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Research Article

FOOD CHEMICAL CONTAMINANTS

Simultaneous Monitoring and Decontamination of Pesticide Residues in Phytomedicine-Enriched Betel Leaf Utilizing QuEChERS-GC-MS/MS Technology to Safeguard Public Health

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Abstract

Background: The presence of undesirable substances, including pesticides (xenobiotics) in betel leaf (*Piper betel*), is a great concern for consumers because it is chewed and consumed directly. To protect the consumer's health, a modified QuEChERS method for monitoring purposes and subsequent decontamination process has been developed.
Objective: The goal of this work was to establish a multi-residue analytical method for monitoring nonpermitted organophosphorus pesticide residues in betel leaf, as well as cost-effective cleaning strategies.
Method: The homogenized 15 g samples (20 betel leaf samples collected in West Bengal, India) were extracted with a modified QuEChERS method using acetonitrile, reconstituted to acetone, and finally analyzed by GC–MS/MS. Possible decontamination techniques (such as tap water washing, 2% saltwater washing, and lukewarm water washing) were evaluated.
Results: The limit of detection ranged from 0.003 to 0.005 mg/kg, and limit of quantification was 0.01 mg/kg. Recoveries ranged from 80 to 120% with RSDr 9%. One sample was found to contain three pesticides 4 to 7 times higher than MRLs. Suggested decontamination methods allowed reducing toxic traces below European limits.
Conclusions: The suggested approach is useful for determining pesticide residues in betel leaves quickly. Traditional techniques of processing betel leaves may reduce pesticide residues below regulatory limits.
Highlights: A multi-residue method and decontamination of pesticides in betel leaf using QuEChERS-GC–MS/MS technology with satisfactory method performance was achieved. Domestic decontamination techniques have a high efficacy in reducing pesticide residues from betel leaves, making them safe for human consumption.

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Betel leaf is an important cash crop in India. Betel vine (*Piper betel* L.) belongs to the family Piperaceae. It has heart-shaped deep green leaves and is an important perennial climber horticultural crop of aesthetic and commercial value (1). Generally, fresh raw leaves are consumed along with betel nuts (*Areca catechu* L.) in Asia and elsewhere in the world by some Asian immigrants. It has many medicinal properties and is used to cure indigestion, stomachaches, diarrhea, and flatulence and to heal wounds, swellings due to sprains, bruises, respiratory disorders, constipation, boils, and gum disorders (2). There are 40 varieties in India, and in West Bengal and Bangladesh, 30 varieties are encountered out of 100 varieties of betel leaf (paan) across the world (3). It is widely cultivated in the states of Assam, Andhra Pradesh, Bihar, Gujarat, Odisha, Karnataka, Madhya Pradesh, Rajasthan, West Bengal, and Maharashtra in India. In 2020–2021, India exported 6159.39 metric tonnes (MT) of betel leaves to the world for a worth of Rs 26.18 crores or $3.55 million (4).

The most common pests reported are mainly scaled insects, bugs, mites, and aphids. The most common diseases of the betel leaf are foot and leaf rot. Farmers use chemical pesticides to control pests and diseases. Consequently, the residues of pesticides remain on the harvested leaves. Despite their advantages, the remaining residues have deleterious effects on humans and the environment. Betel leaf containing pesticide residues is a great concern for the consumer's health as the leaves are chewed directly. To protect consumers' health and to fulfill consumer demand for safe food, Good Agricultural Practice (GAP) should be followed at the time of chemical pesticide application. To ensure GAP, pesticide residue monitoring is the key parameter. A multi-residue method is required to monitor pesticide residues in commercial products. Furthermore, multi-residue methods are essential tools for analysts to determine pesticide residues quickly and easily (5, 6).

A few known techniques of using individual pesticides, such as metalaxyl, mancozeb, and chlorothalonil (7), in and on betel leaves have been documented (8). Additionally, an analytical technique based on gas chromatography (GC) and an electron capture detector (ECD) for a few pesticides have been published (6). Although there are other analytical methods (9, 10) that are available for the analysis of targeted compounds, the scope of the method did not include betel leaf. Therefore, there is a prime need to develop a multi-pesticide residue analysis technique employing GC–MS/MS for routine monitoring and decontamination.

In this experiment, 12 organophosphorus pesticides (4-bromo-2-chlorophenol, phorate, dimethoate, phosphamidon, parathion-methyl, phorate sulfoxide, malathion, phorate sulfone, chlorpyrifos, quinalphos, profenofos, ethion) were incorporated as the most selected pesticides recommended by the Horticulture Department, Tamil Nadu Agricultural University (TNAU) for plant protection in betel vine plantation crops (11). Keeping this view in mind, the present study was aimed at developing a multi-residue analytical method to monitor selected pesticide residues in betel leaf.

Food contaminated with toxic pesticides is likely to be associated with severe effects on human health. Hence, great attention has to be given to standardizing simple, cost-effective methods that can be practiced by farmers to eliminate pesticide residues before marketing. In light of the above facts, a study was carried out to assess the effect of different decontamination techniques on the removal of detected organophosphate pesticide residues.

Experimental

Certified Reference Materials (CRM), Stock, and Working Standards

A total of 12 certified reference materials (CRMs) of the organophosphorus group (4-bromo-2-chlorophenol, phorate, dimethoate, phosphamidon, parathion-methyl, phorate sulfoxide, malathion, phorate sulfone, chlorpyrifos, quinalphos, profenofos, ethion) with a purity of 98% were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and stored in a freezer at a low temperature (−20°C), with light and moisture excluded. An accurate weighed amount of analytical grade material of each pesticide was dissolved in a minimum quantity of distilled acetone and diluted with *n*-hexane : toluene to obtain individual mother stock solutions (500 mg/L) of 12 GC–MS responsive pesticides prepared in a hexane–toluene (1:1) mixture. Mixed standard solutions of different concentrations (10, 1, 0.5, 0.25 mg/L) were prepared by adding the appropriate volume of each individual stock solution using serial dilution techniques. All the stock and working solutions were stored under refrigerated conditions (0–4°C) and protected from the sunlight. The working standard solutions and the matrix-matched calibration standards were prepared daily.

Reagents and Chemicals

Acetonitrile, acetone, hexane, toluene, anhydrous magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), and primary secondary amine (PSA) sorbent were purchased from ThermoFisher Scientific (Maharastra, India).

Equipment

A top-loading balance with a digital display (Sartorius AG, Gottingen, Germany) was used to weigh standards and other materials. A domestic stainless-steel knife, domestic mixer-grinder (M/S Bajaj, India), spatula, Teflon centrifuge tubes (15 and 50 mL) with screw caps (ThermoFisher Scientific), vortex mixture (Spinix, Tarsons, Kolkata, India), centrifuge (Super Spin R-V/FM Plasto Crafts, Mumbai, India), filtration syringe (Hamilton Co., Reno, Nevada, USA), membrane filter paper (Ultipor N66, nylon 6.6 membrane, 13 mm; Pall Life Sciences, USA), micropipets (1–10 mL) (Eppendorf Research plus, Germany), rotary vacuum evaporator with a temperature-controlled water bath (HS 2001 NS, Germany), graduated tube (50 and 100 mL, Borosil), measuring cylinder (100 mL, Borosil), glass tubes, and GC–MS vials were used.

Collection of Samples

The analytical method for the estimation of residues of pesticides in betel leaf has been developed by conducting recovery studies using control leaf samples collected from Boroz, maintained by the All India Coordinated Research Project on Medicinal and Aromatic Plants and Betel Vines, BCKV, Kalyani, Nadia, West Bengal, India. For monitoring purposes, betel leaf samples (200 g) were collected from different markets in South 24 Parganas, Purba Midnapore, Nadia, Hooghly, and Paschim Bardhaman, West Bengal, India at monthly intervals for a period of four months from January 2021 to April 2021 (Figure 1). The samples were collected randomly from different vendors in the markets to get representative samples. To avoid the deterioration or loss of field-incurred residues during transportation,

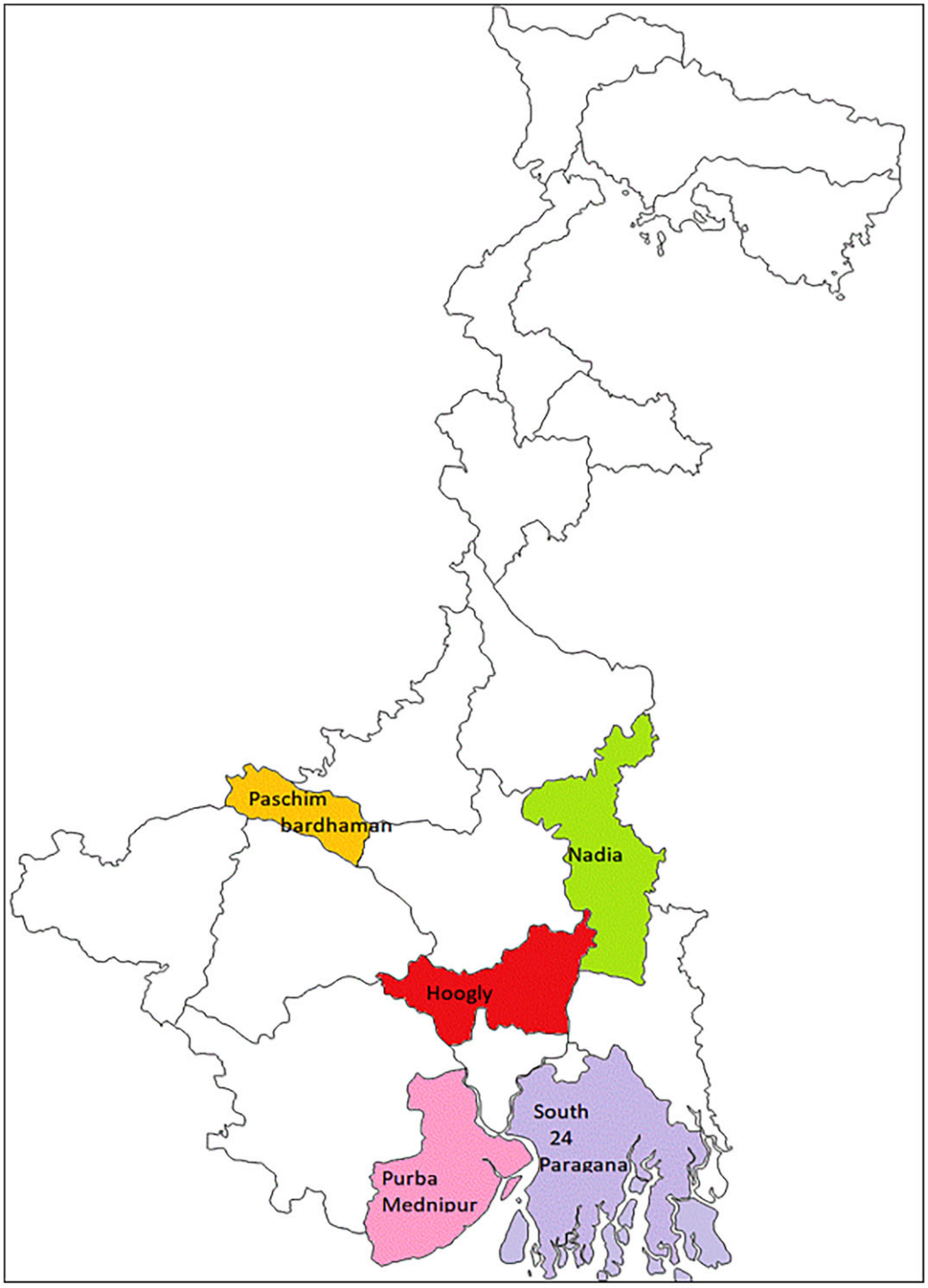

Figure 1. Distribution of monitoring districts in West Bengal, India.

samples were put in low-permeability containers (such as nylon bags) and transported cryogenically using liquid CO_2 (dry ice). These samples were divided into two equal parts to follow monitoring and different decontamination techniques with the intention of removing selected pesticide residues from the betel leaf surfaces. Each sample was processed and analyzed for the determination of the organophosphate group of pesticides. The samples were analyzed within 24 h and stored at −4°C until extraction.

Preparation of Samples

The betel leaves (200 g) collected from markets were brought to the laboratory and chopped with a stainless-steel knife and then ground properly in a domestic mixer-grinder at 1200 rpm for 10 min. Among the ground samples, a representative sample of 15 g was weighed in a polypropylene 50 mL of centrifuge tube. To improve the extraction effectiveness (extractability) of quick techniques such as QueChERS for certain incurred pesticides from low-moisture-content samples such as betel leaves, 5 mL

of water was given to the sample and let to stand for 30 min to rehydrate the sample (12). Then 15 mL of acetonitrile was added to it, and it was closed appropriately and shaken by the vortex mixer for 1 min. After vortexing, it was homogenized in the Silent Crusher M at 12 500 rpm for 1 min to get a fine sample with a greater surface area. Then 6 g of anhydrous magnesium sulfate ($MgSO_4$) and 1.5 g of sodium chloride (NaCl) were added to the homogenized sample and roto-spun for 5 min. After centrifuging for 10 min at 5000 rpm, an aliquot of 5 mL was taken from the supernatant into a 15 mL centrifuge tube containing SPE sorbents in different combinations (PSA + $MgSO_4$/Florisil + $MgSO_4$) and concentrations of primary secondary amine (PSA, 0.1/0.2 g)/(Florisil, 0.025/0.050 g) sorbents and 0.75 g of anhydrous magnesium sulfate ($MgSO_4$). Then it was shaken by the vortex mixer for 30 s and centrifuged at 5000 rpm for 5 min.

After centrifuging, 2.0 mL of the upper layer was evaporated to dryness using a rotary vacuum evaporator at 45°C to evaporate the solvent. The dry residue was reconstituted to 2.0 mL using acetone and filtered through a 0.2 m polytetrafluoroethylene (PTFE) filter in a clean GC vial for the analysis.

GC–MS Operating Conditions

A gas chromatograph (GC-2030 Shimadzu Corp., Kyoto, Japan) coupled to the mass selective detector (MSD) was used for the quantification of selected organophosphorus pesticides (4-bromo-2-chlorophenol, phorate, dimethoate, phosphamidon, parathion-methyl, phorate sulfoxide, malathion, phorate sulfone, chlorpyrifos, quinalphos, profenofos, ethion). Separations were done by the DB-5MS J & W 30 capillary column (30 m long, 0.25 mm i.d., and 0.25 μm film thickness; Agilent, USA). Helium was used as a carrier and argon as makeup gas. The injection volume was 2 μL. The MS conditions include a 6-min solvent delay, a scan rate of 0.50 s, and a scanned mass range of 50–500 *m/z*. All samples were analyzed in the selected ion monitoring (SIM) mode. Data were acquired and processed by GC–MS Lab Solution Software with version 4.45. The compound-specific retention times, *m/z* ions, and molecular masses for the identification, confirmation, and quantification are represented in Table 1. At retention times, selected monitoring ions are used for the identification and confirmation. To find the suitable instrumental parameters, several experiments were conducted, especially to fix the appropriate temperature for the injection port, column oven, and detector, and, finally, the optimized parameters were selected (*see* Supplemental Table 1).

Method Validation

In order to validate the proposed method, the accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), and effect of the matrix were evaluated. The LOD was calculated according to EURACHEM guidelines (13). The LOQ was set following the SANTE 2021 documents (14), which provided the lowest level of fortification for every analyte, providing satisfactory accuracy (average recoveries for individual analytes ranged from 70 to 120%) and precision (RSDr 20%). The five-point (0.01–1.0 mg/L) calibration curve was prepared for checking linearity with the regression coefficient (r^2) for standard mixtures. To determine the potential interferences and cross-contamination, a comparative recovery experiment in three replicates was carried out by spiking 15 g of homogenized blank betel leaf samples with the working mix-standard solution at fortification levels of 1 × LOQ, 5 × LOQ, and 10 × LOQ (Table 3). The accuracy of the method was evaluated based on the average recovery of each xenobiotic from fortified samples. After fortification, a 30 min waiting period was maintained before the extraction procedure, ensuring the proper contact of the analytes with the whole matrix. After that, the samples were prepared following the method described earlier.

The residues of pesticides recovered from the fortified sample were calculated using the following formulas (15, 16):

$$\text{Residues (mg/kg)} = \frac{\begin{array}{c}\textit{Peak height of the sample}\\ \times \textit{concentation of standard in ppm}\\ \times\ \mu\text{L of the standard}\\ \times \textit{Final volume of the sample (mL)}\end{array}}{\begin{array}{c}\text{Peak height with standard}\\ \times\ \text{Mass in g of the sample solution}\\ \times\ \mu\text{L of the sample injected}\end{array}}$$

$$\text{Recovery} = \frac{\text{Observed cncentration} \times 100}{\text{Spiked cncentration}}$$

Development of Decontamination Techniques

Possible decontamination techniques for the elimination of residues of pesticides from betel leaves have been developed by conducting recovery studies using control samples collected from Boroz, maintained by the All India Coordinated Project on Medicinal and Aromatic Plants and Betel Vines, BCKV, Nadia, West Bengal, India. The collected samples were treated with

Table 1. Instrument acquisition parameters of pesticides in GC–MS

Sample no.	Pesticide	RT, min	Target ion	Q1 (% Q1/T)	Q2 (% Q2/T)
1	4-bromo-2-chlorophenol	6.273	99.00	208 (93.90)	231 (94.46)
2	Phorate	10.808	75.00	260 (76.39)	231 (55.12)
3	Dimethoate	11.209	47.00	125 (63.23)	125 (70.66)
4	Phosphamidon	12.685	109.10	127 (40.60)	264 (39.76)
5	Parathion-methyl	12.883	109.00	263 (93.76)	125 (62.81)
6	Phorate sulfoxide	13.534	65.00	97 (80.0)	153 (82.75)
7	Malathion	13.598	99.00	173 (93.83)	173 (81.75)
8	Phorate sulfone	13.736	79.00	153 (52.09)	97 (43.09)
9	Chlorpyrifos	13.821	168.90	125 (60.15)	199 (86.21)
10	Quinalphos	14.681	118.00	146 (36.92)	146 (41.83)
11	Profenofos	15.555	266.90	339 (60.15)	337 (87.76)
12	Ethion	16.476	97.00	153 (59.12)	153 (36.45)

12 organophosphorus pesticides by preparing 2.0 mg/kg solutions in 2 L of water separately and air-drying them. Twenty-four hours after application, each sample was divided into four parts and followed the pesticide reduction procedures as described below.

Treatments

(a) *T1 (tap water washing)*.—Two liters of tap water was taken into the plastic tub, and 0.2 kg of betel leaves were dipped in the tub for 30 min. The leaves were then kept for air-drying on tissue paper for 15 min, followed by extraction and cleanup as described in Figure 2 and analysis.

(b) *T2 (2% saltwater washing)*.—Two liters of 2% salt solution were prepared by mixing 40 g of table salt in 2 L of water in a plastic tub, and 0.2 kg of betel leaves were dipped in the tub for 30 min and then washed in water. Furthermore, the leaves were kept for air-drying on tissue paper for 15 min, followed by extraction and cleanup as described in Figure 2 and analysis.

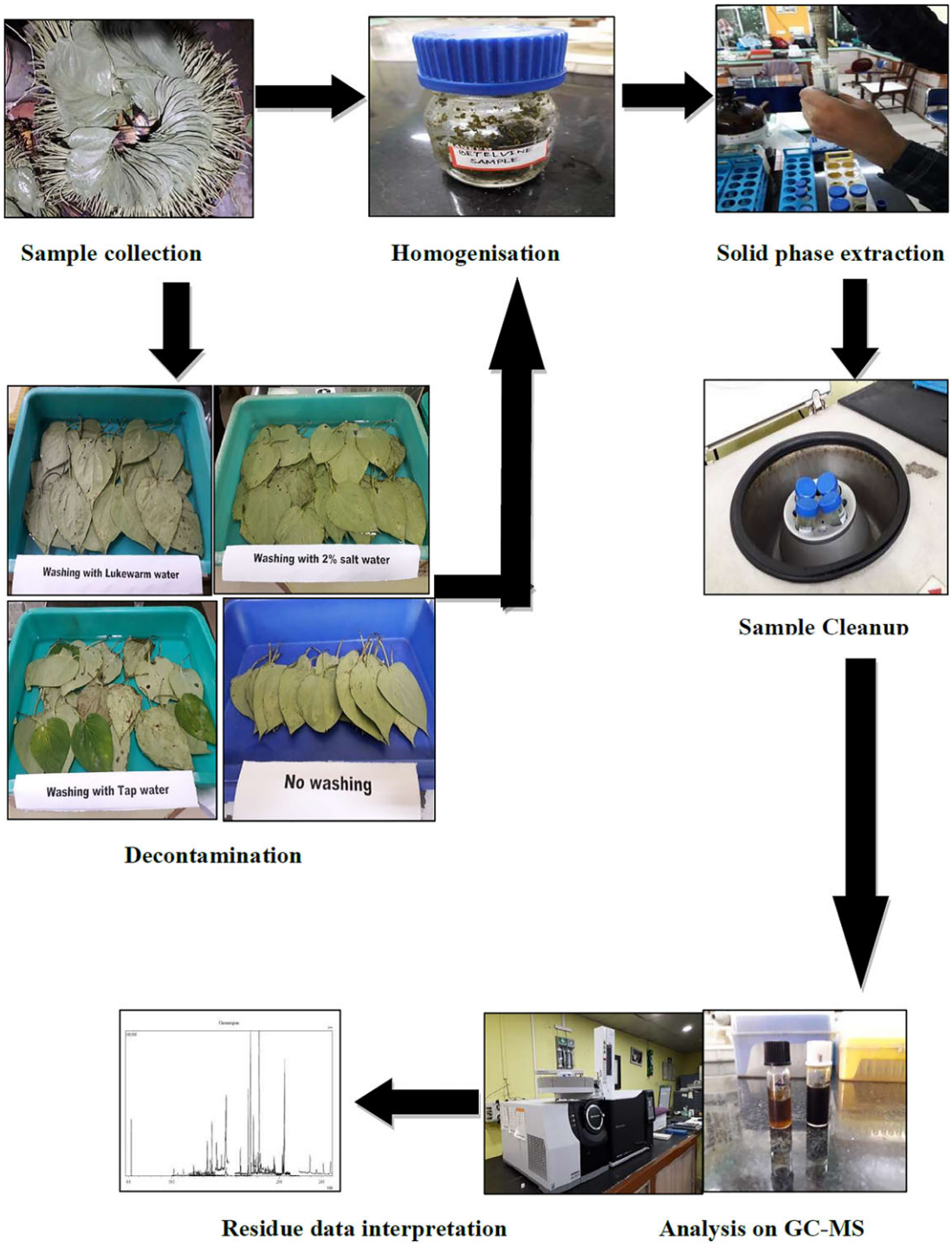

Figure 2. Graphical representation of monitoring and decontamination processes of collected betel leaf samples.

(c) *T3 (lukewarm water washing)*.—For this method, soak 0.2 kg of betel leaves in 2 L of lukewarm (45°C) water for 30 min; the leaves were kept for air-drying on tissue paper for 15 min, followed by extraction and cleanup as described in Figure 2 and analysis.

(d) *T4 (no washing)*.—In this method, 0.2 kg of betel leaves were not treated with any decontamination methods and processed for the analysis. After that, the samples were prepared following the method described earlier. The percentage of residual reduction of pesticide residues was calculated as follows:

$$(R_1 - R_2)/R_1 \times 100 = \% \text{ Residual reduction rate}$$

where R_1 = the amount of pesticide residues present before treatment, and R_2 = the amount of residues present in samples after treatments.

The method was successfully applied to the detected market samples of betel leaves collected from five different districts (South 24 Parganas, Purba Midnapore, Nadia, Hooghly, and Paschim Bardhaman) in West Bengal, India.

Results and Discussion

Optimization of Cleanup

To quantify pesticide residues in agricultural products, cleanup plays an important role in minimizing the matrix effect and increasing the recovery as well. The QuEChERS cleanup method used in this study was modified for the determination of selected pesticides in betel leaf (6).

The optimization (combination and concentrations) of cleanup materials was done based on the previous study conducted by Bhattacharya et al. (17) using the traditional column chromatography method for estimation of single-pesticide residue on betel leaves. Later, Reddy and his co-worker reported the residues of metalaxyl and mancozeb in betel leaf without the SPE cleanup method (8). It is necessary to mention that for the optimization of cleanup materials for betel leaf, PSA was not used. Removal of residual water and cleanup of polar residues were performed simultaneously using a dispersive solid-phase (d-SPE) cleanup. Based on the previous results, a series of experiments were conducted to find the appropriate amount of cleanup sorbents. Dispersive solid-phase extraction (d-SPE) was carried out using PSA (100 mg) + $MgSO_4$ (750 mg), PSA (200 mg) + $MgSO_4$ (750 mg), Florisil (25 mg) + $MgSO_4$ (750 mg), and Florisil (50 mg) + $MgSO_4$ (750 mg) in two combinations and concentrations. These extracting sorbents' proportionate scenario of the average percent recovery is provided in Figure 3. The PSA (200 mg) + $MgSO_4$ (750 mg) sorbent combination was shown to have the highest recovery percentage (80.20–111.20%), while other sorbent combinations failed to recover almost 25% of the pesticides (phorate, quinalphos, profenophos, and ethion) from betel leaves with acceptable recovery percentages (80–120%). The preferential adsorption of polar sample components, which interfered with substances such as pigments on the PSA surface, was thought to be the reason why PSA generated the cleanest chromatographic profiles with lower baselines than those of Florisil. Wahab et al. also discovered that a mixture of sorbents performs a better cleanup of matrix materials than a single sorbent (18). The sorbent combination of PSA (200 mg) and $MgSO_4$ (750 mg) was also shown to be efficient in minimizing the matrix effect. The extraction efficiency of all 12 pesticides was within an acceptable range of 80–120% at three separate levels, with RSD less than 20% with optimized sorbent combination and concentrations (200 mg of PSA + 750 mg of MgSO4). The PSA-based cleanup was chosen to optimize the extraction process since it produced better recoveries than without cleanup.

Instrument Optimization

GC operating parameters have been standardized to increase the sensitivity and selectivity of the instrument. It was found that overall recovery responses had improved by 10.17% when the injector temperature was changed from 230 to 250°C. The organophosphorus compounds were more likely to volatilize at temperatures over 230°C, which removed them from the liquid phase. Additionally, the injector states of splitless and split

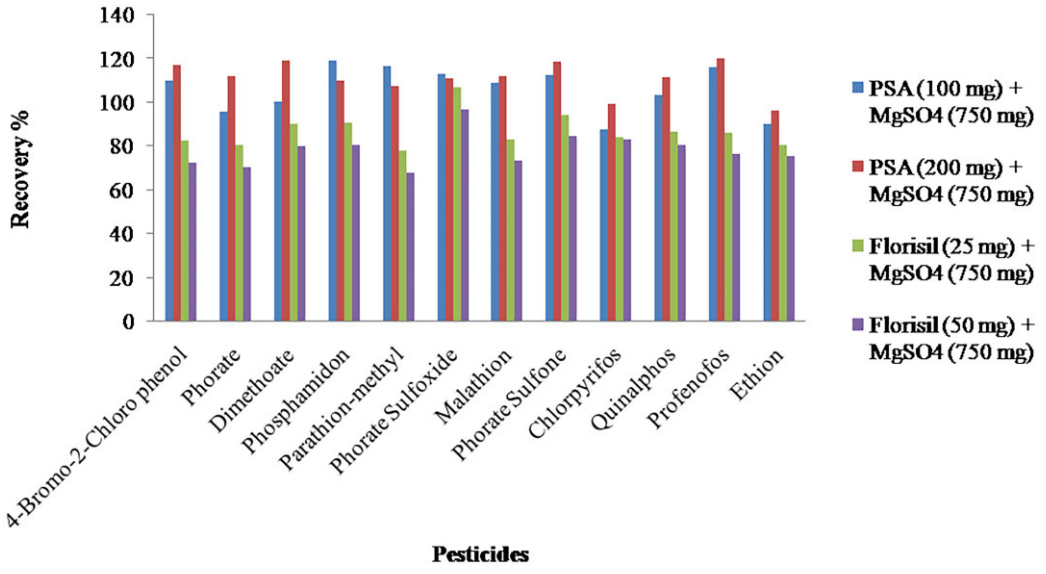

Figure 3. Comparison of percent recovery for various sorbents (combinations and concentrations) in d-SPE for organophosphorus pesticides.

were assessed, and split mode (1:10) resulted in an improvement in the extraction efficiency (13.87%) of all pesticides (Table 2). To avoid target peak overload and to increase the separation efficiency of the column, the split mode has been chosen in GC–MS analysis. Therefore, injector temperature (250°C) and status (split) were the criteria chosen. With these strategies in mind, the reduced runtime (28 min) in this method was determined. El-Hawari (19) and Schwanz (20) reported 60.17 min and 55 min run times for pesticide residue analysis in GC–MS, whereas our method has successfully reduced the runtime to almost half of the reported ones. The MS parameters were standardized for perfect identification in a SIM mode using the retention time (RT) window for each compound from the full scanned chromatogram and the RT of an individual compound.

Method Validation

Validation of the analytical method was performed by observing the linearity, accuracy, and precision as well as the LOD and LOQ. Matrix effects were also considered during method validation. The performance of the chosen technique was determined to be steady since the variation in retention periods (5.78%) and areas (10.23%) of respective compounds after injection matrix-matched standards for a continuous 20 times was within acceptable ranges (RSD less than 20%). Very good accuracy and precision were found for all pesticides at three fortification levels (0.01, 0.05, and 0.1 mg/kg) (Figure 4). The average recoveries ranged from 80 to 120% with relative standard deviations (RSD_r) within 10% (Table 3). Intermediate precision (RSDs), along with accuracy, was found to be very good. Average recoveries ranged

Table 2. Recovery results of 12 OP pesticides in betel leaves at different method parameters including matrix effects

	Injector temp		Injector status		
Pesticides	230°C	250°C	Splitless	Split (1:10)	Matrix effects (%)
4-bromo-2-chlorophenol	90.06 ± 9.28	92.14 ± 4.22	82.34 ± 2.45	90.23 ± 8.67	23.6
Phorate	80.23 ± 10.42	85.03 ± 8.22	79.12 ± 8.53	85.13 ± 3.70	−17.12
Dimethoate	101.03 ± 4.74	106.82 ± 7.32	106.32 ± 1.22	108.24 ± 5.54	12.53
Phosphamidon	90.07 ± 8.25	108.22 ± 7.44	98.12 ± 5.40	109.37 ± 1.53	19.94
Parathion-methyl	78.87 ± 5.41	92.11 ± 8.22	82.03 ± 8.42	96.9 ± 2.91	20.12
Phorate sulfoxide	87.23 ± 7.22	109.25 ± 6.4	109.35 ± 5.02	113.03 ± 3.58	1.69
Malathion	89.3 ± 10.2	92.12 ± 7.08	82.78 ± 3.61	89.07 ± 5.99	−17.07
Phorate sulfone	91.23 ± 6.74	102.33 ± 4.11	102.27 ± 2.06	113.12 ± 3.80	3.18
Chlorpyrifos	93.26 ± 7.12	100.11 ± 8.22	100.19 ± 6.80	108.34 ± 6.55	7.26
Quinalphos	86.36 ± 6.00	96.87 ± 1.32	86.17 ± 4.98	93.25 ± 1.57	22.62
Profenofos	85.42 ± 6.02	98.07 ± 7.11	78.25 ± 2.79	86.64 ± 4.02	20.15
Ethion	78.37 ± 14.02	90.52 ± 7.15	70.22 ± 7.61	90.35 ± 6.82	18.47

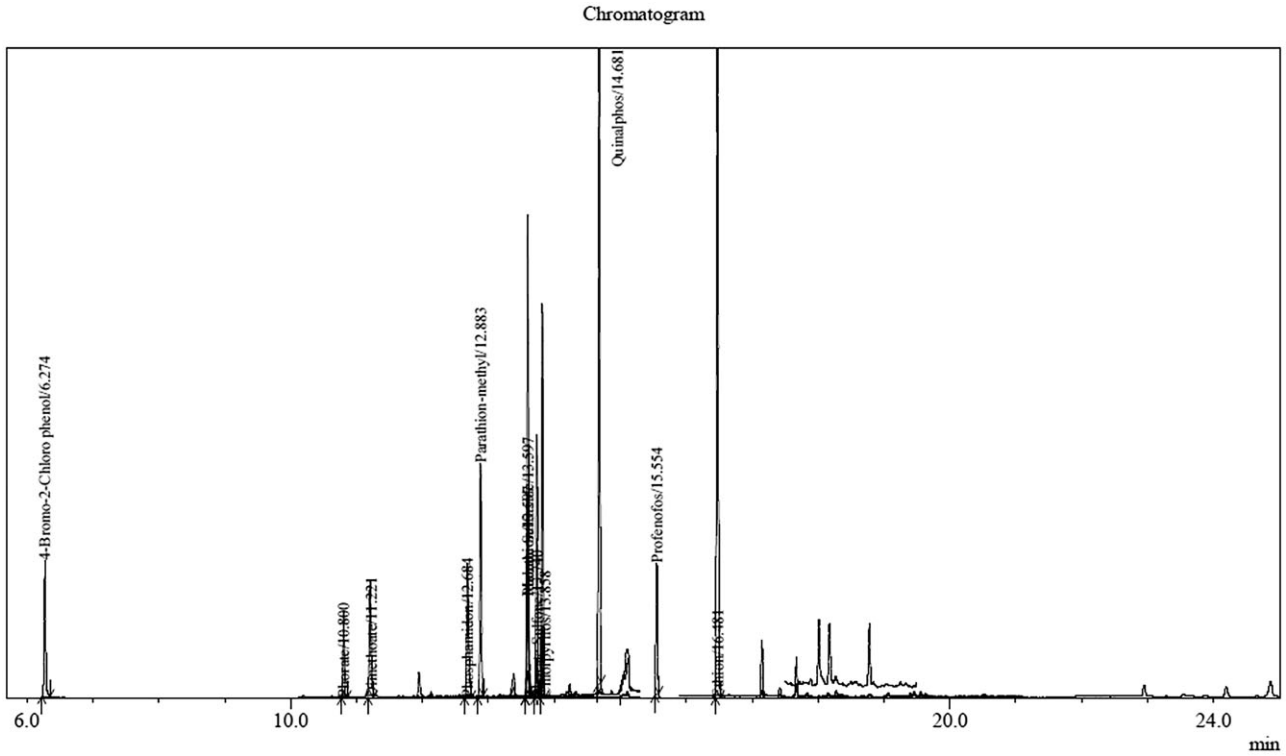

Figure 4. Chromatogram of recovery experiment in betel leaf at 0.1 ppm spiking level.

Table 3. Recovery results of 12 OP pesticides in betel leaves at different fortification levels of LOQ.

	1 × LOQ (0.01 mg/kg)		5 × LOQ (0.05 mg/kg)		10 × LOQ (0.1 mg/kg)	
Pesticides	Avg. recovery, %	RSD, %	Avg. recovery, %	RSD, %	Avg. recovery, %	RSD, %
4-bromo-2-chlorophenol	112	5.02	117	2.45	110	8.67
Phorate	80	6.27	112	8.53	95	3.70
Dimethoate	110	5.46	119	1.22	100	5.54
Phosphamidon	100	7.60	110	5.40	119	1.53
Parathion-methyl	88	8.23	107	8.42	116	2.91
Phorate sulfoxide	117	2.32	111	5.02	113	3.58
Malathion	83	8.16	112	3.61	109	5.99
Phorate sulfone	117	4.29	111	2.06	113	3.80
Chlorpyrifos	114	4.47	100	6.80	88	6.55
Quinalphos	87	8.23	111	4.98	103	1.57
Profenofos	106	9.32	120	2.79	116	4.02
Ethion	80	8.63	96	7.61	90	6.82

from 80 to 120% at three fortification levels of 0.01, 0.05, and 0.1 mg/kg (Table 3).

The five-point calibration curve was prepared by matrix-matched calibration standards in triplicate and was found to have very good linearity with a coefficient of determination (r^2) of >0.98 for all the selected pesticides. The LOD ranged from 0.005 mg/kg, and the LOQ was set to 0.01 mg/kg for all the selected pesticides, achieving acceptable accuracy (mean recoveries for individual pesticides ranging from 80 to 120%) and precision (RSD_r 10%). Therefore, LOD and LOQ were within acceptable ranges of the recommended guidelines such as SANTE (13) and EURACHEM (14).

Matrix Effects

It is critical to evaluate matrix effects during the validation of an analytical method, whether they are present or not, because they have a significant impact on results. The intensity of matrix effects may vary for many reasons. Impurities in the injected samples can cause problems at the detector and also at the injector site. The main source of matrix effect enhancement is the active site in the injection liner, which adsorbs or induces the thermal degradation rate of tested pesticides. Sugar, pigments, and co-eluting in the injected matrixes can enhance or suppress the target response.

Pigments such as chlorophyll, carotenoids, eugenol, chavibetol, and piperols (21) and other co-extracts present in betel leaves at different levels exhibit huge matrix interfaces during analysis. In this study, matrix effects were determined by comparing the slopes of the calibration curves made with matrix-matched standards and solvent-based standards. Eight of the 12 analytes examined showed matrix effects in the range of 1.69 to 17.12%, while four substances (quinalphos, profenofos, parathion-methyl, etc.) showed somewhat greater matrix effects (20.12 to 23.6%), although not significantly (Table 2). Results revealed that the strongest positive matrix effect was found for 4-bromo, 2-chlorophenol (+23.6%), while phorate recorded the maximum negative ME (−17.12%), which was also supported by Menkissoglu-Spiroudi and Fotopoulou (22). They observed that GC-ECD detected pesticides showed pronounced matrix effects (25.3%). Out of the 12 analytes assessed, only two (phorate and malathion) showed around 17% signal suppression effects. If they have the same retention time and MRM transition as the pesticide, some components (secondary metabolites) of betel leaves may be co-extracted with trace amounts of pesticides and result in matrix interference. The adoption of matrix-matched standards is crucial in order to address these issues and account for the matrix impact.

Monitoring of Market Samples

The developed analytical method was successfully applied to monitor 20 betel leaf samples (four samples of each district) collected from different markets of five selected districts (South 24 Parganas, Purba Midnapore, Nadia, Hooghly, and Paschim Bardhaman) in West Bengal, India (Figure 1). Out of 20 analyzed samples, one (5% of the total number of samples) was found to be contaminated with quantifiable residues of chlorpyrifos (0.17 mg/kg), dimethoate (0.09 mg/kg), and malathion (0.08 mg/kg) from the Hooghly District, and 19 (95% of the total number of samples) contained no residues of the sought pesticides (Table 4). Market-monitored leaf samples with pesticide residue levels exceeding EU-MRLs could inadvertently suggest that betel leaf growers are employing dimethoate, chlorpyrifos, and malathion to manage pests.

Despite the fact that only 5% of the samples were contaminated, it is alarming to learn that one sample from the Hooghly District had residue levels that were 4–7 times higher than the EU-MRL. This is significant because betel leaves are commonly chewed by consumers. To prevent the indiscriminate use of synthetic pesticides and to encourage the use of bio-pesticides in betel leaf agriculture for the management of insect pests, policymakers and regulatory organizations should take the necessary steps.

Evaluation of Decontamination Techniques

Indiscriminate and excessive uses of pesticides throughout the globe lead to residues in food materials, water, fruits, and vegetables and in total diets (23). These pesticide residues enter the human body through the consumption of pesticide-contaminated foods, which leads to chronic disorders. Thus, the removal of these residues from food commodities by using different decontamination methods is essential. The effects of decontaminating techniques on the removal of pesticide residues in betel leaves are summarized in Table 5. All the decontaminating techniques significantly removed the residues of target pesticides from betel leaves after treatments in the range of 23 to 54% (Figure 5). It was found that dipping in tap water and 2% saltwater washing for 30 min was more effective in

Table 4. Monitoring scenarios of collected different market samples

Locations	Number of samples		Pesticide detected	Residue, mg/kg (RSD, %)
	Monitored	Detected		
Paschim Burdwan	4	0	–	–
Hooghly	4	1	Dimethoate	0.09 (6.41)
			Chlorpyrifos	0.17 (3.22)
			Malathion	0.08 (5.18)
Purba Midnapore	4	0	–	–
South 24 Parganas	4	0	–	–
Nadia	4	0	–	–

removing the residues, recording a residue removal of 35.02–53.99% and 29.52–50.20%, respectively.

Similarly, lukewarm water washing was found to be the best treatment with a residue load of 22.94–44.93%. From the results, it is presumed that all the decontamination techniques used in the study were more effective in reducing the residues after treatments as the residues were localized on the surfaces of betel leaves, which could be dislodged easily (*see* Supplemental Figure 1). With the passage of time, residues penetrate into the leaves; hence less removal was observed in the market samples. The literature pertaining to the efficacy of the various decontamination treatments used in the present investigation is scanty, especially for betel leaves. However, Varghese and Mathew (24) reported that 2% tamarind was the best decontaminating solution in removing spiromesifen (90.03%) and propargite (96.69%) from green chili. Similar types of observations with phosphamidon and monocrotophos in bitter gourd and cowpea pods have been reported by Kumar (25).

Turmeric was an effective decontaminant in removing chlorantraniliprole residues (79.81–87.40%) from vegetable cowpea on the first and third days after spraying (23). Zohair reported that organophosphorus pesticides (pirimiphos-methyl, malathion, and profenophos) were eliminated more effectively by acidic, neutral, and alkaline solutions, which depended on the kinds and concentrations of solutions (26). Tomatoes contaminated at a level of 1 mg/kg lost 42.90, 46.10, 27.20, 90.80, 82.40, and 91.40% of their HCB, lindane, p, p-DDT, dimethoate, profenophos, and pirimiphos-methyl after washing with a 10% NaCl solution, respectively (27). When cucumbers were dipped in a 2% sodium chloride solution for 20 min, Liang et al. (28) found that the residues of trichlorfon, dimethoate, dichlorvos, fenitothion, and chlorpyrifos were reduced by 63.40, 60.00, 50.00, 31.10, and 66.70%, respectively. All these findings agree with those obtained by Zohair, who reported that soaking contaminated potatoes in neutral (NaCl) solution (5 and 10%) for 10 min resulted in 100% removal of pirimiphos-methyl residues (26).

By processing with 2% saltwater, the detected residues from the market leaf samples were drained out by up to 50%. All the residues were just below MRL levels. The exact cause and effect of residue reduction by 2% saltwater washing solutions are unknown and require further investigation. Because some organophosphates are nonsystemic in nature, they remain attached to surfaces and are easily removed by mechanical stirring with water during washing. Thus, washing has a significant effect on the removal of residues depending on the pesticides because of the less or no penetration of the chemicals into the cuticle layer of the plant surface, resulting in deposits removable by

Table 5. Effects of different decontamination techniques in reduction of pesticides from contaminated betel leaves

Decontamination methods	Detected pesticides	Reduction, %
T_1 (Tap water washing)	Dimethoate	54.0
	Malathion	35.02
	Chlorpyrifos	39.83
T_2 (2% Saltwater washing)	Dimethoate	34.82
	Malathion	50.20
	Chlorpyrifos	29.52
T_3 (Lukewarm water washing)	Dimethoate	40.2
	Malathion	44.93
	Chlorpyrifos	22.94
T_4 (No washing)	Dimethoate	–
	Malathion	–
	Chlorpyrifos	–

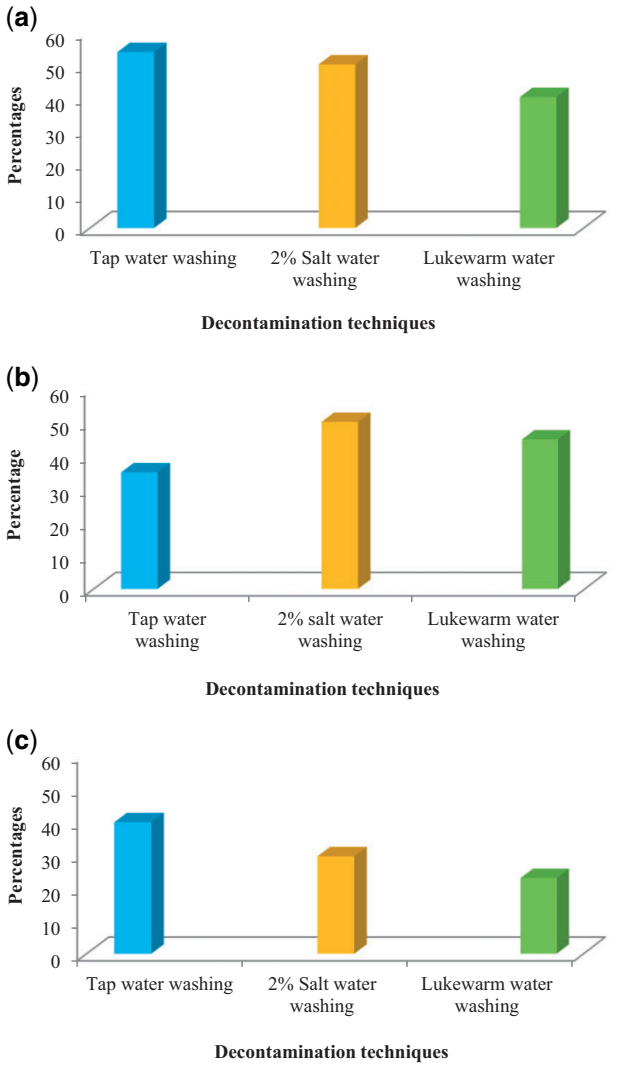

Figure 5. Percent reduction of dimethoate (a), malathion (b), and chlorpyriphos (c) residues from contaminated betel leaves by various decontamination techniques.

washing. There were significant differences in reduction of potential residues among various washing solutions due to their different concentrations. Therefore, ensuring food safety during

processing operations, particularly in washing solutions and cooking, which lead to a high level of residue reduction, may be encouraged (29).

Dimethoate residues on betel leaves were removed significantly when subjected to different contaminations (Figure 5). Betel leaves dipped into the water, which contained tap water in the tub for 30 min, was found to be the most effective of all methods. With this method, residues were reduced by up to 54%. The percentages of dimethoate residues due to various decontamination methods, in descending order, are tap water washing (53.99%), 2% saltwater washing (50.20%), and lukewarm water washing (40.19%). The percentages of malathion residues due to various decontamination methods in descending order are 2% saltwater washing (50.20%), lukewarm water washing (44.93%), and tap water washing (35.02%). Chlorpyrifos is an organophosphorus insecticide that is a derivative of thiophosphoric acid. Betel leaves were washed with tap water for 30 min, and this was found to be the most effective (39.83% removal) of the other methods. The percentages of removal of chlorpyrifos residue due to various decontamination methods were tap water washing (39.83%), 2% saltwater washing (29.52%), and lukewarm water washing (22.94%). Based on the percentages of removal of residue, it is noted that there is a significant difference in decontamination solution in removing chlorpyrifos residues. In the present study, washing with tap water for 30 min followed by washing in the 2% salt water proved to be the most efficient in removing pesticides.

Conclusions

Because consumers chew raw betel leaves, regular monitoring is necessary to check for pesticide residues. The developed and validated QuEChERS technology that follows the standard method is useful for the quick detection of 12 nonapproved pesticide residues in betel leaves. The monitoring results revealed that one sample had pesticide residues over the level deemed acceptable for food (MRLs), including dimethoate, malathion, and chlorpyrifos. After several decontamination approaches were investigated, the capabilities for pesticide removal were as follows: tap water washing > 2% saltwater washing > lukewarm water washing. The results of the experiment suggest that preparing betel leaves using conventional techniques may lower pesticide residues below MRL levels and render them safe for ingestion by humans. In order to reduce the amount of pesticide residues in food, effective agricultural practices must be used during the production, post-harvest, and marketing of food products, especially those intended for raw consumption. Furthermore, it is crucial to standardize easy-to-implement, affordable pesticide-removal methods that farmers or other end users might apply.

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Conflict of Interest

All authors declare no conflict of interest.

Supplemental Information

Supplemental information is available on the *J. AOAC Int.* website.

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