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Research Article

Effect of simple processing on selected pesticide residues in cottonseed (*Gossypium* spp.)

Thirukkumar Subramani*
Department of Food Science and Nutrition, Community Science College and Research Institute, Tamil Nadu Agricultural University, Madurai- 625104 (Tamil Nadu), India
Hemalatha Ganapathyswamy
Department of Food Science and Nutrition, Community Science College and Research Institute, Tamil Nadu Agricultural University, Madurai- 625104 (Tamil Nadu), India
Vellaikumar Sampathrajan
Center of Innovation, Department of Biotechnology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai- 625104 (Tamil Nadu), India
Amutha Sundararajan
Department of Food Science and Nutrition, Community Science College and Research Institute, Tamil Nadu Agricultural University, Madurai- 625104 (Tamil Nadu), India
Murugan Marimuthu
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore- 641003 (Tamil Nadu), India

*Corresponding author. Email: psthirukumar@gmail.com

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Abstract
Food processing techniques reduce pesticide residue accumulation in food components. The present study investigated the effectiveness of simple processing techniques such as roasting, soaking, autoclaving and storage conditions on twenty-seven selected pesticides belonging to the classes of organophosphates (OPs), organochlorines (OCPs) and pyrethroids (PPs) in pesticide-fortified cottonseed (*Gossypium* spp.). The residue concentration was analysed by GC–MS/MS from the extract of different treated samples as untreated and pesticide free (T_1), pesticide treated (T_2) and pesticide treated cum processed (R_1-Roasting 5 min; R_2-Roasting 10 min; S_1-Soaking 6 hours; S_2-Soaking 12 hours; AC_1-Autoclaving 5 min; AC_2-Autoclaving 10 min; ST-Storage) cottonseed. The recovery values of the residues ranged from 78.20 to 114% with the application of 1, 5 and 10 µg/g pesticide in pesticide-free cottonseed. The concentration of pesticide residues is presented for nondetected levels in pesticide-free samples. Otherwise, pesticide-treated samples contained all pesticide residues ranging from 856 to 1138 ng/g OP, 782 to 1058 ng/g OCPs and 857 to 1140 ng/g PP, which are higher than the maximum residue limits (MRLs) set by The Japan Food Chemical Research Foundation (FFCR). Among the different processing methods, autoclave samples had fewer residues detected (3 compounds), followed by stored (5 compounds), soaked (7 compounds) and roasted (8 compounds) samples. The stored cottonseeds contained residues for phorate, total lindane excluding δ-lindane and deltamethrin at the end of storage. Nevertheless, the residue from phorate, δ-lindane and deltamethrin exceeded the concentration of MRLs. This finding indicated that the most effective method for reducing pesticide residues was autoclaved treatment from the respective pesticide-fortified cottonseed sample.

Keywords: Cottonseed, Organochlorines, Organophosphates, Pesticide Residue, Processing, Pyrethroids

INTRODUCTION

Cotton is one of the major economic cash crops, and the leading cottonseed cultivated countries worldwide are India (6.42 MMT) followed by China (5.93 MMT), USA (4.33 MMT), Brazil (2.91MMT) Pakistan (1.35 MMT) in 2019-2020 and accounts for 77% of global output in the form of fiber and animal feed (Statista, 2021; Thirukkumar *et al.,* 2021). Cotton cultivation practices are largely dependent on the application of pesticides to minimize pest attacks with a focus on yield and storage quality. Cotton production losses account for 82% worldwide due to the absence of pest management in cotton. Overall, owing to agricultural

practices, the sales of agricultural input products such as insecticides, herbicides, fungicides and plant growth regulators are 55%, 29%, 11% and 5%, respectively, in India (Insecticides India, 2019). According to the International Cotton Advisory Committee (Kabissa, 2019), before introducing *Bacillus thuringiensis* (*Bt*) cotton in 1996 worldwide, 50% of chemical insecticides were used to suppress bollworm infestation in cotton crops annually. Wherever *Bt* cotton is toxic to bollworms, it does not guarantee any other pest attack or develops resistance to bollworms or pink bollworms.

There are 1326 insects and other arthropod species linked with cotton cultivation worldwide (Hargreaves, 1948), and the most frequently attacked pests include a complex group of Lepidoptera, including *Helicoverpa armigera, Helicoverpa zea, Helicoverpa punctigera, Heliothis virescens, Earias spp., Diparopsis spp.* and *Pectinophora gossypiella*. Currently, sucking pests such as mirids, silver leaf whiteflies, stinkbugs and aphids are major issues in *Bt* cotton and are controlled by applying insecticides with profound influence on the economic pattern of cotton production. The development of *Bt* cotton was fully emphasized for the *Bt* toxin against lepidopteran larvae and not for sucking pests. The presence of emerging pest problems such as mealy bugs and pink bollworms in cotton, have been reflected in the surge in the usage of insecticides from 14.8% to 16.1% from 2010 to 2014 (Ferrigno *et al.,* 2017; Kranthi, 2014). Overall, for agricultural chemical consumption, cotton is at the fourth-largest place among countries, namely India, China, Brazil, the USA and Pakistan, which face the problems of 'boll weevil, cotton bollworm, pink bollworm, whitefly and leaf curl virus worsening'. Different dosages of OPs control these pests, OCPs and PPs although disturbing acetylcholinesterase enzyme activities in the nervous system, resulting in impaired nerve coordination (Pakravan *et al.,* 2016).

In India, *Bt* cotton was introduced in 2002, leading to tripling cotton production and contributing to 90% of the total cotton production in the country. The *Bt* cotton cultivated area increased from 45% in 2006 to 95% in 2013 and reflects insecticide usage, which is estimated at 11.598 M tonnes (0.9 kg/ha) (Kranthi, 2014). The insecticide market value slowed from 2002 to 2006 and boomed from 2006 to 2013 from 2200 to 11600 metric tonnes of insecticides used to control sucking pests and 4400 to 11800 metric tonnes of insecticides for bollworms. India has been placed in the second position for cotton export worldwide, accounting for 5% of the gross cropped area. Additionally, the total pesticide consumption status has risen from 36 to 50% in the country (Statista, 2021).

Increased usage of insecticides has also contributed to the increase in cotton yield as well as the economic status of cotton-growing farmers and countries. From the farmer's perspective, spraying high dosages of pesticides at shorter interval periods or mixing their own levels of insecticides can be used to control the pests. This indiscriminate and injudicious usage of pesticides might cause contamination with high pesticide residues in cottonseed products, which are consumed in the form of oil, deoiled cake, meal, flakes and milk for human consumption in addition to animal feed due to the availability of good-quality protein, fatty acids, fibers and vitamins (Thirukkumar *et al.,* 2021; Balasubramanya and Shaikh, 2007).

At the time of postharvest processing, such as mechanical harvesting, drying and dehulling, the nature of the final product quality, such as nutrients, moisture content, foreign matter and storage conditions, such as temperature, humidity and type of storage, may affect the growth of insects and influence end product quality. Common insects such as *T. castaneum, O. surinamensis, T. granarium and S. oryzae* lead to deterioration of oilseed quality at the time of prolonged storage of cottonseeds in godown or bin (Rajendran and Chayadevi, 2004). The application of insecticides can control this, *viz.,* malathion, primiparous-methyl, chlorpyriphos-methyl, dichlorvos and deltamethrin (Dauguet, 2009).

Despite the numerous advantages of pesticide usage in the economic development of the country, huge consumption has affected the environment, ecology and health. The long-term consumption of pesticide residues results in immunotoxic, neurotoxic and carcinogenic effects in humans (Gupta, 2006). OP and OCPs are lipophilic in nature and easily accumulate in extracted edible oil at different concentration levels (Bajpai *et al.,* 2007 and Qin *et al.,* 2011; He *et al.,* 2017), and long-term deposition of their residuals can cause various problems to the environment and human life.

The physicochemical properties of pesticides can be changed by the oxidation and reduction, pH, temperature, hydrolysis, and metabolism of plants, animals, and microbes present in the environment and processing conditions. Different simple and modern processing techniques include washing, soaking, peeling, drying, blanching, cutting, cooking, baking, milling, canning, pressure cooking, microwave cooking, fermentation, pasteurization, and sterilisation ozonization, are available for dissipating the pesticide residue concentration to food products. On the other hand, molecular and processing factor interactions contribute to an increase in the residue level (Bajwa and Sandhu, 2014).

However, there is little literature available for estimating the pesticide residue content in raw or processed vegetables, fruits and their byproducts (Nguyen *et al.,* 2020; Chen *et al.,* 2014; Kiwango *et al.,* 2020). Thus, this work aimed to reveal the processing factors and the effect of selected simple food processing techniques on the selected organophosphates (OPs), organochlorines (OCPs) and pyrethroids (PPs) residue in pesticide-

fortified cottonseed samples.

MATERIALS AND METHODS

Mature, dust and pesticide residue-free cottonseed (*Gossypium* spp.) variety MCU 5 was procured from the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore. The sample was packed in an air-tight container and stored at below 4°C for further use. The 100 ppm of three mixed standard solutions, OPs - solution with mixture of OPs compounds (9 no's), OCPs - solution with mixture of OCPs compounds (9 no's) and PPs - solution with mixture of PPs compounds (9 no's) with 27 compounds, were obtained from Toxicology laboratory, TNAU, Coimbatore and the chemical names, molecular weight and formula are listed in Table 1. The working standards (0.2 to 1 ppm) were prepared from the above standard by diluting with hexane and stored in the dark at -20°C. These working standards were used to determine the retention time of the pesticide compounds and to quantitatively determine the residues in the samples.

Chromatographic chemicals, *viz.,* acetronitrile, ethyl acetate, anhydrous magnesium sulfate (MgSo4) and sodium chloride (NaCl), were purchased from Merck, USA, and primary secondary amine and 0.22 µm polypropylene filters were procured from Agilent Technologies.

Sample preparation and processing

An untreated and pesticide-free cottonseed sample (T_1) was selected for pesticide-fortified and pesticide-fortified cum process treatments. Furthermore, the sample (T_1) was treated with 1mg/ml/g pesticide mix solutions to 5 g of sample and designated a pesticide-fortified sample (T_2). They were kept for 1 hour at ambient temperature in closed conditions and further analysed for residue concentration. For other processing treatments, T_2 samples were adapted to the different methods of processing, such as (i) roasting, (ii) soaking, (iii) autoclaving and (iv) storage. Roasting was performed at 80°C for 5 min (R_1) and 10 min (R_2) for sample T_2. After completion of the roasting process, the samples were immediately cooled at room temperature and further extracted and analysed for residue level as per the extraction process. Soaking was performed by the sample (T_2), which was immersed in distilled water (1:5 w/v) for 6 hours (S_1) and 12 hours (S_2) under ambient conditions (30±2°C). After soaking, the residue was extracted as per the extraction process from the water filtrate samples. The autoclaving process was carried out by the sample (T_2), which was kept in an autoclave at processing conditions of 120°C for 5 min (AC_1) and 10 min (AC_2). Under storage conditions, a pesticide-fortified sample (T_2) was packed in a high-density polyethylene (HDPE) container, and the sample was stored at ambient temperature (30±2°C) for up to 120 days. This sample was named ST. In ST sample residues, the concentration level was analysed at the end of the storage period. All the samples were analysed for OP, OCP and PP residue concentrations as per the extraction process. Each treatment was processed as triplicate samples for analysis.

Sample extraction

Pesticide residue extraction was carried out as per the QuEChERS extraction method (Wilkowska and Biziuk, 2011). Five grams of each treated sample was transferred into a 50 ml centrifuge tube and mixed well in a vortexer for 1 min. after adding 15 ml of actronitrile. Approximately 4 g of anhydrous $MgSO_4$ and 2 g of NaCl were added, shaken well by vortexing for 1 min, and centrifuged at 4200 rpm for 5 min.

After centrifugation, 1 ml supernatant acetronitrile layer was transferred into a 2 ml Eppendorf tube containing 50 mg primary secondary amine and 150 mg anhydrous $MgSO_4$. The mixture was vortexed for 1 min and centrifuged at 4°C for 5 min at 1000 rpm. The upper extract was collected and filtered through a 0.22mm polypropylene filter and transferred into a 1.5 ml glass vial for GC–MS/MS analysis.

GC–MS/MS analysis

The pesticide residue analysis was carried out in Shimadzu GC–MS-TQ8040. An Rxi-5 Sil MS fused silica column was used for separation. The analysis conditions were as follows: the injection, transfer line and ion source temperatures were 250°C, 240°C and 230°C, respectively, and ultrahigh purity helium (99.99%) was used as the carrier gas at a total flow rate of 4.1 ml/min and a constant flow rate of 1.15 ml/min. One microliter of the sample was injected into the system in splitless mode. The oven temperature program was 110°C at hold time for 5 min and then increased up to 280°C at hold time for 10 min. Mass spectrometry conditions were performed at an interface temperature of 230°C and a source temperature of 200°C. The solvent cut time was set to 2 min, and the injection volume was 1ml.

The components were identified by the NIST 11 mass spectral library. The quantification of the components was calculated from the reference standard concentration of the calibration curve, and for confirmation and quantification of components, MRM experiments were conducted for 27 compounds. The ion values are shown in Table 1, and the spectrum is presented in Fig. 1.

Residues and recovery efficiency

The untreated and pesticide-free cottonseed sample (T_1) was fortified at concentrations of 1, 5 and 10 µg/

ml/g pesticide mix solution in 5 g of sample. They were kept for 1 hr at ambient temperature under closed conditions, and then residues were extracted according to the above extraction procedure. The amount of pesticide residues recovered was quantified by comparison of the peak area of the standard with that of the treated cottonseed sample under identical conditions. The amount of residues recovered in ppm and recovery percentage were calculated using Eq. 1.

$$\text{Residue (mg/g)} = \frac{A_s}{A_{std}} \times \frac{W_{std}}{W_s} \times \frac{A_{stdj}}{A_{sj}} \times V_s \quad \text{.....Eq. 1}$$

Where:

A_s = Peak area of the sample;

A_{std} = Peak area of the standard;

W_{std} = Weight of the standard in ng;

W_s = Weight of the sample in g;

V_s= Volume of the sample (final extract in ml);

A_{sj} = Aliquot of the sample injected in µl; and

A_{stdj}= Aliquot of the standard injection in µl

$$\text{Recovery (\%)} = \frac{\text{Residue}}{\text{Spiked level concentration}} \times 100 \quad \text{……Eq. 2}$$

Quality validation

The average residue content and recovery percentage of the 27 pesticide compound residues at the three concentration levels are shown in Table 2. The standard curve of each pesticide was retrieved by using 27 pesticide mix components of concentrations between 0.2 and 1.0 µg/ml. Their determination coefficient (R^2) values ranged from 0.993 to 0.999, with a relative standard deviation (RSD) below 20% at three spiking levels. The detection limit of the pesticide compounds was obtained from a 3:1 signal-to-noise ratio, and the values were 0.01 to 0.02 µg/g. The blank sample did not appear in any significant peaks. The average recovery values of the residues ranged from 78.20 to 114% (n=3), representing an acceptable level of 70 to 120% (AOAC, 2000). The limit of detection (LOD) and limit of quantification (LOQ) of the method ranged from 0.05 to 0.005 ng/g for all pesticide components.

Statistical analysis

The statistical analysis was performed by SPSS 17.0 for ANOVA. The results were represented based on the mean value from three analytical values and the standard deviations. The significance of the difference (p<0.05) was evaluated by the influence of the processing factor and pesticide residue content.

RESULTS AND DISCUSSION

Pesticide residue of unfortified and pesticide-fortified samples

The OP, OCP and PP compounds were analysed in pesticide-free (T_1) and pesticide-fortified (T_2) samples, and the effects of processing, *viz.,* R_1, R_2, S_1, S_2, AC_1, AC_2 and ST, on pesticide-fortified cottonseed samples are shown in Table 3. The T1 sample showed a nondetectable concentration level for all selected pesticide compound residues, in which the T_2 samples had all pesticide residues with concentrations ranging from 856 to 1138 ng/g, 782 to 1058 ng/g and 857 to 1140 ng/g in the selected OP, OCP and PP compounds, respectively. Additionally, this residue resulted in more MRL in all OP OCP and PP compounds and was thus not suitable for consumption and may cause illness to humans and the environment.

Effects of processing on pesticide compounds

Organophosphates (OP's) compounds

The selected OP compound residue was present in T_2, and its concentration accounted for a maximum in methyl parathion (1138 ng/g), followed by dimethoate (992 ng/g), ethion (991 ng/g) and profenofos (856 ng/g). The selected OP compounds, such as phorate, were detected in treatments R_1, R_2 and ST at 56.45, 88.93 and 53.32 ng/g, respectively. Methyl phorate residues in S_1 (115.77 ng/g), chlorpyrifos residues in R_1 (18.64 ng/g) and R_2 (25.49 ng/g) and ethion residues were present in the roasting process at 8.42 and 9.68 in R_1 and R_2,

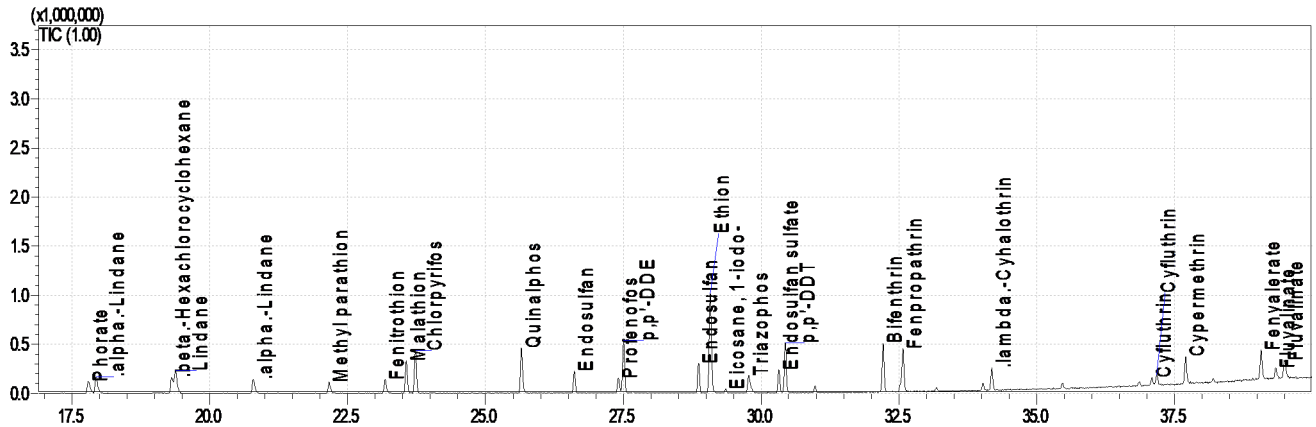

Fig. 1. *GC–MS/MS Spectrum for selected pesticide standard*

respectively, and the autoclaving process at 0.89 and 0.67 ng/g in AC_1 and AC_2, respectively, by pesticide-treated cottonseed samples. The OP compound residues, *viz.,* phorate, chlorpyrifos and ethion residue concentrations, were higher in the R_2 process than R_1 (p<0.05). S1 and S2 showed a nondetectable limit of OP residues in the soaking treatment, except for methyl parathion in S_1 (115 ng/g). Autoclaving processes inhibited the selected OP residue accumulation except for ethion in pesticide-treated cottonseed samples. In storage treatments (ST), phorate residue was present at a concentration of 53.32 ng/g compared with other OP compounds. By considering the MRL of OP compounds set by FFCR, the processed sample from R_1, R_2 and ST treatments showed excess phorate residue than MRL (0.05 mg/kg), and the other OP compound residues were present from all processed samples that had a nondetectable or low concentration level than MRL (Table 3).

Organochlorines (OCP's) compounds

The OCP residues accumulated in the T_2 sample at 91.91%, ranging between 782 ng/g in β-endosulfan and 1058 ng/g in δ-lindane, including total lindane (α-lindane, β-hexachlorochlorocyclohexane, lindane and δ -lindane), total endosulfan (α-endosulfan, β-endosulfan and endosulfan sulfate) and total DDT (*p,p'*-DDE and *p,p'*-DDT), which were 42.33, 28.88 and 20.7%, respectively. The total lindane compound residue was not detected in the processed sample for R_1 and R_2; otherwise, α-lindane in ST (2.06 ng/g), β-hexachloroclohexane in S_2 (38.34 ng/g) and ST (4.72 ng/g), lindane in ST (0.58 ng/g) and δ-lindane in S_1 (30.84 ng/g), AC_1 (69.32 ng/g) and AC_2 (41.25 ng/g) were present. Endosulfan isomers (α and β) were not detected in any processed samples except α-endosulfan, which was 25.32 ng/g in the 12-hour soaked pesticide-treated sample (S_2). Another preliminary compound for endosulfan sulfate was detected at concentrations of 56.65, 36.45, 152.29, 55.17 and 4.28 ng/g in the R_1, R_2, S_1, S_2 and ST samples, respectively. From the total DDT, the *p,p'*-DDE residue was present in the concentration of 12.58, 7.66 and 2.93 ng/g in R_1, R_2 and AC_1 samples and other *p,p'*-DDT residue was leading in soaking processed sample as 4.38 ng/g in S_1 and 3.94 in S_2. In the cottonseed sample, the residue

Table 1. Molecular weight and MRM parameters for selected OPs, OCPs and PPs from GC–MS/MS

S. No	Compound name	Formula	Molecular weight	MRM 1	MRM 2
Organophosphates (OP's)					
1	Phorate	$C_7H_{17}O_2PS_3$	260.4	260.00 >75.10	231>128.80
2	Dimethoate	$C_5H_{12}NO_3PS_2$	229.26	93.0>63.0	125.0>78.9
3	Methyl parathion	$C_8H_{10}NO_5PS$	263.21	263.0>109.0	125.0>79.0
4	Malathion	$C_{10}H_{19}O_6PS_2$	330.358	127.0>99.0	173.0>99.0
5	Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	350.59	197.0>169.0	199.0>171.0
6	Quinalphos	$C_{12}H_{15}N_2O_3PS$	298.3	146.0>118.10	146.0>91.10
7	Profenofos	$C_{11}H_{15}BrClO_3PS$	373.63	97.0>65.0	139.0>97.0
8	Ethion	$C_9H_{22}O_4P_2S_4$	384.48	235.0>165.10	237.0>165.10
9	Triazophos	$C_{12}H_{16}N_3O_3PS$	313.31	161.0>134.1	77.0>51.10
Organochlorines (OCP's)					
10	α-Lindane	$C_6H_6Cl_6$	290.83	219.0>182.9	181.0>145.1
11	β-Hexachlorocyclohexane	$C_6H_6Cl_6$	290.83	181.0>145.10	219>183.10
12	Lindane	$C_6H_6Cl_6$	290.83	219.0>183.0	181.0>144.9
13	δ-Lindane	$C_6H_6Cl_6$	290.83	219.0>183.0	181.0>144.9
14	alpha-Endosulfan	$C_9H_6Cl_6O_3S$	406.95	195.0>160.0	160.0>125.20
15	*p,p'*-DDE	$C_{14}H_8Cl_4$	318.025	246.0>176.10	248.0>176.1
16	β-Endosulfan	$C_9H_6Cl_6O_3S$	406.95	207.0>172.10	159.0>123.1
17	Endosulfan sulfate	$C_9H_6Cl_6O_4S$	422.925	272.0>236.80	274.0>238.90
18	*p,p'*-DDT	$C_{14}H_9Cl_5$	354.486	235.0>165.10	237.0>165.1
Pyrethroids (PP's)					
19	Fenitrothion	$C_9H_{12}NO_5PS$		125.0>79.0	277.0>260.0
20	Bifenthrin	$C_{23}H_{22}ClF_3O_2$	422.9	181.0>166.10	182.00>167.10
21	Fenpropathrin	$C_{22}H_{23}NO_3$	349.4	97>55.10	181.0>152.1
22	λ-Cyhalothrin	$C_{23}H_{19}ClF_3NO_3$	449.85	181.0>152.10	197.0>141.2
23	Cyfluthrin	$C_{22}H_{18}Cl_2FNO_3$	434.3	163.0>127.0	163.0>91.0
24	Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	416.3	163.0>127.1	163.0>91.10
25	Fenvalerate	$C_{25}H_{22}ClNO_3$	419.9	167.0>125.10	125.0>89.1
26	Fluvalinate	$C_{26}H_{22}ClF_3N_2O_3$	502.913	250>200.1	142>94.5
27	Deltamethrin	$C_{22}H_{19}Br_2NO_3$	505.21	181.0>152.0	181.0>150.8

MRM - Multiple Reaction Monitoring

concentrations of β-hexachlorocyclohexane in S2 and δ -lindane in S_1, AC_1 and AC_2 were greater than the MRL of 0.01 mg/kg.

Pyrethroids (PP's) compounds

Among the samples, T_2 samples resulted for all PP compounds, whose concentration ranged from 857 ng/g for cyfluthrin to 1140 ng/g for fenpropathrin. Among the PP compounds, the residues from fenitrothion, cyfluthrin, cypermethrin and fluvalinate were not detected in any pesticide-fortified processed samples. In the roasting process, the bifenthrin residue concentration was not significant in R_1 and R_2 (5.54 ng/g in R_1 and 5.32 ng/g in R_2); alternatively, fenvalerate and deltamethrin were significant in R_1 (7.41 and 128.64 ng/g) and R_2 (8.41 and 106.33 ng/g). Bifenthrin and fenpropathrin residues were detected in S_1 and S_2 samples; otherwise, deltamethrin and λ-cyhalothrin were present in S_1 and S_2 samples, respectively. Among processing treatments, autoclaving (AC_1 and AC_2) and 90-day stored (ST) pesticide-fortified cottonseed samples resulted in a nondetectable limit of residues from any PP compounds except deltamethrin at 46.14 ng/g in ST. With the exception of deltamethrin, the concentrations of all PP residues from pesticide-fortified processed samples did not exceed the MRL (0.05 to 10 mg/kg) of PP (Table 3). These results were in conformation with the effects of various pesticide residues in different processed foods as per earlier studies are discussed as follows:

Various factors, including species, pesticide compound formulation, spiking method, analytical methods, processing methods, environmental conditions, and the interaction of molecules and physicochemical properties of the food and pesticide sample, contribute to the dissipation of the residues. Upon applying heat during roasting, sterilization and pasteurization processes have modified the evaporation, distillation and degradation activities of pesticide components. Wherever higher degradability appeared in the sterilization and baking

Table 2. Residue concentration and recovery percentage for 1, 5 and 10 µg/g pesticide component-fortified samples

S. No	Pesticide name	Average Residue (mg/g)			Average Recovery (%)		
		1	5	10	1	5	10
Organophosphates (OP's)							
1	Phorate	0.925	4.642	7.351	92.50	92.84	73.51
2	Dimethoate	0.992	4.982	8.929	99.20	99.64	89.29
3	Methyl parathion	1.14	4.358	8.151	114.00	87.16	81.51
4	Malathion	0.892	4.507	7.064	89.20	90.14	70.64
5	Chlorpyrifos	0.938	3.956	8.413	93.80	79.12	84.13
6	Quinalphos	0.961	4.058	7.320	96.10	81.16	73.20
7	Profenofos	0.856	4.983	9.483	85.60	99.66	94.83
8	Ethion	0.991	4.861	9.329	99.10	97.22	93.29
9	Triazophos	0.948	4.449	8.561	94.80	88.98	85.61
Organochlorine (OCP's)							
10	α-Lindane	0.887	4.985	9.827	88.70	99.70	98.27
11	β-Hexachlorocyclohexane	0.983	5.014	10.782	98.30	100.28	107.82
12	Lindane	0.882	4.824	9.413	88.20	96.48	94.13
13	δ-Lindane	1.058	4.698	8.364	105.80	93.96	83.64
14	α-Endosulfan	0.964	4.950	10.621	96.40	99.00	106.21
15	*p,p'*-DDE	0.991	5.637	12.002	99.10	112.74	120.02
16	β-Endosulfan	0.782	4.921	9.583	78.20	98.42	95.83
17	Endosulfan sulfate	0.854	3.868	9.796	85.40	77.36	97.96
18	*p,p'*-DDT	0.872	3.954	9.681	87.20	79.08	96.81
Pyrethroids (PP's)							
19	Fenitrothion	0.952	5.014	8.582	95.20	100.28	85.82
20	Bifenthrin	0.948	4.015	8.473	94.80	80.30	84.73
21	Fenpropathrin	1.140	4.821	9.358	114.00	96.42	93.58
22	λ-Cyhalothrin	0.873	4.225	8.153	87.30	84.50	81.53
23	Cyfluthrin	0.857	3.985	6.470	85.70	79.70	64.70
24	Cypermethrin	0.948	4.418	9.127	94.80	88.36	91.27
25	Fenvalerate	0.881	5.004	9.387	88.10	100.08	93.87
26	Fluvalinate	0.938	4.983	9.158	93.80	99.66	91.58
27	Deltamethrin	0.995	4.875	11.254	99.50	97.50	112.54

Table 3. Pesticide residues in pesticide-free, pesticide-fortified, and pesticide-fortified cum processed cottonseed samples (ng/g) and MRL (mg/kg)

S. No	Pesticide name	T_1 (ng/g)	T_2 (ng/g)	R_1 (ng/g)	R_2 (ng/g)	S_1 (ng/g)	S_2 (ng/g)	AC_1 (ng/g)	AC_2 (ng/g)	ST (ng/g)	MRL* (mg/kg)
Organophosphates (OP's)											
1	Phorate	nd	925.0±13.21	56.45±0.84	88.93±2.60	nd	nd	Nd	nd	53.32±0.07	0.05
2	Dimethoate	nd	992.0±5.37	nd	nd	nd	nd	Nd	nd	nd	1.0
3	Methyl parathion	nd	1138.0±8.80	nd	nd	115.77±2.91	nd	Nd	nd	nd	1.0
4	Malathion	nd	892.0±7.89	nd	nd	nd	nd	Nd	nd	nd	20
5	Chlorpyrifos	nd	938.0±8.29	18.64±0.12	25.49±0.65	nd	nd	Nd	nd	nd	0.05
6	Quinalphos	nd	961.0±3.07	nd	nd	nd	nd	Nd	nd	nd	0.02
7	Profenofos	nd	856.0±8.15	nd	nd	nd	nd	Nd	nd	nd	3.0
8	Ethion	nd	991.0±8.09	8.42±0.21	9.68±0.21	nd	nd	0.89±0.01	0.67±0.02	nd	0.3
9	Triazophos	nd	948.0±9.67	nd	nd	nd	nd	Nd	nd	nd	0.2
Organochlorine (OCP's)											
10	α-Lindane	nd	887.0±9.65	nd	nd	nd	nd	Nd	nd	2.06±0.05	0.01
11	β-Hexachlorocyclohexane	nd	983.0±9.36	nd	nd	nd	38.34±1.25	Nd	nd	4.72±0.10	0.01
12	Lindane	nd	882.0±11.40	nd	nd	nd	nd	Nd	nd	0.58±0.01	0.01
13	δ-Lindane	nd	1058.0±4.58	nd	nd	30.84±0.52	nd	69.32±0.99	41.25±0.75	nd	0.01
14	α-Endosulfan	nd	964.0±6.55	nd	nd	25.32±0.03	nd	Nd	nd	nd	0.3
15	*p,p'*-DDE	nd	991.0±8.43	12.58±0.27	7.66±0.15	nd	nd	2.93±0.08	nd	nd	0.05
16	β-Endosulfan	nd	782.0±15.43	nd	nd	nd	nd	Nd	nd	nd	0.3
17	Endosulfan sulfate	nd	854.0±9.75	59.65±0.52	36.45±0.96	152.29±5.07	55.17±0.48	Nd	nd	4.28±0.39	0.3
18	*p,p'*-DDT	nd	872.0±7.71	nd	nd	4.38±0.09	3.94±0.02	Nd	nd	nd	0.05
Pyrethroids (PP's)											
19	Fenitrothion	nd	952.0±9.42	nd	nd	nd	nd	Nd	nd	nd	0.8
20	Bifenthrin	nd	948.0±2.19	5.54±0.14	5.32±0.13	2.50±0.05	3.64±0.07	Nd	nd	nd	0.5
21	Fenpropathrin	nd	1140.0±8.53	nd	nd	28.71±0.93	5.92±0.18	Nd	nd	nd	0.15
22	λ-Cyhalothrin	nd	873.0±7.72	nd	nd	nd	16.45±0.33	Nd	nd	nd	0.05
23	Cyfluthrin	nd	857.0±10.49	nd	nd	nd	nd	Nd	nd	nd	1.0
24	Cypermethrin	nd	948.0±2.58	nd	nd	nd	nd	Nd	nd	nd	0.1
25	Fenvalerate	nd	881.0±5.64	7.41±0.01	8.41±0.12	nd	nd	Nd	nd	nd	0.2
26	Fluvalinate	nd	938.0±6.38	nd	nd	nd	nd	Nd	nd	nd	0.1
27	Deltamethrin	nd	995.0±16.24	128.64±2.10	106.33±1.66	72.56±1.08	nd	Nd	nd	46.14±4.09	0.04

*Pesticide Maximum Residue Limit (MRL) for cottonseed set by The Japan Food Chemical Research Foundation (FFCR); T_1- Untreated pesticide free; T_2-Pesticide treated sample; R_1- Roasting 5 min; R_2-Roasting 10 min; S_1-Soaking 6 hours; S_2-Soaking 12 hours; AC_1-Autoclaving 5 min; AC_2-Autoclaving 10 min; ST-Storage; nd-non detectable

process, it was due to the penetration rate of steam, which was higher than other heat processes (Sengupta *et al.,* 2010). However, a prolonged drying process has increased the residue level due to moisture loss of the sample (Holland, 1994). OCP (γ-BHC, chlordane and heptachlor) compounds were spiked at concentrations of 0.2 and 1 µg/g in roasted green coffee beans at 250° C for 210 seconds. γ-BHC was not detectable at all in the sample, and chlordane also lost up to 90% after the roasting process. The high vapour pressure of the γ-BHC compound could be the reason for its disappearance during the roasting process, even as chlordane has a lower vapour pressure (Sakamoto and Manabe, 2012). Similar studies were observed in residue reductions for hexachlorobenzene (99.8%), heptachlor epoxide (97.9%), pp-DDE, pp-DDD (97.6) and op-DDT (96%) and not detectable residues in cypermethrin and endosulfan isomers (α and β) from roasted (240°C for 14 min) coffee beans (Mekonen *et al.,* 2015).

The efficiency of the selected OP and OCP residue reduction or elimination processes was determined by soaking potato pieces in acidic, alkaline, neutral and tap water solutions for 10 min. The results showed that tab water was less effective than acidic (radish) solutions for residue elimination (Zohair, 2001). Chlorpyrifos at the levels of 10, 15 and 25 PPM were spiked and soaked for 24, 48 and 72 hours at 25°C, respectively, in chickpea samples. It was evident that the 24-hour soaking process led to the complete dissipation of chlorpyrifos residues without producing metabolites such as oxon and 3,5,6-trichloro-2-pyridinol (Kaushik et al., 2016). Considering the different cooking methods of rice, the presoaking process causes lead residue reductions for compounds such as deltamethrin, penconazole, kresoxim-methyl, cyproconazole, epoxiconazole and azoxystrobin of 87.98%, 73.69%, 85.93%, 71.31%, 78.18%, and 90.33%, respectively (Medina, et al 2021). Soaking crushed sorghum with boiled water (90°C) for 22 hours showed a significant reduction in dichlorvos, fenitrothion, pirimiphos-methyl, and malathion deltamethrin by 87%, 36%, 28%, 32% and 29%, respectively. The presence of temperature in water increases volatilization or degradation, thus leading to an increase in the reduction of dichlorvos due to its high vapour pressure (Han *et al.,* 2016). These losses accounted for the addition or dilution of water at the time of soaking for grains or samples, which could dilute the concentration of the pesticide components in the water.

Chlorpyrifos are highly degraded in pressure cooking and microwave cooking (Kaushik et al., 2016). DDT and its metabolites were decreased in the range of 15.6 to 58.8%, while malathion, chlorpyrifos, pp-DDT and lindane were reduced by 51.9, 44.7, 29.8% and 73% in milk pasteurization at 65°C for 30 min (Abou-Arab, 1999a). In the sterilization process (121°C for 15 min), the residues from HCH, DDT, endosulfan and lindane were reduced by 19, 13, 11 and 84.4%, respectively, in tomatoes (Abou-Arab, 1999b). DDT and its derivative residues had a greater reduction in pressure cooking at 3 min for 15 psi with microwave cooking for 6 min in green beans (Kaushik et al., 2016). Cooking spinach at 122°C for 66 min resulted in a 100% reduction in azinphos-methyl, 96% malathion and 100% methyl parathion, and cooking tomato at 100°C for 30 min resulted in 71 to 81.6% dissipation of selected OPs (Kaushik et al., 2016 and Abou-Arab, 1999b). The above studies revealed that applying heat and pressure in the pressure cooking process can modify cottonseed and pesticide component interactions through increases in volatilization and degradation, thus resulting in reduced residue deposition in the end products.

Storage temperature, medium and structure of the compounds could influence the residue accumulation in the samples, and the lipophilic pesticide residues were more penetrated with oilseeds (Bajwa and Sandhu, 2014). Deltamethrin was found to be 0.03 to 0.2 mg/kg and 0.4 to 1.5 mg/kg after 180 and 240 days of stored wheat flour at 20±2°C, respectively. The results indicated that deltamethrin was long stable at the storage time of the sample (Balinova et al., 2007). Similar results were observed after 180 days of storage of pesticide-treated cottonseed samples. The recommended concentration level of malathion (2% dust) was applied to pesticide-free wheat, and its initial concentration was 8.89 mg/kg. After 127 days of storage at room temperature, the residue (4.28 mg/kg) was found to be more abundant than MRL (Uygun et al. 2005). Chlorpyrifos (dose level - 6 g a.i m^{-2}) applied to chickpea seeds resulted in residue levels below 0.5 ppm and produced metabolite components at the end of 150 days of storage (Kaushik et al., 2016). L. Zhao et al. (2014) revealed that the concentration of chlorpyrifos in soybean storage at periods of 0, 15, 31, 60, 90, and 112 days was reduced by 0, 4.2, 26.9, 36.2, 38.1 and 62.3%, respectively. This can be attributed to the volatility characteristics of chlorpyrifos.

Conclusion

The study on residues from pesticide-free, pesticide-fortified and pesticide-fortified cum simple processing on selected pesticide compounds from OPs, OCPs and PPs in cottonseeds (*Gossypium* spp.) indicated that 1 µg/ml/g of mixed pesticide-fortified sample contained all residues, which showed an exceeded concentration of MRLs, and the processing techniques showed an enhanced reduction of residues from pesticide-fortified samples. The R_2 process increased the residues for phorate, chlorpyrifos and ethion in OPs, alternatively

reduced *p,p*-DDE and endosulfan sulfate in OCPs and significantly changed bifenthrin, fenvalerate and deltamethrin in PPs compared with R_1. During soaking, the S_2 process showed a nondetectable concentration level for all OPs and improved the reduction in selected OCPs and PPs with S_1. Most of the selected pesticide compounds, except for ethion, δ-lindane and pp-DDE residues were not detectable in the pressure cooking process. The pesticides fortified cum 180 days of stored cotton seeds contained residues for phorate, total lindane excluding δ-lindane and deltamethrin. The findings of this work indicate that most pesticide residues are reduced through simple processing techniques and that autoclaving eliminates most of the residue in pesticide-fortified samples. However, pesticide residues such as phorate, δ-lindane and deltamethrin concentrations were found in excess of MRLs in a few processed samples, which the applicant should consider in food products, and people will be aware of residue -reducing techniques at the industrial or household level for achieving better health.

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Conflict of interest

The authors declare that they have no conflict of interest.

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