrazine	Herbicide	20	0.05-2.55	19	0.31
Carbofuron	Insecticide	13	0.14-1.61	13	0.22
Malathion	--do--	15	0.02-1.69	14	0.23
Chlorpyrifos	--do--	10	0.07-1.89	08	0.25
Cypermthrin	--do--	8	0.02-0.6	07	0.0425
Profenofos	--do--	14	0.02-1.43	11	0.15
Tricyclazole	Fungicide	19	0.05-1.14	18	0.27
Difenoconazole	--do--	20	0.05-1.52	13	0.41
Tebuconazole	--do--	20	0.05-2.9	13	0.36
Azoxystrobin	--do--	21	0.05-1.12	0	0.25

Real sample analysis

Among these 40 samples pesticide residues were most often found. All the samples contained multiresidues of different pesticides. The percentage of samples having pesticides residues are, Carbofuron (50 %), Malathion (37.50 %), Profenofos (32.50 %), Chlorpyrifos (25 %), Cypermethrin (20 %), Difenoconazole in (47.50 %), Tricyclazol in (52.50 %), Tebuconazole in (50.00 %), and Azoxystrobin in (20 %) of the samples analyzed shown in Figure 3.

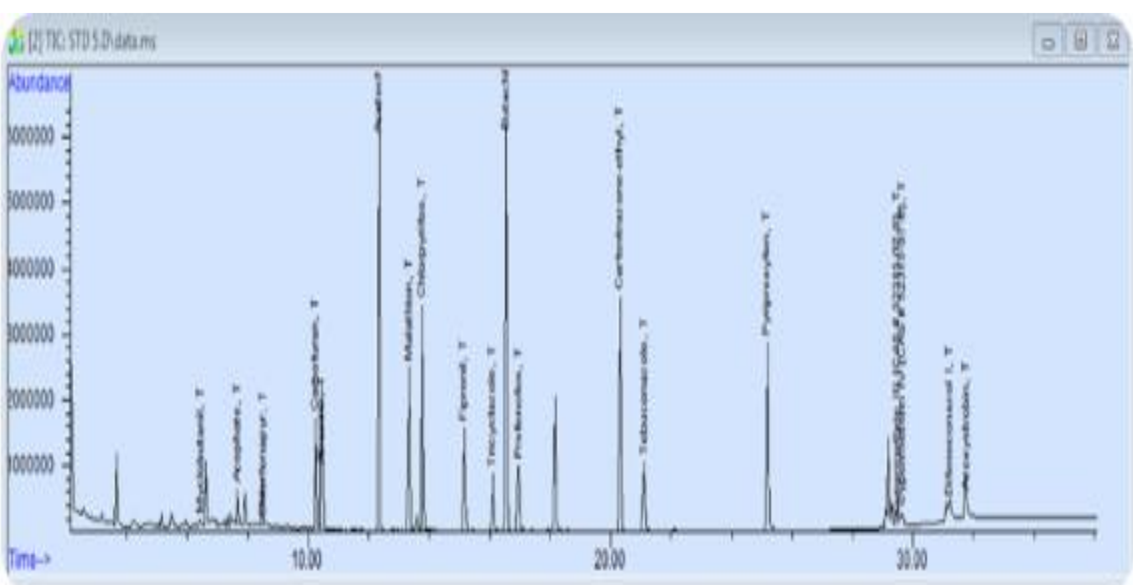

Figure 1. GCMS Chromatogram of 10 pesticide standards under study.

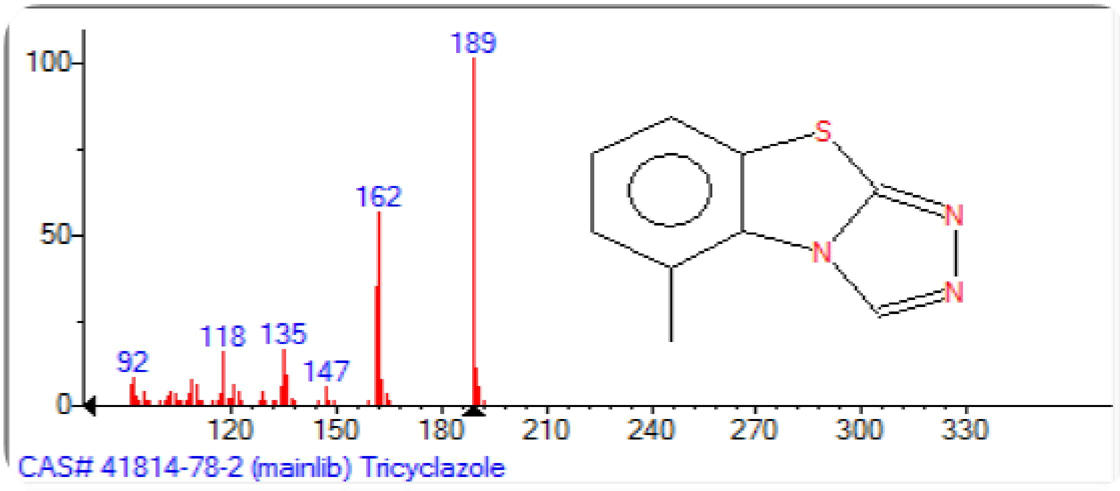

Figure 2A. Tricyclazole GCMS Spectra match with NIST Library.

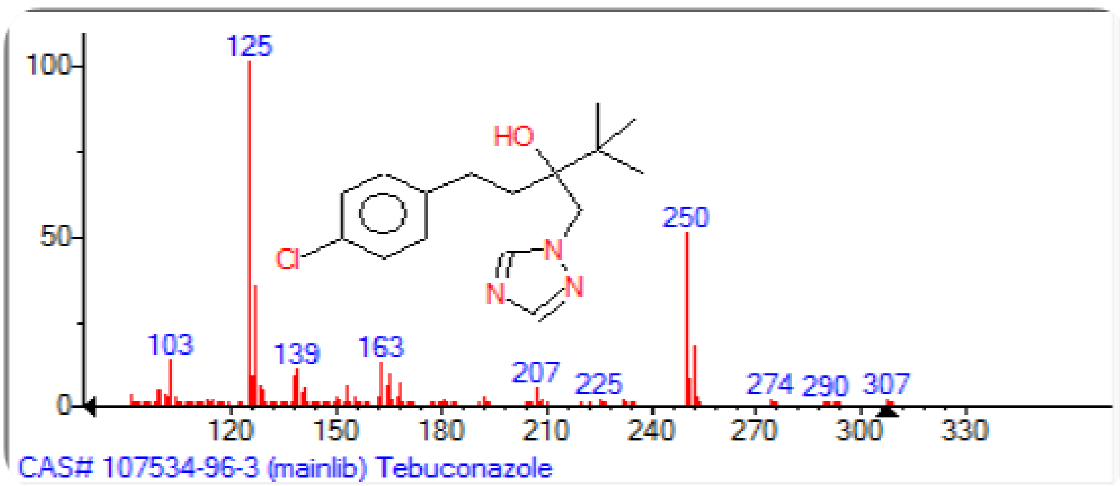

Figure 2B. Tebuconazole GCMS Spectra match with NIST Library.

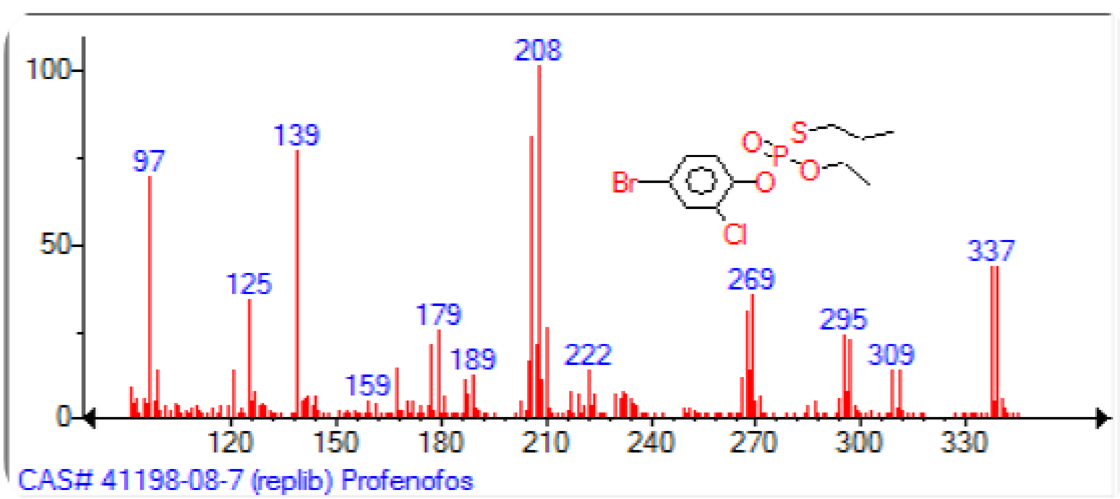

Figure 2C. Profenofos GCMS Spectra match with NIST Library.

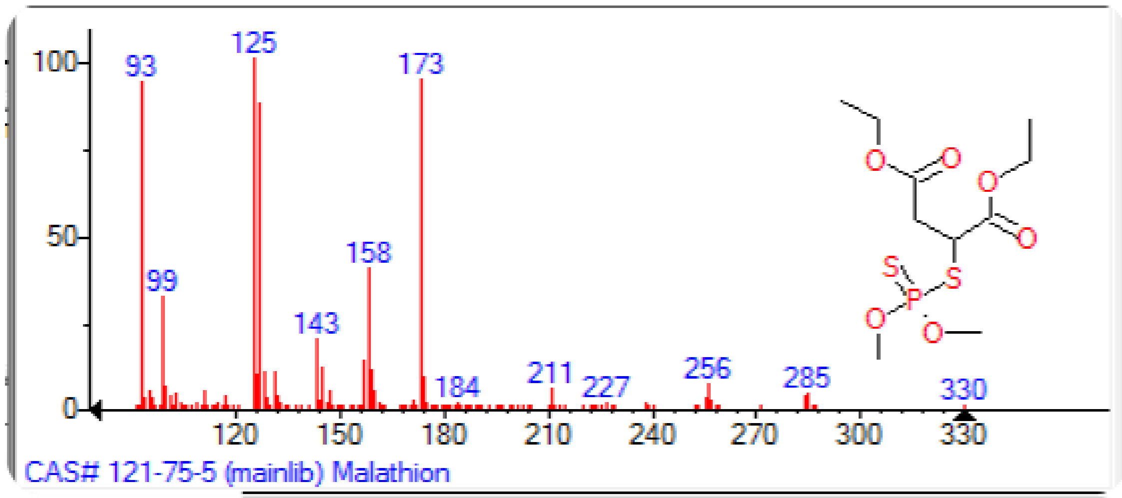

Figure 2D. Malathion GCMS Spectra match with NIST Library.

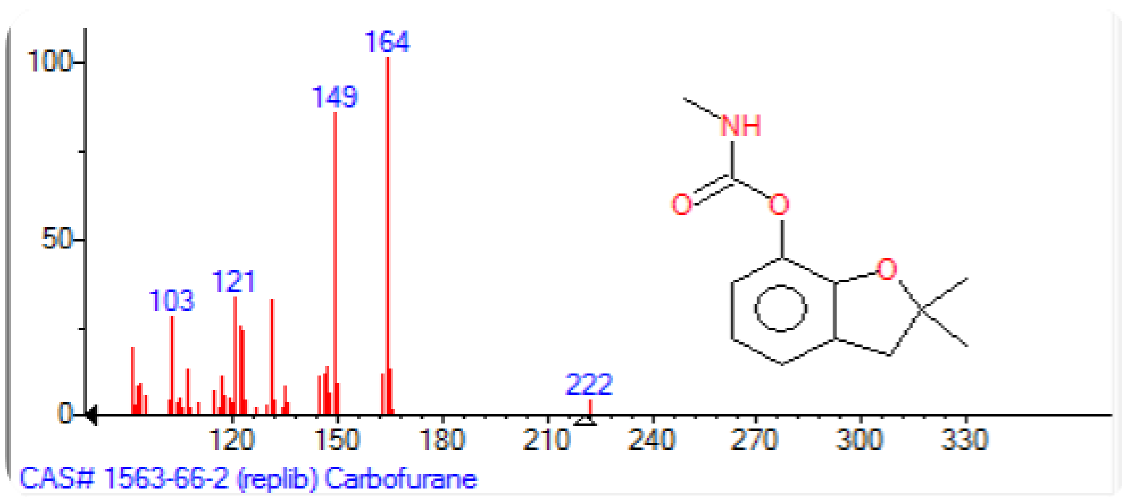

Figure 2E. Carbofuron GCMS Spectra match with NIST Library.

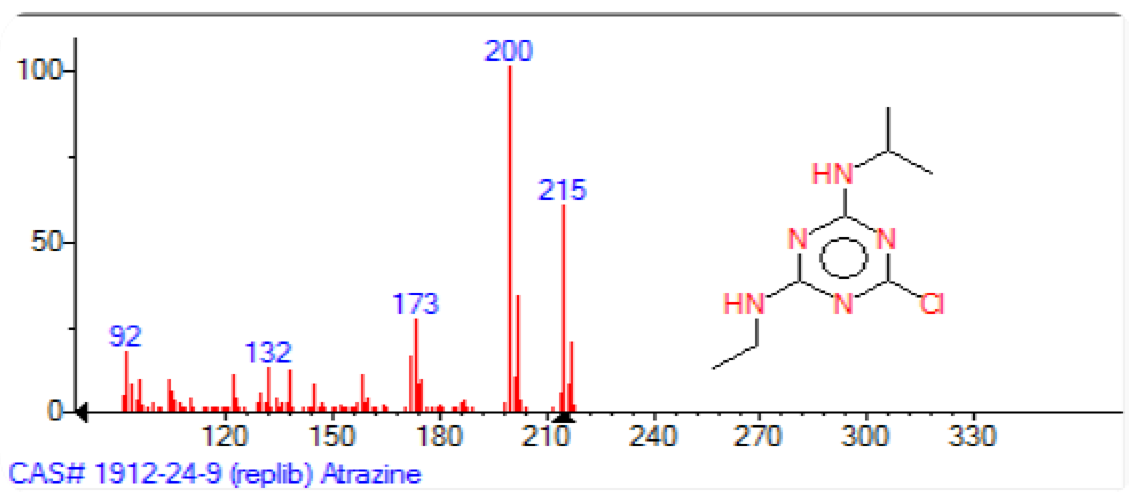

Figure 2F. Atrazine GCMS Spectra match with NIST Library.

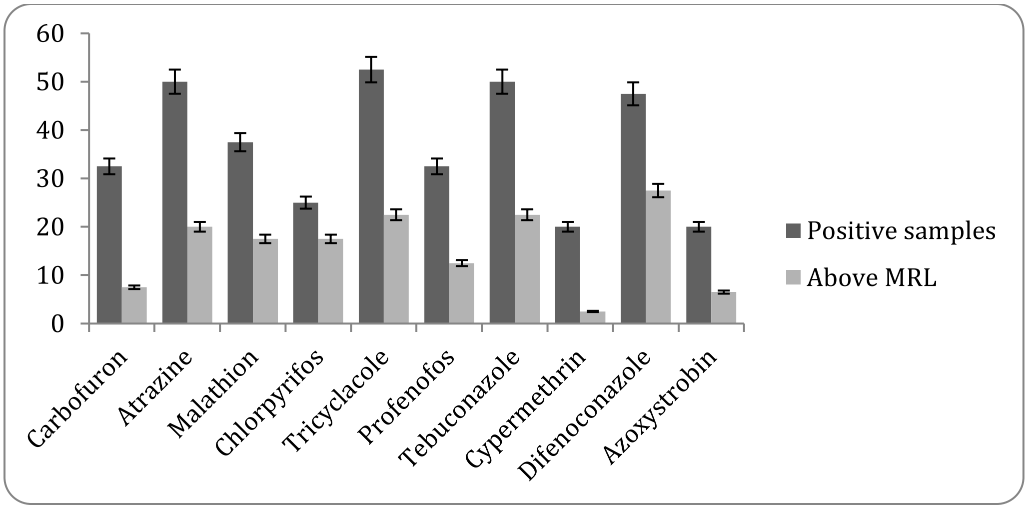

Figure 3. Graphical Representation of results of different pesticide residues.

DISCUSSION

Results of the present study showed four fungicides (Difenoconazole, Tricyclazole,Tebuconazole and Azoxystrobin are present in almost 50 % of the samples analyzed from these four districts. Among these Azoxystrobin residues were under MRL, while Difenoconazole, Tricyclazole, Tebuconazole were present above MRLs in almost all the samples. Grey mould (Botrytis cineria) and Powdery mildew (Sphaerotheca macularis f. sp. Frgariae) are two common diseases of strawberry that infects the fruit and leaves hence cause significant yield losses (Petrasch *et al.,* 2019). High amounts of fungicides are sprayed on strawberries to control these two diseases. Previous studies also support this fact as Malhat *et al*., 2019 reported that residues of botrytis fungicides are frequently found in marketed strawberry fruit. Moreover, distributors usually do not wash strawberries prior to distribution, growers also apply fungicides shortly before harvest to lengthen the consumer /retailer shelf life. Fungal attack becomes severe with increase in temperature and rainfall during fruiting stage. The outer surface of strawberry is rough which may also account for higher levels of fungicides, because rough surfaces slowdown degradation process. Our results are in line with previous studies showing fungicide residues in strawberries as reported by Sojka *et al*., 2015 that fungicide residues azoxystrobin, boscalid, difenoconazole, tebuconazole, tetraconazole, pyrimethanil, fenhexamid, pyraclostrobin, thiophenate methyl fludioxanilfolpet, dithiocarbamate, cyprodinil, and insecticide chlorpyrifos residues are found in strawberries. Pesticide residues found in banana during a study were mostly fungicides like thiabendazole, propiconazole, and imazalil; the insecticide Chlorpyrifos (Gomes *et al*., 2020). The main insect of strawberry is the strawberry weevil '(*Anthonomus signatus*) (Mozuraitis *et al.,* 2020). Carbofuron is a carbamate applied for the control of weevil Profenofos, Malathion and Chlorpyrifos are all organophosphates applied for the insect control. Similarly, Cypermethrin is a synthetic parathyroid. Parathyroid and carbamates are more persistent than organophosphates.

CONCLUSION

Results of this study were interpreted in relation to Maximum residue levels (MRLs) requirements set in legal European Union (EU) regulation for products present in the market. Results of the study indicated that strawberries analyzed during the study have high levels of different pesticides in almost all samples. This is due to indiscriminate pesticide use and lack of awareness over fruits and vegetables. It is highly dangerous as theses commodities are consumed fresh. Due to less time interval given between their harvest and consumption risk of pesticide poisoning is high. Lack of decision maker and employee's training make it more adverse. Managers and all the employees should be trained properly for different operations. This information could be useful for policy makers, government agencies and the public at large.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors have equal contributions.

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