Original Research Article

Pesticide Residues in Vegetables Collected from Different Markets of Navsari District of India

ABSTRACT

A study was conducted during 2018-19 in Navsari district of Gujarat, India to investigate the presence of pesticide residues in vegetables *viz.* brinjal, cabbage, chilli, okra, and tomato. The analytical scope of pesticide residues comprised of Gas Chromatography (GC) and Liquid Chromatography (LC) amenable 111 pesticides. A total of 180 samples of five vegetables collected from six different talukas during pre and post-monsoon season of which 75 (41.67%) were positives samples, 25 (13.89%) samples with multiple pesticide residues, 20 (11.12%) samples with pesticide residues above MRL (Maximum Residue Limit) and 105 (58.34%) samples were found residues free. Among the studied talukas, maximum positive vegetable samples of pesticide residues were found in Navsari (22) followed by Jalalpore (16), Chikhli (13), Vansda (11), Gandevi (9) and Khergam (4). In case of vegetables, maximum positive samples to pesticide residues were found in chilli (31) followed by okra (18), cabbage (12), brinjal (7) and tomato (7). The study revealed that the vegetables producing farmers of the Navsari region of India were not following the standard pre-harvest intervals, proper instructions concerning the application of pesticides in appropriate concentration, time and type of pesticides used. It has also thought that there is a need for measures to be implemented towards educating farmers against harmful effects of using the pesticides that may not only harm the health of the human beings but also damage the environment.

Keywords: MRL, Navsari, pesticide residues, vegetables

1. INTRODUCTION

Vegetable cultivation is an important part of agricultural activities because majority of the Indian population is vegetarian which creates a higher demand. To satisfy these demand farmer communities bend toward pesticide application to overcome crop pests. To eliminate pest attack and maintain the yield of the crop the application of the pesticides increases in modern agriculture. In this way, to increase both animals and crop outputs, improve the quality of products and decrease the incidence of illnesses propagated by insects [1]. Approximately one-third of the agricultural products are produced and protected by using pesticides [2]. The approximate loss in yield of fruits, vegetables, and cereals from pest injury would reach 78%, 54%, and 32% respectively [3]. The other side of the coin represents that pesticides cause many problems by contamination and direct exposure to the non-targeted organism. The effect of pesticides on human health are impaired memory and concentration, disorientation, severe irritability, confusion, headache, difficulties, insomnia, dizziness, tremor, nausea, breathing and abnormal cramps and sweating, *etc*. [4]. Comparatively, children are more susceptible to pesticide exposure [5]. Anyway, our reliance on pesticides is difficult to sustain because of unintended long-term negative impacts on the environment and human health in particular neurological effects, respiratory and reproductive effects, and cancer [6]. Thus, total control over pesticide residues is not possible. So, the governments of different countries had been trying enacted legislation to minimize the consumer exposure to harmful pesticides, and regulate the appropriate use of pesticides in terms of that grant, different type of registration (application rates and pre-harvest intervals) by an authorized agency and allowing for free deliberation as to which products are to be treated with pesticides as long as the treatment complies with the established maximum residue limits (MRLs) [7]. To protect consumers from exposure to violate levels of pesticide residues in food commodities, the European Commission and CODEX have established MRLs [8, 9].

A few studies for monitoring of pesticide residues in vegetables have been conducted to know contamination in daily basis food, pesticide application pattern, their environmental load, and health risk assessment. However, monitoring studies of pesticide residues in vegetables in India were insufficient. In the production of fruits and vegetables, insecticides are used to control pests and

fungicides to control diseases. Pesticides are applied directly to the crops and some of the pesticides and their metabolites may still be present as residues in the fruits and vegetables after their harvest. It is a universal truth that most insecticides and fungicides are toxic in nature but when used properly they account for an important input in fruits and vegetable production to produce economically marketable products. However, their improper and unintended use creates a problem for human being and the environment [2]. There was a scarcity of information about pesticide contamination in agricultural products, particularly in vegetables, with pesticide residues in the Navsari district, Gujarat, India. Fruits and vegetables have been given a lot of attention in monitoring programs because most of them are eaten raw and it is expected that they contain higher pesticide residue levels as compared to other food groups of plant origin [10]. The fruits and vegetables are highly susceptible to pest attacks because of higher moisture content and tender nature. Thus, the predominant purpose of this present study was to determine the status of pesticide residues in commonly used annual vegetable samples collected from different talukas of Navsari district of Gujarat, India to establish a database that includes the levels of these pesticide residues in vegetables of the particular region and the obtained findings will provide the scientific evidence for agricultural authorities to control and implements recommendation for pesticides use pattern and management of pesticide residues in food commodities to escape human and the environment from their hazardous effects.

2. MATERIALS AND METHODS

2.1 Chemical and reagents

Analytical grade reagents used in the study had purity ≥ 99%. UHPLC-grade acetonitrile, acetone, and hexane were procured from MERCK, Germany. Anhydrous magnesium sulphate and sodium acetate were obtained from MERCK, Germany, and Fisher Chemical, UK, respectively. The certified reference materials of pesticide active ingredient were taken from Sigma-Aldrich, Belgium. Primary and Secondary Amine (Ethylenediamine-N-propyl, particle size 40 µm) was obtained from SUPELCO, USA. Water was produced locally through a Milli-Q water purification system.

2.2 Sampling design

A total of 180 samples of vegetables including brinjal, cabbage, chilli, okra, and tomato collected from six different talukas (a subdivision of a district) *viz.,* Navsari, Jalalpore, Gandevi, Chikhli, Khergam and Vansda of Navsari district (Fig. 1). Samples were collected twice in a year (pre-monsoon and post-monsoon) and a total of five commodities (1.0 kg each from 3 different randomly selected vendors in the market of each taluka in two seasons). Samples were enclosed in a clean blotting paper and placed in plastic bags. The interview with the local vendors revealed that most of the vegetables originating from different farmers of the neighbouring villages. Therefore, they were considered as representative samples of this region. The collected samples were coded separately and brought to the Food Quality Testing Laboratory, N.M. College of Agriculture, Navsari Agricultural University, Navsari, and kept at 4°C later were subjected to homogenization.

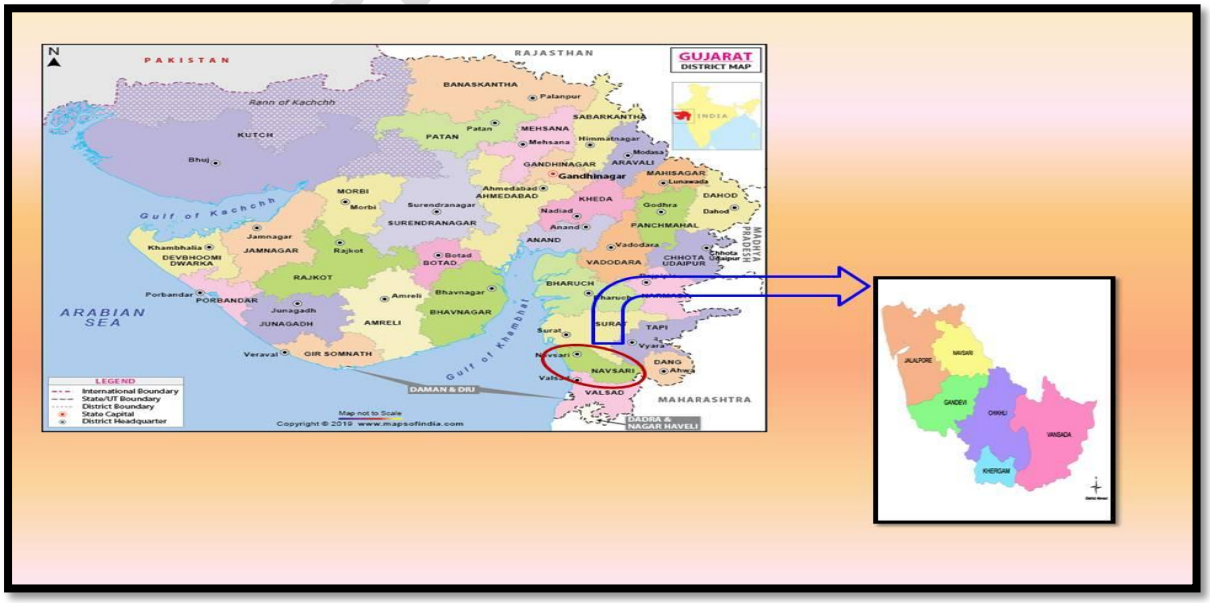

Fig. 1: Pesticide residue survey location of six talukas of Navsari District, Gujarat [11]

2.3 Pesticides extraction and clean up

The AOAC official method 2007.01 [12] used for the multi-residue extraction and clean-up were performed using the QuEChERS method commonly used in the analysis of food matrices. Each sample (approx. 500 g) was homogenized with a robot coupled homogenizer at 14000-15000 rpm for 2-3 minutes and avoided cross-contamination between samples thoroughly washing the grinder every time. Precisely taken 15.0±0.1g representative sample from homogenized vegetable (brinjal, cabbage, chili, okra, and tomato) sample having water ≥ 80% in 50 mL capacity of polypropylene tube followed by adding 15 mL of 1% Acetic Acid in Acetonitrile (v/v) and shaken it vigorously for 2 minutes and kept into the deep freezer for 20-30 min. at temp. -20°C. A mixture of 6 g Magnesium Sulfate and 1.5 g Sodium Acetate added to the extract in the tube which was centrifuged for 3 min at 3500 rpm. Transferred the 6.0 mL of an aliquot in 15 mL capacity centrifuge tube having 0.900g $MgSO_4$ and 0.300 g PSA (Primary Secondary Amine) and again, centrifuged it for 2 minutes at 2500 rpm to get clean supernatant. Taken 2-2 mL of an aliquot in two different 15 ml capacity test tubes (one tube for GC-MS and one tube for LC-MS/MS injection) and evaporated both tubes to near dryness with nitrogen gas by Turbo Vap at 40°C. For LC-MS/MS injection made up the volume to 4 mL using acetonitrile (HPLC grade) and the residue was reconstituted and extract filtered it into the glass vial through PTFE filter (0.2µm) and Quantified on LC-MS/MS and similarly, for GC-ECD injection made up the volume to 2 mL using n-Hexane: Acetone (9:1) and the residue was be reconstituted and extract filtered it into the glass vial by PTFE filter (0.2µm) and quantified on LC-MS/MS or GC-ECD.

2.4 Scope of pesticide residues analysis

A total of 111 pesticides were analyzed in vegetable samples. Out of a total of 111 pesticides, 85 pesticides were analyzed on LC-MS/MS and remainder 26 pesticides were on GC-ECD. GC-MS was used for ion confirmation for 26 analytes.

2.4.1 Gas Chromatography-Electron capture detector (GC-ECD) Analysis

A total of 26 compounds were analyzed with the help of a Thermo Scientific Trace 1310 gas chromatograph with an auto-sampler (AS 1310), coupled with an Electron Capture Detector (ECD). The separation was performed on a Restek- Rxi-5ms capillary column (30 m long × 0.25 mm internal diameter × 0.25 µm film thickness). Sample injection was operated in the splitting mode 1:10, with an injector temperature of 250 ºC and the detector temperature was 300 ºC. The oven temperature was held initially at 110 ^{o}C for 1 minute, programmed from 110 to 210 ºC at 10 ºC/min. and held for 1 minute at 210 ºC, further programmed from 210 to 300 ºC at 5 ºC/min. and finally held at 300 ºC for 6 minute. Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min. and nitrogen used as a makeup gas with a flow of 25.0 mL/min. The Thermo Scientific Chromeleon 7.2 version software was used for system control, data analysis and acquisition.

2.4.2 Liquid Chromatography-Tandem Mass Spectrometry Analysis

A total of 85 pesticides were analyzed by LC-MS-MS. The analysis was operated using a liquid chromatograph (Thermo Scientific Dionex UltiMate-3000) coupled with triple quadrupole mass detector (QqQ Thermo Scientific Quantum Access Max) and analytes separation was carried out with the help of an AccucoreC_{18} Column (100 mm × 2.1 mm × 2.1µm). The mobile phase-A consisted of Deionized water + 0.1% Formic acid + 5mM Ammonium Formate and mobile phase-B had Methanol water + 0.1% Formic acid + 5mM Ammonium Formate and the flow rate was set at 0.3 mL/min. The injection volume was 5 µL. The column temperature was set at 25 ºC, and the injection volume was kept 5 µL. The ion spray voltage capillary needle was maintained at 4500 V and Vaporizer temperature kept at 350 ºC with the capillary temperature of 325 ºC. Each compound was monitored and quantified by the multiple reaction monitoring (MRM) transitions. Prior MS/MS conditions were optimized with identification of the parent and product ions, as well as the selection of the tube lens and collision energy.

2.5 Method validation

Validation is the process of verifying that a method is fit for the intended purpose. The analytical method was validated according to the single laboratory validation approach of the SANTE (2017) [13]. The validation parameters were including linearity, the limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision (Table: 1 & 2). A linearity study was performed to determine the performance of GC-ECD. To establish the linearity in GC-ECD, five different concentrations of the standards *viz*., 100, 300, 400, 500 and 600 ng/mL and for LC-MS/MS, six *viz*., 2.5, 5, 7.5, 25, 50, and 75 ng/mL were injected. The data obtained in the linearity study were used to work out the regression equation and coefficient of determination (R^2). The LOD and LOQ were determined based on signal to noise ratio (S: N) of 3 and 10, respectively. The accuracy (% recovery) and precision (% RSD) of the analytical method was conducted for two different matrices *i.e.* tomato

and okra at two fortification levels with three replications and an untreated sample and reagent blank. The accuracy (% recovery) was calculated by dividing the recovered concentrations by fortified concentration, and precision (within-laboratory reproducibility, % RSD) was obtained by dividing the standard deviation of response by the mean response.

3. EXPERIMENTAL RESULTS

3.1 Method Validation

In the method validation study of the extraction and analysis methods five parameters were validated: linearity, the limit of detection (LOD), the limit of quantification (LOQ), accuracy (% recovery), and precision (%RSD) [13]. The linearity dynamic range of 26 pesticides on GC-ECD was in the range of 100 to 600 ng/mL and 85 pesticides on LC-MS/MS were obtained between 2.5 to 75 ng/mL with the coefficient of determination (R^2) in the range of 0.986 to 0.999 and 0.981 to 0.999, respectively. The LOD and LOQ for 26 pesticides on GC-ECD ranged from 15.67 to 48.8 ng/mL and 52.23 to 161.9 ng/mL, respectively (Table 1). Similarly, LOD and LOQ for 85 pesticides on LC-MS/MS worked out in the range of 0.95 to 3.03 ng/mL and 3.174 to 10.124 ng/mL (Table 2). The % recovery of 26 pesticides on GC-ECD for tomato and okra matrices at 200 ng/g was obtained in the range of 88.57±1.78 to 105.52±3.15% and 74.97±1.30 to 102.97±1.85%, respectively, and similarly for both matrices at 400 ng/g were 88.31±3.96 to 100.49±2.32% and 81.19±2.10 to 99.40±1.30%, respectively. The % RSD of 26 pesticides on GC-ECD for tomato and okra matrices at 200 ng/g were obtained in the range of 0.62 to 3.96% and 0.59 to 4.21%, respectively, and similarly for tomato and okra at 400 ng/g were 1.16 to 4.49% and 0.68 to 4.19%, respectively (Table: 1). The % recovery of 85 pesticides on LC-MS/MS for tomato and okra matrices at 10 ng/g was obtained in the range of 75.46±3.57 to 101.41±7.22% and 75.83±4.15 to 93.86±3.95%, respectively, and similarly for both matrices at 25 ng/g were 77.11±5.50 to 107.8±5.15% and 75.89±4.79 to 103.70±8.62%, respectively. The % RSD of 85 pesticides on LC-MS/MS for tomato and okra matrices at 10 ng/g were obtained in the range of 1.21 to 8.74% and 1.27 to 11.56%, respectively, and similarly for tomato and okra at 25 ng/g were 1.40 to 9.37% and 1.13 to 9.86%, respectively (Table 2).

Table 1. Characteristics and method validation parameters of 26 pesticides on GC-ECD

Pesticides	R^2	LOD (ng/mL)	LOQ (ng/mL)	Accuracy (% recovery) and Precision (% RSD)							
				Tomato (ng/g)				Okra (ng/g)			
				200		400		200		400	
				Mean Rec. (%)	% RSD	Mean Rec. (%)	% RSD	Mean Rec. (%)	% RSD	Mean Rec. (%)	% RSD
Metolachlor	0.998	28.48	94.96	101.0	1.96	95.37	1.58	102.97	1.80	99.40	1.48
HCH-alpha	0.992	25.34	84.46	92.31	0.84	90.97	2.28	78.86	1.65	81.40	1.99
HCH-beta	0.998	29.52	98.40	95.16	1.94	96.32	1.35	79.85	3.15	83.60	2.59
HCH-gamma	0.991	28.89	96.30	105.52	2.99	93.46	2.85	80.29	2.65	84.48	2.35
Fluchloralin	0.998	19.33	64.45	94.59	1.33	99.21	1.21	80.15	0.96	81.19	2.59
Chlordane	0.997	29.90	99.67	97.54	0.62	96.18	1.38	84.88	3.59	88.34	1.57
HCH-delta	0.999	22.04	73.49	88.57	2.01	90.51	1.69	77.19	1.50	85.20	2.34
Metribuzin	0.997	24.66	82.23	101.0	1.09	96.80	1.34	86.74	1.43	84.57	1.82
Methyl-parathion	0.991	29.18	97.28	97.43	2.17	93.83	1.65	79.90	1.60	83.41	1.07
Fipronil-desulfinyl	0.996	28.45	94.83	95.84	0.77	91.15	1.74	85.04	1.24	88.13	3.09
Aldrin	0.994	17.50	58.36	93.25	3.96	96.18	2.66	87.46	1.77	91.45	1.39
Heptachlor	0.986	19.36	64.56	95.49	3.09	93.05	2.89	83.99	0.59	92.74	0.68
Fipronil	0.988	29.89	99.63	98.23	2.51	97.24	1.88	97.31	3.90	96.56	2.70
Butachlor	0.987	40.88	136.8	99.79	3.75	93.37	3.89	96.58	3.61	94.46	1.56
Hexaconazole	0.997	21.63	72.10	99.69	2.27	93.82	1.46	82.90	1.65	91.02	2.77
P - P' – DDE	0.999	28.25	94.17	94.32	3.77	97.85	1.28	87.25	1.10	91.02	2.77
Fipronil-sulfone	0.993	29.61	98.70	97.12	3.72	92.32	1.26	87.04	0.83	92.22	2.05
P - P' – DDD	0.998	15.67	52.23	97.28	2.52	96.15	1.23	79.84	2.94	84.89	2.30
Edifenphos	0.992	18.87	62.93	98.66	1.86	95.75	1.38	79.20	3.04	82.99	2.45
P - P' – DDT	0.998	22.58	75.29	94.16	3.68	97.82	1.16	84.10	2.66	82.49	3.25

Fluopicolide	0.995	27.85	92.86	97.63	212	100.4	2.30	86.49	1.39	90.90	1.42
Bifenthrin	0.992	38.18	127.3	93.18	2.33	88.31	4.49	75.02	4.21	83.61	3.64
Lambda-cyhalothrin	0.996	18.70	62.36	92.21	1.88	95.71	1.25	74.97	1.74	82.69	2.70
Permethrin	0.987	48.58	161.9	97.84	3.34	94.01	1.80	87.40	3.64	83.66	4.19
Cypermethrin	0.990	18.71	62.36	93.36	2.45	95.99	1.64	75.75	2.43	83.00	2.76
Deltamethrin	0.998	23.79	79.32	95.12	3.83	95.44	1.64	76.86	2.46	82.12	3.30

Table 2. Characteristics and method validation parameters of 85 pesticides on LC-MS/MS

Pesticides	R^2	LOD (ng/mL)	LOQ (ng/mL)	Accuracy (% recovery) and Precision (% RSD)							
				Tomato (ng/g)				Okra (ng/g)			
				10		25		10		25	
				Mean Rec. (%)	% RSD	Mean Rec. (%)	% RSD	Mean Rec. (%)	% RSD	Mean Rec. (%)	% RSD
Cyromazine	0.999	2.668	8.893	86.96	3.30	84.61	5.83	82.39	7.90	87.61	5.29
Acetamiprid	0.999	3.037	10.124	92.54	2.56	94.22	3.78	86.10	5.11	91.43	1.42
Pymetrozine	0.996	1.220	4.065	75.46	4.61	80.15	4.73	83.03	4.75	84.47	2.47
Carbendazim	0.993	1.307	4.356	82.60	6.39	86.34	9.37	80.33	6.79	79.79	7.09
Mevinphos	0.996	1.095	3.649	93.79	7.91	87.16	8.76	87.27	2.23	86.66	1.81
Pirimicarb	0.996	1.498	4.992	91.72	3.81	80.65	1.46	86.30	4.24	89.61	3.13
Ethirimol	0.994	1.575	5.250	79.90	3.70	86.59	1.79	84.72	3.04	89.30	3.92
Oxamyl+ NH_4	0.997	1.360	4.532	93.26	4.69	88.19	2.91	88.11	7.50	92.25	4.32
Oxadixyl	0.991	1.214	4.047	82.94	6.95	78.48	6.37	89.91	5.86	91.46	3.74
Carbetamide	0.996	1.015	3.382	89.61	6.89	94.37	8.69	85.41	2.50	88.86	3.91
Metribuzin	0.996	1.133	3.776	87.90	3.60	87.75	1.40	91.68	2.90	91.62	4.43
Propoxur	0.999	1.576	5.252	78.25	8.69	84.66	6.04	81.73	7.77	86.54	6.45
Carbofuran	0.998	1.541	5.136	89.51	7.34	86.38	8.92	88.46	7.84	86.29	7.23
Bendiocarb	0.995	1.623	5.411	77.44	5.10	80.56	3.80	79.51	6.39	85.52	5.13
Carbaryl	0.997	1.081	3.602	90.34	5.65	91.43	8.55	83.18	4.46	81.73	5.97
Monolinuron	0.998	2.052	6.840	80.27	7.11	86.75	7.30	88.88	7.10	88.96	3.22
Prometon	0.994	1.559	5.196	80.59	2.78	82.36	4.51	89.77	2.31	89.77	2.75
Secbumeton	0.993	2.373	7.909	75.46	4.93	78.83	7.10	86.47	3.40	82.50	1.38
Terbumeton	0.998	1.015	3.382	87.10	4.58	86.11	5.27	78.99	6.98	84.90	6.12
Metobromuron	0.999	2.511	8.370	85.19	3.48	83.61	5.58	85.55	6.45	83.84	6.50
Propham	0.998	0.957	3.187	91.90	4.55	83.99	2.74	91.99	5.74	85.21	7.01
Ametryn	0.990	2.178	7.260	84.09	8.02	85.20	9.34	87.25	4.25	88.10	1.13
Methoprotryne	0.999	0.976	3.253	79.73	4.16	86.75	3.61	82.19	10.51	80.15	4.73
Imazalil	0.993	2.945	9.818	81.74	4.43	86.42	2.25	89.34	2.21	86.96	4.01
Cycluron	0.996	2.775	9.249	85.07	6.00	92.35	5.23	84.86	5.02	92.82	4.14
Isoproturon	0.999	2.919	9.730	89.25	6.34	89.25	5.44	82.31	8.79	92.96	5.94
Metalaxyl	0.986	2.923	9.742	77.78	6.09	80.78	1.60	89.88	2.06	80.46	4.25
Forchlorfenuron	0.991	2.555	8.515	83.42	5.30	94.62	5.85	86.90	5.82	84.86	7.62
Chlorantraniliprole	0.993	2.347	7.824	92.63	7.98	88.15	4.34	88.01	9.68	92.01	5.15
Phenmedipham	0.990	2.718	9.060	85.54	8.30	86.66	7.14	82.48	3.74	84.34	1.92
Diethofencarb	0.991	2.812	9.373	87.27	6.65	93.19	6.11	84.72	9.24	83.59	7.01
Linuron	0.991	2.701	9.002	99.43	4.68	107.8	4.78	80.90	5.58	86.81	3.25
Prometryn	0.988	1.610	5.367	91.09	5.23	92.16	6.32	89.94	1.65	83.08	3.22
Fenobucarb	0.990	1.514	5.248	80.79	6.38	88.45	5.54	82.47	6.67	84.90	4.77
Terbutryn	0.992	2.736	9.120	91.57	5.53	90.94	4.42	98.17	8.78	103.7	8.32
Fenamidone	0.990	2.740	9.133	83.12	8.74	90.50	7.95	84.08	8.36	81.87	6.18
Mandipropamid	0.989	2.643	8.810	84.87	2.00	85.40	5.12	88.46	5.68	87.17	6.52
Flutolanil	0.990	2.889	9.628	83.16	6.67	79.65	6.28	86.39	2.58	89.46	1.27
Promecarb	0.999	1.554	5.179	75.49	4.93	80.71	7.84	82.52	3.33	80.15	4.79
Dimethomorph	0.993	2.873	9.576	90.65	7.46	90.51	5.54	86.75	4.84	91.05	6.70
Methoxyfenozide	0.993	2.021	6.738	85.85	4.28	86.83	7.32	77.06	1.67	86.41	4.43
Spiroxamine	0.996	1.481	4.936	83.26	6.11	83.43	2.08	91.27	3.76	92.91	6.54
Bromuconazole	0.995	2.581	8.602	88.78	6.85	94.13	7.10	89.45	7.44	91.63	6.50

Mefenact	0.995	2.110	7.035	82.00	6.15	83.27	6.46	82.04	5.18	88.12	7.04
Iprovalicarb	0.992	1.429	4.762	79.39	7.32	84.72	8.34	81.95	2.26	83.34	2.16
Cyproconazole	0.998	2.007	6.691	84.62	4.65	80.63	8.24	80.93	2.17	83.01	1.62
Mepanipyrim	0.988	2.298	7.659	86.18	6.72	85.24	6.22	82.58	5.22	77.47	4.47
Chloroxuron	0.991	1.821	6.069	81.15	6.27	87.46	8.69	77.16	6.11	78.31	8.76
Fenhexamid	0.994	2.482	8.274	84.33	2.85	89.98	3.11	84.67	3.97	84.02	5.36
Myclobutanil	0.984	1.600	5.335	83.03	4.36	82.25	1.78	89.34	5.71	85.20	9.48
Spirotetramat	0.998	1.471	4.921	101.4	7.12	98.09	6.04	90.94	4.54	89.67	6.53
Fluoxastrobin	0.993	1.868	6.225	81.74	3.02	84.13	2.64	85.07	4.34	81.17	9.52
Butafenacil [M+NH_4]	0.992	1.575	5.250	89.42	4.19	91.44	5.65	93.86	4.21	94.40	6.90
Flufenacet	0.995	2.263	7.544	84.99	4.71	84.57	4.41	93.68	5.86	95.24	5.24
Triticonazole	0.996	1.922	6.406	85.22	3.36	86.43	2.29	86.55	4.54	89.19	4.08
Epoxiconazole	0.992	2.538	8.461	80.77	5.18	89.16	6.08	89.83	6.46	91.66	5.20
Tetraconazole	0.991	1.685	5.617	81.79	5.11	82.36	2.81	82.12	2.02	82.15	2.16
Fenbuconazole	0.993	2.629	8.763	78.08	5.35	89.02	4.53	85.88	2.27	88.42	3.92
Dimoxystrobin	0.998	2.515	8.383	81.69	3.01	87.14	5.76	77.57	7.58	84.64	4.47
Fenoxycarb	0.998	2.762	9.207	90.96	5.45	86.05	3.23	81.77	8.09	84.84	5.68
Kresoxim methyl	0.983	1.636	5.452	88.41	5.71	86.62	6.12	90.61	6.89	93.84	8.29
Diclobutrazole	0.995	1.248	4.162	84.49	7.05	80.93	1.88	86.76	5.21	88.99	7.42
Tebufenozide	0.997	2.232	7.438	77.33	5.16	83.09	4.46	75.83	5.47	80.15	1.82
Picoxystrobin	0.999	2.808	9.359	90.78	7.59	94.57	6.04	88.33	6.70	86.27	6.98
Penconazole	0.982	1.693	5.645	83.57	5.44	87.49	9.23	86.96	8.85	90.86	8.21
Tebuconazole	0.998	2.687	8.957	87.17	5.21	85.62	4.53	85.94	3.76	87.89	8.58
Propiconazole	0.994	2.312	7.707	85.73	5.22	84.47	2.05	83.31	8.52	85.42	6.32
Metaconazole	0.999	2.250	7.50	76.69	7.43	77.11	7.14	76.84	6.86	75.89	6.31
Pyraclostrobin	0.995	2.490	8.299	82.93	3.49	84.73	1.43	86.02	7.30	86.78	4.61
Thiobencarb	0.999	2.403	8.011	85.30	4.80	83.43	2.72	90.39	7.99	93.90	4.34
Pencycuron	0.998	1.266	4.219	85.13	2.56	85.29	3.26	89.05	4.70	92.47	5.96
Diniconazole	0.991	1.189	3.962	83.75	1.80	84.12	3.10	79.52	4.01	88.04	7.69
Spinosad-D	0.997	1.500	5.001	83.55	8.05	83.02	4.76	87.03	1.27	90.07	3.55
Trifloxystrobin	0.995	2.767	9.222	85.66	4.57	93.14	5.39	83.39	6.08	83.10	6.56
Triflumizole	0.991	1.566	5.222	79.64	4.68	81.69	7.85	80.42	10.84	87.14	7.22
Buprofezin	0.990	1.529	5.095	84.81	2.82	91.65	5.24	85.25	6.59	83.50	4.37
Tebufenpyrad	0.998	2.460	8.199	84.04	3.40	79.42	6.08	83.18	7.04	86.43	5.03
Piperonyl-butoxide [M+NH_4]	0.994	2.456	8.188	89.05	5.00	86.34	6.07	82.28	8.87	82.46	3.89
Emamectin B1b benzoate	0.996	1.535	5.118	81.43	4.12	80.75	6.94	87.92	4.84	92.85	5.19
Quinoxyfen	0.992	1.556	5.188	85.16	1.21	84.46	3.66	83.51	7.38	90.79	5.10
Hexythiazox	0.989	2.074	6.913	80.58	7.54	84.25	4.08	83.45	5.59	85.08	6.51
Emamectin B1a benzoate	0.999	1.571	5.238	80.19	5.42	78.33	4.44	82.80	6.43	89.17	7.93
Etoxazole	0.996	2.328	7.758	85.66	4.39	82.77	7.18	84.13	6.34	88.69	8.04
Propargite [M+NH4]	0.998	0.952	3.174	82.24	4.49	85.94	5.05	83.04	11.56	87.69	7.73
Spirodiclofen	0.981	2.081	6.937	87.25	3.64	86.57	2.74	81.56	4.29	80.71	9.86

3.2 Status of pesticide residues in different talukas of Navsari District

A total of 180 samples of different vegetables were collected from six talukas of Navsari district in pre- and post-monsoon season. So, a total of 30 samples were collected from each taluka and each talukas result was presented in the Table 3. Among the talukas, highest positive vegetable samples were found in Navsari 22 (73.34%) followed by Jalalpore 16 (53.34%), Chikhli 13 (43.34%), Vansda 11 (36.67%), Gandevi 9 (30.0%) and Khergam 4 (13.34%). However, maximum samples with multiple residues were found in Navsari 9 (30.0%) followed by Chikhli 8 (26.67%), Jalalpore 2 (6.67%), Gandevi 2 (6.67%), Khergam 2 (6.67%) and Vansda 2 (6.67%). The positive samples >MRL values were also maximum in Navsari 8 (26.64%) followed by Chikhli 3 (10.0%), Khergam 3 (10.0%), Jalalpore 2 (6.67%), Gandevi 2 (6.67%) and Vansda 2 (6.67%). Out of total 180 samples 75 (41.67%) were found positive with pesticides residues, 25 (13.89%) were found with multiple residues and 20 (11.12%) were had residues >MRL value.

Table 3. Status of pesticide residues in different talukas of Navsari district

Taluka	Commodity	Sample analyzed	Positives sample	Samples with Multiples residues	Positives samples >MRL
Navsari	Brinjal, cabbage, chilli, okra and tomato	30	22 (73.34%)	9 (30.0%)	8 (26.64%)
Jalalpore		30	16 (53.34%)	2 (6.67%)	2 (6.67%)
Gandevi		30	9 (30.0%)	2 (6.67%)	2 (6.67%)
Chikhli		30	13 (43.34%)	8 (26.67%)	3 (10.0%)
Khergam		30	4 (13.34%)	2 (6.67%)	3 (10.0%)
Vansda		30	11 (36.67%)	2 (6.67%)	2 (6.67%)
Total		**180**	**75 (41.67%)**	**25 (13.89%)**	**20 (11.12%)**

3.3 Status of pesticide residues in different vegetables

This study aimed to determine the degree of pesticide residues in annually available vegetables of Navsari district. A total of 180 samples of five vegetables were collected from different talukas of Navsari. However, a total of 36 samples of each vegetable were collected from two seasons. Among the vegetables maximum positive samples with pesticide residues were in chilli (31), followed by okra (18), cabbage (12), brinjal (7) and tomato (7). However, maximum samples with multiple residues was found in chilli (15) followed by okra (5) and cabbage (5). The higher positive sample had residues greater than MRL values were found in chilli (8) followed by cabbage (6), brinjal (4) and okra (2). The status of pesticide residues in different vegetables was presented in Table 4.

Table 4. Status of pesticide residues in different vegetables

Vegetables	Samples analyzed	Positives sample	Samples with multiples residues	Positives samples > MRL	Pesticide Detected (ng/g)
Brinjal	36	7	0	4	Acetamiprid, Hexaconazole, Propoxur
Cabbage	36	12	5	6	Acetamiprid, Chlordane, HCH-beta, Heptachlor, Hexaconazole, Fluchloralin, Metribuzin
Chili	36	31	15	8	Acetamiprid, Cyproconazole, Hexaconazole, Fipronil, L-cyhalothrin, Propargite $[M+NH_4]$, Propoxur, Tebuconazole
Okra	36	18	5	2	Acetamiprid, Chlordane, Fipronil, Fluchloralin, Piperonyl-

					butoxide [M+NH_4], Tebuconazole
Tomato	36	7	0	0	Acetamiprid, Tebuconazole
Total	**180**	**75 (41.67%)**	**25 (13.89%)**	**20 (11.12%)**	

3.4 Frequency and classification of detected pesticides from vegetables

The scope of experiment had total 111 pesticides. Out of them, 85 pesticides analyzed on LC-MS/MS and remainder 26 pesticides on GC-ECD. Out of the total of 111 pesticides, 14 type pesticides were detected in vegetable samples of Navsari district. Out of 14 pesticides, 7 (50.0%) were insecticides, 3 (21.42%) fungicides, 2 (14.28%) herbicides, 1 miticide (7.14%) and 1 (7.14%) pesticides synergist. The different classes and their chemical group are presented in Table 5.

Table 5. Frequency and classification of detected pesticides by their class and chemical composition.

Sr. No.	Detected pesticides	Frequency of detection in samples	Class	Chemical composition
1	Acetamiprid	27	Insecticide	Neonicotinoid
2	Chlordane	5	Insecticide	Organochlorine
3	Cyproconazole	1	Fungicide	Triazole
4	Fipronil	3	Insecticide	Phenyl pyrazole
5	Fluchloralin	3	Herbicide	Chloroaniline
6	HCH-beta	3	Insecticide	Organochlorine
7	Heptachlor	3	Insecticide	Organochlorine
8	Hexaconazole	8	Fungicide	Triazole
9	L-cyhalothrin	9	Insecticide	Synthetic pyrethroid
10	Metribuzin	5	Herbicide	Triazinone
11	Piperonyl-butoxide [M+NH4]	1	Pesticide synergist	Benzodioxole
12	Propargite [M+NH4]	6	Miticide	Organosulfite
13	Propoxur	5	Insecticide	Carbamate
14	Tebuconazole	31	Fungicide	Triazole

4. DISCUSSION

The present experiment shows the scientific evidence of the presence of pesticide residues in different vegetable samples of Navsari district. Based on the European Commission, if the recovery (%) and RSD (%) of pesticides in different matrices are consistently variable in replicate tests, this outcome is acceptable [13]. The present methods showed the per cent recovery and per cent RSD are within the range of 70–120% and ≤ 20%, respectively which reflect the trueness and precision of the analytical method and it appeared to be suitable for detecting almost all the targeted compounds in different vegetables. The more variability in recovery was obtained for polar pesticides because, in LC-MS/MS analysis, it may be due to the presence of matrix components that coelute with the compounds of interest and can interfere with the ionization process in the mass spectrometer, causing either ionization suppression or enhancement and this phenomenon is called the matrix effect [14].

In the present study, pesticide residues were found in 41.67% vegetable samples of Navsari district. The present study of pesticide residues in vegetable samples was also similar to the preliminary studies [15-19]. However, this study result was comparatively somewhat more than the previous studies [20-22]. In the present experiment, the percent of positives samples were comparatively lower than previous experiments [5, 23-27]. The present finding revealed that 11.12% of the samples have pesticide concentration violated the MRLs values established by the EU and CODEX [8,9]. The result of samples > MRLs percent in this study comparable to the findings by a previous studies [15,28]. However, the samples > MRLs result was comparatively higher than the preliminary studies [22, 21]. Similarly, the result of the samples > MRLs percent obtained comparatively lower than the previous studies [5, 16, 18, 23, 26]. The 13.89% samples of a total of 180 vegetable samples were found with

multiple pesticide residues and that result was somewhat lower to previous studies [25]. The fourteen pesticides detected from a total of 111 analyzed pesticides of which 7 (50.0%) were insecticides, 3 (21.42%) fungicides, 2 (14.28%) herbicides, 1 miticide (7.14%) and 1 (7.14%) pesticide synergist which was somewhat justified by previous done by Ramadan *et al.* [26].

Based on the above result it is verisimilitude to state that the vegetable farmers of the Navsari region of India were not following the standard pre-harvest intervals, proper instructions concerning the application of pesticides in appropriate concentration, time and type of pesticides used. Therefore, a large number of vegetable samples were found positively contaminated with any active ingredients of pesticides. The manifestation of the indiscriminate use of pesticides in vegetables could create heath hazardous for human and the environment. The widespread and overuse of pesticides could increase cost of farming, relines of the farmer on chemical control, and faced with several pest complexes like resurgence and resistance. It is due to lack of alternative methods, awareness in farmer community, competition among farmers, easy and cheap of the pesticides used. The government should give attention to develop strategies for reduction in injudicious pesticide use by the farmers through training focusing on timing and safe pesticide use and encourage alternatives to chemical control like biological, physical, and mechanical methods. Consumers should be aware of household measures of reduction in the contamination of pesticides in raw plant origin products, particularly vegetables, and fruits because that mostly consumed freshly. Different practical methods to reduce pesticides like washing, salted water wash, peeling, boiling and processing can be practised [29,30].

5. CONCLUSION

Intensive cultivation practices enhance the infestation of crops by different pests and diseases, and for the control of these pests heavy uses of pesticides are adopted. From the present study it can be concluded that out of 180 vegetable samples collected from different talukas of Navsari district, 75 (41.67%) samples were found with pesticide residues, 25 (13.89%) samples with multiple residues and only 20 (11.12%) samples had residues above their MRL. Among 111 pesticides, total fourteen pesticides were detected in vegetable samples. The main aim of this study was to generate the alertness among the people about the impact of pesticides used. In future, this study may be helpful in taking necessary steps to mitigate such problems raised due to improper use of pesticides.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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