Journal of Food Composition and Analysis 148 (2025) 108499

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Analytical method development and multi-residue analysis of pesticides in Indian Black Tea with implications for food safety and consumer protection

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ARTICLE INFO

Keywords:
Indian black tea
LC-MS/MS
GC-ECD
Pesticide residue analysis
Food composition
Modified QuEChERS method

ABSTRACT

A modified QuEChERS method was developed and validated for efficient extraction and accurate quantification of pesticide residues in black tea using GC-MS/ECD and LC-MS/MS techniques. Optimization of the clean-up sorbent composition, particularly the proportion of graphitized carbon black, significantly improved recovery efficiency. The method showed acceptable linearity, selectivity, trueness, precision, recovery, limits of quantification, and measurement uncertainty, meeting internationally recognized validation criteria. Using this approach, 36 commercial black tea samples from India were analyzed for 78 pesticide residues, including those regulated by FSSAI. Eleven pesticides were detected in 28 samples, with monocrotophos most frequent, followed by acetamiprid, acephate, imidacloprid, cypermethrin, and captafol. Methomyl, dinotefuran, simazine, 4,4′-DDT, and γ-BHC appeared in fewer than 10 % of samples. Although 26 samples exceeded Indian MRLs, none surpassed Codex Alimentarius limits. Dietary risk assessment based on Estimated Daily Intake, Hazard Quotient, and Hazard Index values indicated negligible acute risk for adults and children. However, the recurring detection of multiple residues, even at trace levels, suggests potential chronic exposure concerns. Continuous monitoring and the adoption of safer pest management strategies are essential to safeguard consumer health.

1. Introduction

Tea (*Camellia sinensis*) holds a significant place in global beverage consumption, often referred to as the “queen of beverages” due to its therapeutic properties, pleasant aroma, and complex flavour profile. Globally, it is the most consumed beverage after water, driven by its rich content of health-promoting bioactive compounds such as catechins, gallic acid, theanine, epigallocatechins, chlorogenic acid, and various micronutrients (Chen et al., 2017). India is a major contributor to global tea production, accounting for approximately 23.34 % of total output, with prominent cultivation regions in the northeastern states (Assam, West Bengal, Sikkim), the Western and Eastern Ghats (Tamil Nadu, Kerala, Karnataka), and northern hills (Uttarakhand, Himachal Pradesh) (Tea Board of India, 2021). However, its cultivation is highly susceptible to biotic stress, particularly from insect pests and fungal diseases, due to favourable climatic conditions in tea-growing regions. More than a thousand species of phytophagous pests, weeds, and pathogens are known to affect tea crops, necessitating the routine use of chemical pesticides for crop protection (Chen et al., 2017; Seenivasan and Muraleedharan, 2011).

Insecticides such as monocrotophos, acephate, acetamiprid, imidacloprid, and cypermethrin are commonly applied against major tea pests, including the tea mosquito bug (*Helopeltis theivora*), aphids, thrips, jassids, and caterpillars (FAO, 2020; Roy et al., 2016). These pesticides, belonging to classes such as organophosphates, neonicotinoids, and synthetic pyrethroids, offer broad-spectrum efficacy and rapid action. However, their frequent use and persistence raise concerns about pesticide residues in finished tea products. The detection of multiple pesticide classes organophosphates, organochlorines, carbamates, neonicotinoids, and herbicides has been widely reported in commercial teas (Sharma et al., 2008; Cajka et al., 2012), highlighting the need for robust residue monitoring and improper or excessive application can result in the accumulation of pesticide residues in tea leaves, which is especially concerning as freshly picked leaves are directly processed without washing. These residues have been associated with a range of adverse health effects in humans, including nausea, neurological disorders, endocrine disruption, and increased cancer risk (Jaggi et al., 2001; Jaga and Dharmani, 2006; Karthika and Muraleedharan, 2009).

In recent years, methods like enzyme-linked immunosorbent assay (ELISA) and biosensors have gained attention for being simple, fast, and

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https://doi.org/10.1016/j.jfca.2025.108499
Received 9 July 2025; Received in revised form 15 October 2025; Accepted 19 October 2025
Available online 24 October 2025

useful for on-site monitoring. Among them, electrochemical biosensors are especially promising due to their sensitivity, portability, and ability to provide real-time results with minimal sample preparation (Singh et al., 2020). In several tea-producing nations, systematic studies have reported the presence of both authorised and banned pesticides in tea products. For instance, recent Chinese and Taiwanese investigations found multiple residues in retail tea samples, some exceeding acceptable limits and posing chronic dietary risks (Wu et al., 2022; Lu et al., 2020).

In this study, we optimised and modified the QuEChERS protocol by introducing a pre-extraction hydration step and employing modified matrix specific QuEChERS extraction method, optimised with sorbents like PSA, C18, GCB, along with $MgSO_4$ to remove tea pigments and polyphenols. In the QuEChERS cleanup step, both Graphitised Carbon Black and C18 sorbents were utilised to effectively address the complex black tea matrix. GCB is crucial for removing planar interfering compounds, such as pigments, polyphenols, and sterols, which are abundant in tea and can cause significant matrix effects. Concurrently, C18 targets non-polar interferences like lipids and waxes, ensuring a more comprehensive purification of the extract. The synergistic use of these two sorbents allows for the removal of a wider range of matrix components, leading to cleaner extracts, improved analytical performance, and enhanced accuracy and reproducibility for multi-residue pesticide analysis in black tea. Detection is commonly performed using gas chromatography with electron capture or mass spectrometric detectors (GC-ECD/MS/MS) for volatile pesticides, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for polar and thermally labile compounds (Cajka et al., 2012; Chen et al., 2017). Considering the widespread consumption of tea in India, with an average per capita intake of approximately 6 g per day (Jaggi et al., 2001; JMPR, 2020), the detection and dietary risk assessment of pesticide residues are essential for protecting public health. However, national-scale monitoring of banned or unapproved pesticide residues in commercially available Indian black tea are currently lacking. In order to address this concern, the Food Safety and Standards Authority of India (FSSAI) issued a directive in March 2024 mandating all notified laboratories to screen for a wide range of pesticide residue in tea, including compounds that have not been monitored previously (FSSAI, 2024).

This present study was conducted based on this directive and aims to address the gap by evaluating 78 pesticides commonly used in Indian agriculture, particularly in major tea-growing areas, using a novel matrix-specific modified QuEChERS method to support national monitoring efforts and to evaluate potential consumer health risks based on residue levels observed in samples collected from major tea-producing regions across India. Unlike previous studies, our approach provides matrix-specific validation, selective extraction efficiency, and essential post-regulatory baseline data to inform implementation and guide future policy development and addresses the lack of nationally representative pesticide residue data for Indian black tea by employing a validated, matrix-specific modified QuEChERS method.

2. Materials and methods

2.1. Chemicals and reagents

All reagents and chemicals employed in this study were of analytical grade. Anhydrous magnesium sulphate ($MgSO_4$ of 98.5 %) and sodium acetate (CH_3COONa) of ACS grade were purchased from Merck, India. Primary secondary amine (PSA) and Graphitised carbon black (GCB) were purchased from Agilent Technologies, USA. Acetonitrile and methanol, used as solvents in this study, were of LC-MS/MS grade ($\geq$99.9 % purity) and sourced from Biosolve, Netherlands. Ultrapure water used in the study was obtained from a Milli-Q water purification system (Millipore, Merck, USA). High-purity gases (>99.99 %) were utilised across all analytical instruments. All chemicals, reagents, and gases were verified to be free from any potential interferences before use.

2.2. Certified reference materials (CRMs) and standard stock solutions

In this study, a multi-residue method was developed to detect a wide range of pesticide residues in tea. Since several target pesticides were only available as individual single certified reference materials (CRMs), a combination of mixed-standard solutions and high-purity single standards were procured. Highly pure pesticide standard of an Organochlorine pesticide mix, containing Alpha BHC, Beta BHC, Gamma BHC, Delta BHC, Heptachlor, Aldrin, Heptachlor epoxide, Gamma Chlordane, Alpha Endosulfan, Alpha Chlordane, Dieldrin, Endrin, Beta Endosulfan, Endrin aldehyde, 4,4-DDT, 4,4-DDE, 4,4-DDD, Endosulfan sulfate, Endrin ketone, Methoxychlor, and individual certified reference materials like Thiamethoxam, Ethofenprox, Propazine, Malathion, Captafol, Chlorpyrifos, Dinotefuran, Fenitrothion, 2,4-DDT, 2,4-DDD, Ethyl paraoxon, with purity levels ranging from 97 % to 99 % were also obtained from Sigma Aldrich (Switzerland). Imidacloprid, Carbendazim, Dichlorvos, Phorate, Acephate, Dimethoate, Fipronil, Atrazine, Diazinon, Methyl parathion, Quinalphos, Profenofos, Aldicarb, Methomyl, Carbofuran, Fenthion, Simazine, Ethyl parathion, Chlorfenvinphos, Difenoconazole, Hexaconazole, Tebuconazole, Spiromesifen, Fenpropathrin, Captan, Trifloxystrobin from HPC (Germany) and Monocrotophos, Phosphamidon, Ethion, Disulfoton, Phosalone, and a pesticide mix containing L-Cyhalothrin, Cypermethrin, Pendimethalin, Cyfluthrin, Permethrin, Fenvalerate, Deltamethrin, Tefluthrin, Tetrachlorvinphos, Dichloran were obtained from AccuStandard (USA) and Aldicarb sulfone, Aldicarb sulfoxide, Fenthion sulfone, Fenthion sulfoxide, 2,4-DDE, Acetamiprid from Dr. Ehrenstorfer GmbH (Germany), were used for comprehensive pesticide residue analysis. Each standard mixture contained a different number of pesticides representing various chemical groups Supplementary Table S2.

2.3. Preparation of working standards

Individual stock solutions (1000 mg/kg) were prepared by accurately weighing 10 $\pm$ 0.1 mg of each pesticide. Each pesticide was dissolved in a few drops of acetone and then made up to 10 mL in a standard flask. Solvents were selected according to the analytical technique: n-hexane for GC-ECD analysis and LC-grade methanol for LC-MS/MS analysis. Stock solutions were combined to prepare intermediate mixtures containing multiple compounds. Two separate groups were made: 21 pesticides in methanol at 10 mg/kg for LC-MS/MS analysis and 57 pesticides in n-hexane at 1 mg/kg for GC-ECD analysis. The intermediate mixtures were stored at –20 °C (Samsung refrigerator) for further use. Working standard solutions were prepared by diluting the intermediate mixtures to the required concentrations: LC-MS/MS: 0.5, 1, 2, 5, 10, 20, 25, and 50 μg/kg, GC-ECD: 10, 20, 50, 100, 150, and 200 μg/kg. These solutions were used to evaluate linearity and for spiking samples during recovery studies. All standard solutions were stored in a deep freezer maintained below −20 °C.

2.4. Equipment

An analytical balance AUX 220 (Shimadzu) with 0.01 g accuracy was used for the preparation of the standard mixture solution and for weighing tea samples. Solvents were taken using a 200 μL and 1 mL Trans™ micropipette (Tarsons, India). The homogenization was performed using a mixer (Prestige Deluxe LS). The samples were centrifuged using an R-24C (Remi, India) centrifuge and were vortexed using a Spinix (Tarsons, India) vortex mixer. The samples were concentrated using a Turbo Vap-LV (Caliper, USA) before the chromatographic analysis.

2.5. Sample collection

To ensure a complete representation of the Indian black tea market, thirty-six commercially available black tea samples were randomly collected from markets of major tea-growing regions across India between June and December 2024 to give a good national representation. This helps ensure that the results apply broadly to Indian black teas, following the sampling procedures outlined by FAO/WHO Codex guidelines (FAO, 1999) for monitoring pesticide residues and conducting risk assessment. Eighteen samples were sourced from Assam. Five samples were collected from Chikmagalur in Karnataka. Another four samples were collected from Kerala, specifically the Munnar region situated in the Western Ghats. Four samples were from the Nilgiris of Tamil Nadu. Furthermore, five samples were obtained from the Darjeeling region of West Bengal. Each sample, weighing approximately 50 g, was transferred into sterile 50 mL centrifuge tubes to prevent contamination. All samples were properly labelled with relevant details, including location, date of collection, and sample code. The samples were then stored under optimal conditions at 26.5 °C and 56 % relative humidity in a dry and dark environment until analysis. The tea samples were randomly collected from major tea-growing regions across 2.6. Extraction and cleanup

2.6. Extraction and cleanup

The conventional QuEChERS (Anastassiades et al. 2003) method was modified and evaluated for the determination of pesticide residues in the tea samples. The samples (1 ± 0.1 g) were weighed in a 50 mL centrifuge tube, and the samples were hydrated for 30 min by adding 10 mL of MilliQ water, followed by the addition of 10 mL of acetonitrile with 1 % acetic acid. The mixture was vortexed for 1 min to ensure thorough mixing, after which anhydrous magnesium sulphate (4 g) and sodium acetate (1 g) were added. The sample was then centrifuged at 5000 rpm for 5 min to separate the organic layer. The cleanup was employed using a combination of PSA, $MgSO_4$, C18, and GCB to improve extract purity and matrix removal efficiency. PSA and $MgSO_4$ were used for sugar and water removal, respectively, while both C18 and GCB were included to target lipophilic interferences and pigments commonly found in black tea. (Agilent Technologies, 2014).

For dispersive cleanup, PSA sorbent (0.4 g), C18 sorbent (0.4 g), GCB (0.045 g) and anhydrous magnesium sulphate (1.2 g) were weighed separately in a 15 mL centrifuge tube. To this, 6 mL of the supernatant from previously centrifuged tea samples was added. The mixture was first vortexed and then centrifuged at 5000 rpm for 5 min to obtain a purified extract. For sample preparation for LC-MS/MS analysis, 1 mL of the supernatant obtained after centrifugation was filtered through a PTFE (0.25 μm) syringe filter into a clean vial and injected into LC-MS/MS. For GC-ECD analysis, 2 mL of the obtained supernatant was transferred into a clean and dry test tube and subjected to solvent evaporation using a TurboVap evaporator, then they are reconstituted with 1 mL of n-hexane, and filtered through a PTFE (0.25 μm) syringe filter into a clean vial and injected on GC-ECD system for further analysis.

2.6.1. GC-ECD/MS conditions

For the GC analysis of the pesticide residues, a Gas chromatography coupled with an electron capture detector (GC-MS/ECD) was performed using an Agilent 7890B system fitted with an HP-5 capillary column (30 m × 320 μm × 0.25 μm) for the efficient separation of the analytes.

A split injection liner was used for uniform vaporisation and consistent sample introduction. The ECD as detector, Nitrogen gas having a column flow rate of 0.75 mL/min[1] , was used as the gaseous medium. The temperatures of the injector, ion source, and interface were maintained at 160 °C, 290 °C, and 325 °C, respectively. The column temperature program was optimised for sharp peak elution. An injection volume of 1 μL was used with a split ratio of 1:10, while the run took a total of 35 min to complete. The GC run was carried out at varying oven temperatures, followed by a temperature gradient programme. The oven temperature was initially maintained at 160 °C, where it was held for two minutes. Next, the oven temperature was raised to 290 °C, where it was held for seven minutes. The maximum oven temperature is 325 °C. The temperature of the oven was configured from 60 °C to 325 °C. The ionisation source was electron ionisation (EI), and column head pressure was maintained at 7.6 psi and each sample, along with spiked samples, was injected into the GC-ECD system in duplicates.

2.6.2. LC-MS/MS conditions

For LC analysis of pesticide residues, a Liquid Chromatography system (Xevo TQ-S, Waters, USA) coupled with a triple quadrupole mass spectrometer equipped with an electron spray ionisation (ESI) source operating in positive mode was employed. An ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 ×50 mm; Waters, USA) was used for efficient analyte separation. The mobile phase consists of a binary solvent system: 5 mM ammonium acetate in 0.1 % acetic acid in water (Solvent A) and 5 mM ammonium acetate in 0.1 % acetic acid in methanol (Solvent B). Sample injection volume was 1 μL, and analyte elution occurred under gradient mode over a 17-minute runtime. The elution gradient programme is given in Table 1.

Mass detection was carried out using electrospray ionisation (ESI) in positive mode. The ion source and interface temperatures were maintained at 526 °C and 300 °C, respectively. Dwell time for each MRM transition was set at 33 ms to ensure sufficient data points and precise quantification. Nitrogen served as both the desolvation and cone gas, while argon was used as the collision-induced dissociation (CID) gas. The nebuliser gas flow rate was maintained at 1.0 L/min, and the heating gas flow rate was set at 15 L/min to ensure efficient ionisation and desolvation.

2.7. Analytical method development and validation

LC-MS/MS was used for the analysis of pesticides that are thermally labile, polar, and non-volatile, which degrade at high temperatures or cannot be vaporised easily. GC-ECD was employed for volatile, thermally stable, and halogenated pesticides because it is highly sensitive to compounds with electronegative elements. Hence detection method was selected based on the chemical nature, volatility, thermal stability, and detector compatibility of the pesticide. For MS methods, identification is often confirmed using ion ratios and multiple reaction monitoring (MRM) and for ECD, retention time is considered. Table 2 shows the Multiple Reaction Monitoring (MRM) parameters for selected pesticides, including precursor/product ion transitions, cone voltage, collision energy, and retention time (RT), optimised for LC-MS/MS analysis and Table S1 in the supporting information shows the retention times (RT) of various pesticides analysed using GC-ECD detection. In compliance with SANTE/11312/2021 guidelines, the analytical method was validated by evaluating key performance parameters, including linearity, retention time (RT), specificity, recovery (trueness and precision), limit of quantitation (LOQ), reproducibility, repeatability, and robustness/ruggedness. Measurement uncertainty (MU) at the LOQ level was estimated following the EURACHEM/CITAC Guide (2012). System suitability tests, such as signal-to-noise and retention time checks, were carried out specifically for the tea matrix. The method's robustness could be further tested with more pesticides and reagents, which was outside the scope of this study. The reliability of the laboratory data was ensured through the implementation of a quality assurance (QA) program, comprising proficiency testing, spike testing, and intra-laboratory comparisons, in line

Table 1
Elution gradient programme in LC-MS/MS.

Time (min)	Flow (mL/min)	% of solvent A	% of solvent B	curve
Initial	0.45	98	2	Initial
0.25	0.45	98	2	6
12.25	0.45	1	99	6
13	0.45	1	99	6
13.1	0.45	98	2	6
17	0.45	98	2	6

Table 2
Multiple Reaction Monitoring (MRM) parameters for selected pesticides, including precursor/product ion transitions, cone voltage, collision energy, and retention time (RT), optimized for LC-MS/MS analysis.

Pesticide	MRM Transition (*m/z*) Precursor > Product 1 / Product 2	Cone (V)	Collision energy (eV)	RT (min)
Monocrotophos	224.10 > 127.10 / 192.94	30	15	3.21
Fenthion Sulfone	311.00 > 109.10 / 125.00	32	24	5.89
Imidacloprid	256.10 > 174.90 / 209.00	25	20	3.57
Acetamiprid	223.00 > 56.10 / 126.00	30	20	3.98
Aldicarb sulfone	223.00 > 86.00 / 148.00	35	14	2.55
Aldicarb sulfoxide	207.00 > 89.00 / 132.00	20	15	2.37
Fenthion Sulfoxide	295.00 > 109.10 / 280.10	20	36	5.71
Dinotefuran	203.00 > 113.00 / 129.00	15	10	2.31
Aldicarb	213.10 > 89.10 / 116.10	35	20	4.71
Carbofuran	222.11 > 123.00 / 165.10	10	20	5.47
Methomyl	162.90 > 88.00 / 105.90	15	10	2.82
Acephate	183.90 > 94.60 / 142.80	20	10	1.84
Dimethoate	230.00 > 124.80 / 198.8	20	22	3.84
Tebuconazole	308.20 > 70.10 / 124.00	30	40	8.05
Carbendazim	192.10 > 132.10 / 160.10	10	30	4.69
malathion	331.00 > 98.90 /126.90	30	25	7.28
Thiamethoxam	292.00 > 132.00 / 211.20	25	20	2.99
Ethofenprox	394.30 > 135.16 /177.19	10	28	5.06
Simazine	202.10 > 96.10 / 104.10	20	30	5.35
Fenthion	279.00 > 105.20 / 169.00	20	24	6.3

with (ISO/IEC 17025,2017) standards.

2.8. Risk assessment

The long-term (chronic) dietary risk associated with pesticide residues in tea samples was evaluated using three key parameters: estimated daily intake (EDI), hazard quotient (HQ), and hazard index (HI). The EDI was calculated based on the average pesticide residue concentrations (mg/kg) detected in tea samples, the average daily consumption of tea (mg/kg body weight per day) was obtained from Food cluster diets for Group G05/Global Environment Monitoring System (WHO/ GEMS /FOODS, 2003), and standard body weights of Indian adults (60 kg) and children (25 kg) as reported by the National Institute of Nutrition (NIN, 2010).

$$\text{Estimated Daily Intake(EDI)} \quad \left(\text{mg} \Big/ \text{kg bw} \Big/ \text{day}\right) = \frac{C \times F}{BW}$$

Where,
C - concentration of pesticides in tea (mg/kg),
F - daily tea consumption rate (kg/person/day), and
W - average body weight (kg) of the consumer.

To address non-quantifiable pesticide residues, those below the limit of quantification (LOQ), two scenarios were considered in accordance with international guidelines (EFSA, 2021). In the lower bound (LB) scenario, non-detects were treated as zero, while in the upper bound (UB) scenario, non-detects were set equal to the LOQ. The HQ for each pesticide was derived by dividing the EDI by the acceptable daily intake (ADI). ADI values were obtained from the Joint FAO/WHO Meeting on Pesticide Residues (JMPR, 2020), which is fixed by the WHO (World Health Organisation, 2018). HQs were calculated separately for adults and children according to their body weight and exposure levels.

$$\text{Exposure} = \frac{\text{Residue level(LB or UB)} \quad \times \text{Tea Intake(kg/day)}}{\text{Body Weight(kg)}}$$

$$HQ = \frac{\text{Exposure}}{\text{ADI}}$$

As tea samples frequently contain multiple pesticide residues, a cumulative risk assessment was performed by summing the HQs of all detected pesticides to compute the hazard index (HI), which provides an estimate of overall chronic dietary exposure.

$$HI = \sum HQ_i$$

An HI value greater than 1 indicates a potential health concern, whereas a value less than or equal to 1 suggests that the risk is within acceptable limits (Lozowicka et al., 2014; Gad Alla et al., 2015).

2.9. Statistical analysis

For statistical analysis, Microsoft Excel 2021 (Microsoft Corporation, USA) was used. The analyses were performed in three replications, and the results were expressed as mean ± SD wherever applicable.

3. Results and discussion

3.1. Development and optimisation of extraction methods

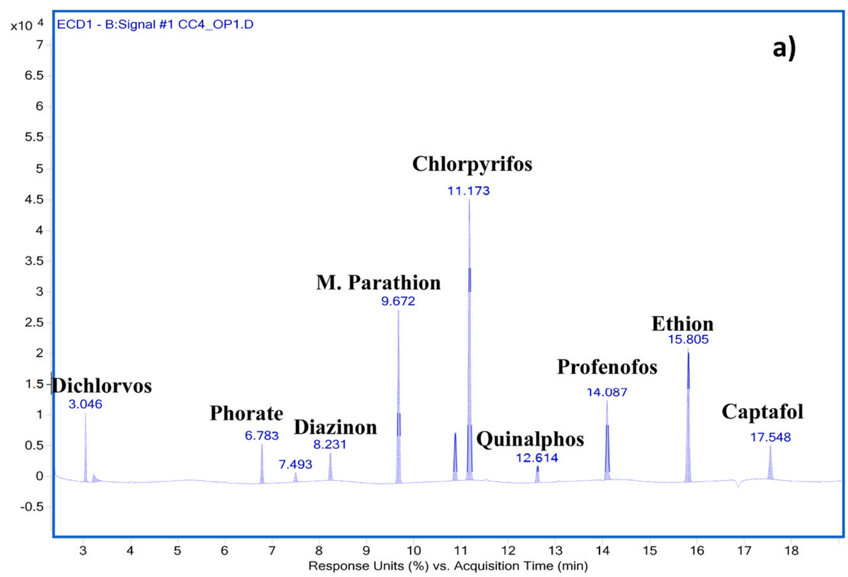
The QuEChERS method has been optimised for extracting and analysing pesticide residues in tea samples using GC-MS/ECD and LC-MS/MS techniques. Previous studies (Huang et al., 2019; Ly et al., 2020) have identified acetonitrile as a preferred extraction solvent owing to its broad compatibility with various classes of pesticides and its capacity to enhance recovery rates while reducing interference from co-extracted matrix components. The impact of sample hydration on pesticide extraction efficiency was assessed by pre-soaking tea samples in water for 30 min before acetonitrile extraction. Instead of brewing the tea where heat could potentially degrade or alter some pesticide residues, we opted for room temperature hydration using 10 mL of Milli-Q water for every 1 g of tea. In order to improve the extraction of pesticides from black tea, the dry tea samples were soaked in water for 30 min before adding acetonitrile as the extraction solvent. Since tea is a dry matrix, directly adding organic solvent like acetonitrile before extraction often leads to low recoveries because the dry matrix does not allow proper solvent penetration or release of analytes. We include this hydration step based on the previous studies. Paya et al. (2007) highlighted that water addition improves analyte release from dry plant matrices, making it a critical step for enhancing extraction efficiency in methods like QuEChERS. More recently, Zhao et al. (2022), studied the addition of different volumes of water before extraction and found that using 10 mL of water significantly increased the pesticide recovery compared to extraction with no hydration. This step proved valuable in improving the overall recovery of pesticides. In our study, this hydration step made it more reliable and gave consistent results, supporting the effectiveness of our modified QuEChERS method for pesticide residue analysis in dry Indian black tea samples. To establish an optimised extraction protocol for pesticide residue analysis in black tea, six different extraction protocols based on QuEChERS method were performed. These variations involved differences in sample weight of tea (0.5–1 g), extraction salt composition ($MgSO_4$ and CH3COONa), and clean-up sorbents used in the dispersive solid-phase extraction (d-SPE) step. Notably, the amount of graphitised carbon black (GCB) used in the clean-up ranged from 25 mg to 60 mg across the methods, which significantly influenced pigment removal and analyte recovery. The comparison of the standard QuEChERS method and modified QuEChERS method has been shown in Table 3. The modified QuEChERS method is specifically optimised for complex matrices like tea, which contain high levels of pigments and polyphenols that can interfere with pesticide analysis. Unlike the standard method, sodium acetate is used instead of NaCl for better buffering and addition of GCB and C18 during clean-up will remove pigments and lipids effectively. A hydration step before extraction further improves analyte recovery. These modifications result in reduced matrix interference, higher recovery rates (70–120 %), and greater suitability for

Table 3
Comparison of Standard and modified QuEChERS method for Tea.

Parameter	Standard QuEChERS (Anastassiades et al., 2003)	Modified QuEChERS (Specifically for tea)
Sample Matrix	For food matrices	Complex matrix like tea (high pigments, polyphenols)
Extraction Salts	$MgSO_4$ + NaCl	$MgSO_4$ + Sodium acetate
Clean-up Sorbents	PSA, $MgSO_4$	PSA + $MgSO_4$ + GCB/C18 for pigment/lipid removal
Recovery Range	60–110 %	70–120 %
Matrix Interference	Higher	Reduced
Suitability for Tea Matrix	Moderate	High
Hydration Step	No hydration	30 min hydration before extraction

accurate pesticide residue analysis in tea Supplementary Table S3. After cleanup, 1 mL of each sample was filtered through a 0.25 µm PTFE filter into LC vials. Subsequently, 2 mL was transferred into clean glass test tubes, subjected to nitrogen evaporation, reconstituted with 1 mL of n-hexane, and then filtered through a 0.25 µm PTFE filter into GC vials for GC-ECD analysis. Fig. 1 shows a GC-ECD chromatogram of a standard tea pesticide mixture (100 µg/kg) and an LC-MS/MS chromatogram of an LC tea pesticide mix (1 µg/kg). The individual compound names along with their respective MRM transitions and retention times have been provided in Tables S1 and S2 for GC-ECD and LC-MS/MS analyses, respectively.

Optimisation of modified QuEChERS parameters for pesticide recovery from tea samples is shown in Table 4. Among the tested conditions, Method 2 (using 1 g tea sample, 4 g $MgSO_4$ + 1 g sodium acetate, and higher amounts of clean-up sorbents) yielded the highest recovery range (90–115 %), indicating better extraction efficiency and matrix clean-up. Increasing the sample amount and adjusting the extraction salt ratio, along with optimised sorbent quantities, significantly better pesticide recovery. Methods 3 and 6 also showed acceptable recoveries (80–100 %), whereas methods 1, 4, and 5, with lower sample mass and varied GCB amounts, exhibited relatively lower recoveries. Thus, method 2 appears to be the most effective for pesticide residue analysis in tea. This method provided the highest overall recovery of pesticides, with minimal matrix interference and better reproducibility. Based on these results, this method was selected as the optimal procedure for routine quantification of pesticide residues in tea samples. Tea being a



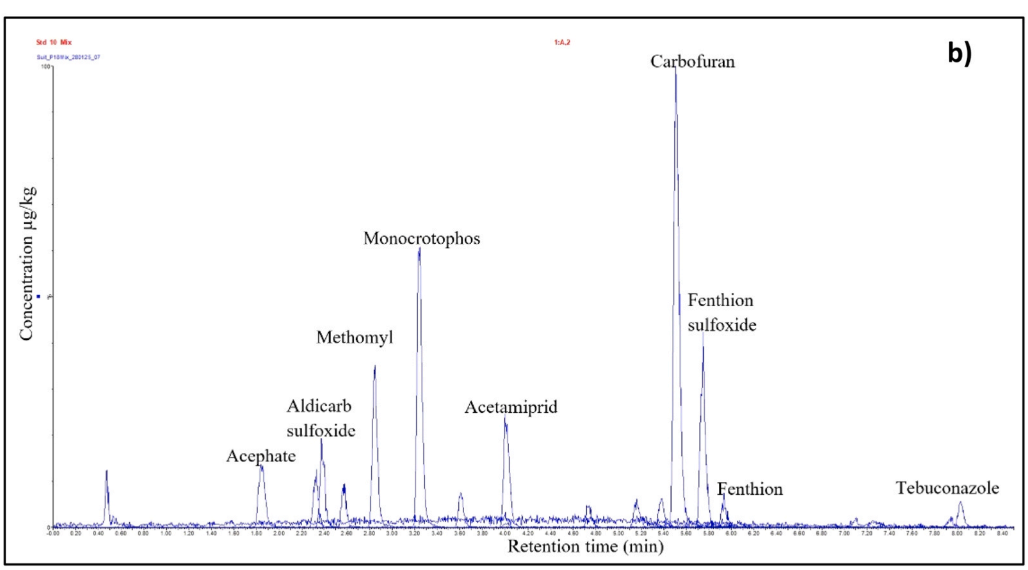


Fig. 1. a) GC-ECD chromatogram of standard tea pesticide mixture (100 µg/kg) and b) LC-MS/MS chromatogram of LC tea pesticide mix (1 µg/kg).

Table 4
Optimization of modified QuEChERS parameters for pesticide recovery from tea samples.

Method	Tea Sample	Extraction Salts	Supernatant Collected	Clean-up Sorbents (mg)	Final Reconstitution Volume	Recovery %
1	0.5 g	6 g $MgSO_4$ + 1.5 g Sodium Acetate	4 mL	0.2 g PSA, 0.2 g C18, 25 mg GCB, 0.6 g $MgSO_4$	1 mL n-Hexane	65–72
2	1 g	4 g $MgSO_4$ + 1 g Sodium Acetate	6 mL	0.4 g PSA, 0.4 g C18, 45 mg GCB, 1.2 g $MgSO_4$	1 mL n-Hexane	90–115
3	0.5 g	6 g $MgSO_4$ + 1.5 g Sodium Acetate	6 mL	0.2 g PSA, 0.2 g C18, 40 mg GCB, 0.6 g $MgSO_4$	1 mL n-Hexane	80–90
4	0.5 g	6 g $MgSO_4$ + 1.5 g Sodium Acetate	6 mL	0.2 g PSA, 0.2 g C18, 50 mg GCB, 0.6 g $MgSO_4$	1 mL n-Hexane	65–78
5	0.5 g	6 g $MgSO_4$ + 1.5 g Sodium Acetate	6 mL	0.2 g PSA, 0.2 g C18, 60 mg GCB, 0.6 g $MgSO_4$	1 mL n-Hexane	55–82
6	0.5 g	4 g $MgSO_4$ + 1 g Sodium Acetate	6 mL	0.4 g PSA, 0.4 g C18, 45 mg GCB, 1.2 g $MgSO_4$	1 mL n-Hexane	80–100

complex matrix containing polyphenols, fatty acids, and other pigments, four different clean up sorbents PSA, C18, GCB in combination with $MgSO_4$ and sodium acetate gave acceptable recoveries (70–120 %) of all the pesticides in the testing range as per SANTE (SANTE/11312/2021).

$$\%\text{Recovery} = \frac{\text{Obtained Concentration}}{\text{Spiked Concentration}} \times 100$$

3.2. Method validation

The following analytical parameters were studied to assess the validity of the methodology: LOQ, trueness, precision, recovery, repeatability, robustness/ruggedness, RT, MI Specificity, linearity of the calibration curves, and MU. The minimum detectable concentrations were established as three times the standard deviation of the blank, while the lowest concentration on the calibration curve of the neat standard was designated as the LOQ. For all the pesticides tested, the determined LOQs were at or below the respective MRLs for tea as set by Codex (Codex, 2022), and a few exceeded the FSSAI (FSSAI, 2018). Precision was also assessed at 10 times the LOQ. No matrix interference was detected in chromatograms for the targeted pesticides. Extraction efficiency was evaluated over the LOQ using four replicates on different days at varying spiking concentrations one being below the LOQ 0.5, 1, 10, and 25 μg/kg for LC-MS/MS and 10, 25, 50, and 100 μg/kg for GC-ECD, using blank tea matrices that were analytically confirmed to be free of detectable pesticide residues (Supplementary Table S4). The percentage recovery, ranging between 72 % and 112 % and 72 and 110 %, respectively, for LC-MS/MS and GC-MS/ECD amenable pesticides, was at acceptable levels. Good precision of the methodology was demonstrated with % RSD ranging between 1.43 % and 2.82 % for GC-MS/ ECD and 0.46–14.72 % for LC-MS/MS. To minimise matrix effects, quantification was performed using matrix-matched calibration standards (Codex, 2022). Good linearity with six-point calibration was obtained for 57 pesticides using GC-ECD in the range of 10, 20, 50, 100, 150, 200 μg/kg and eight-point calibration was obtained for 21 pesticides using LC-MS/MS in the range of 0.5, 1, 2, 5, 10, 20, 25, 50 μg/kg with a correlation coefficient of $R^2 \geq 0.999$. In this study, there was no significant deviation in recoveries based on any specific pesticide classes. Based on the chemical property of the pesticides under study, the detection method (LC-MS/MS or GC-ECD) was selected accordingly so as to get maximum recovery by using suitable detectors. Hence the overall recoveries for most pesticides remained within the acceptable range of 70–120 % across all spiked levels. However, some matrix effects were seen in few compounds during quantification, particularly in LC-MS/MS. To account for this, we employed matrix-matched calibration in both GC-ECD and LC-MS/MS which helped in signal enhancement and detector response accurately. For example, Fig. 2 shows the GC-ECD chromatograms of tea pesticide mix showing neat standard and matrix-matched standard, highlighting the influence of matrix effects on analyte response. To illustrate the analytical sensitivity and emphasize the importance of matrix correction, a comparison plot of detected pesticide concentrations relative to the LOQs (1 μg/kg for LC-MS/MS and 10 μg/kg for GC-ECD) is provided. The limits of detection for all analysed compounds were below the maximum residue limits established by the EU, ensuring reliable quantification of targeted pesticide concentrations in the samples (European Commission, 2024). Method specificity was demonstrated by ensuring that no interfering peaks appeared in the blank sample runs (Codex, 2022). Measurement

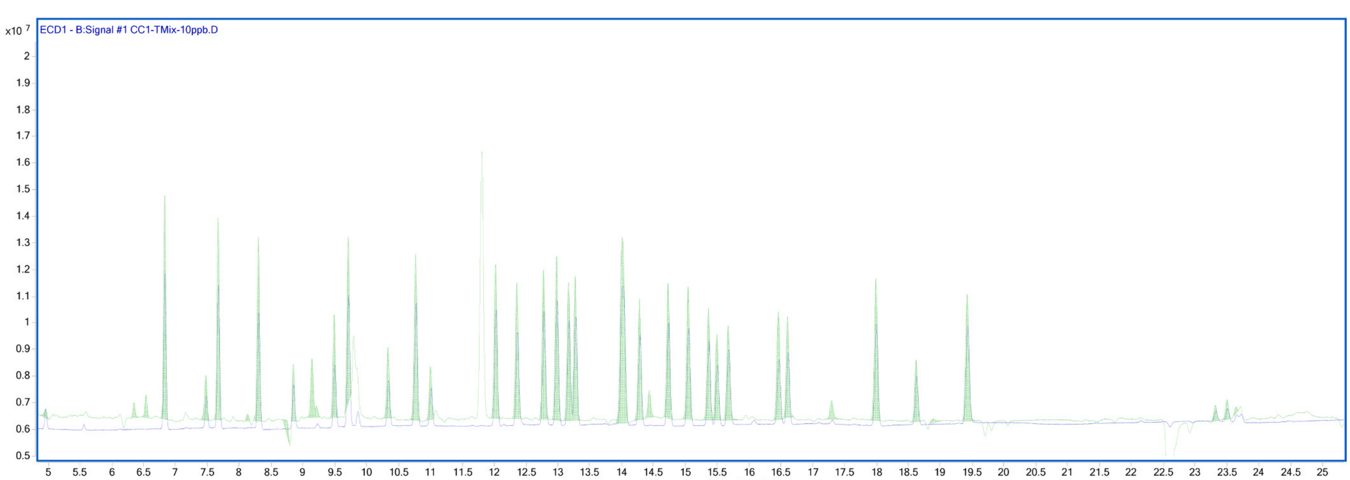


Fig. 2. GC-ECD chromatograms of Tea Pesticide mix showing neat standard (green), and matrix-matched standard (blue), highlighting the influence of matrix effects on analyte response.

uncertainty (MU) was calculated by considering all relevant sources of uncertainty, including analytical balance, micropipette accuracy, standard purity, glassware error, recovery, repeatability, and homogeneity. The combined standard uncertainty was calculated using the root sum of squares (RSS) method. This value was then expanded using a coverage factor of 2 to reflect a 95 % confidence level. For example, at a 25 μg/kg spiking level, the combined standard uncertainty was calculated as 0.0535 μg/kg, and the final uncertainty budget was 22.43 ± 2.40 μg/kg, with the major contributions coming from recovery performance and the uniformity of the spiked samples. The expanded measurement uncertainty (MU) values for all the pesticides at LOQ lie in the range of 5.94–35.19 %, which fall within the acceptable limits as per SANTE/11312/2021 guidelines, particularly considering the complexity of the matrix and the low analyte concentrations. The precision and trueness of the method were in agreement with the previously reported studies (Huang et al., 2019; Ly et al., 2020).

3.3. Monitoring of pesticide residues

In the present study, a total of 36 black tea samples collected from different regions of India were analysed for residues of 78 pesticides, including those listed by the Food Safety and Standards Authority of India (FSSAI, 2024), using GC-ECD and LC-MS/MS techniques. Fig. 3 shows chromatograms of neat standard monocrotophos (1 μg/kg) and neat & matrix matched standard of cypermethrin (100 μg/kg). Comparison of detected pesticides with respect to LOQ 0.001 mg/kg for LC and 0.01 mg/kg for GC. Also comparison of detected pesticides against LOQ 0.001 mg/kg for LC and 0.01 mg/kg for GC was plotted. Out of the 36 samples analysed, 11 different pesticide residues were detected in 28 samples (77.78 %), with monocrotophos being the most frequently detected (63.89 %), followed by acetamiprid (47.22 %), acephate (27.78 %), imidacloprid (22.22 %), cypermethrin (22.22 %), and captafol (13.89 %). Other detected pesticides included methomyl, dinotefuran, simazine, 4,4'-DDT, and Gamma BHC, each found in less than 10 % of the total samples. Notably, only 2 samples (5.56 %) were found to contain residues below the Maximum Residue Limits (MRLs), while 26 samples (72.22 %) exceeded the respective MRLs, indicating a potential public health concern. Importantly, all five detected pesticides (monocrotophos, acetamiprid, acephate, imidacloprid and cypermethrin) are classified by the FSSAI as either banned or not approved for use in tea. Fig. 4 shows the calibration curve for monocrotophos in the range 0.5, 1, 2, 5, 10, 20, 25 μg/kg by LC-MS/MS. Monocrotophos is explicitly banned due to its high toxicity, while acephate, cypermethrin, imidacloprid, and acetamiprid are not approved and are subject to a default maximum residue limit (MRL) of 0.01 mg/kg in tea (FSSAI, 2024). Table 5 shows the detection, concentration range, and estimated exposure levels (lower bound, upper bound, and mean values) of various pesticide residues identified in tea samples, along with their corresponding FSSAI maximum residue limits (MRLs) and analytical limits of quantification (LOQs). The detection of these pesticides above this

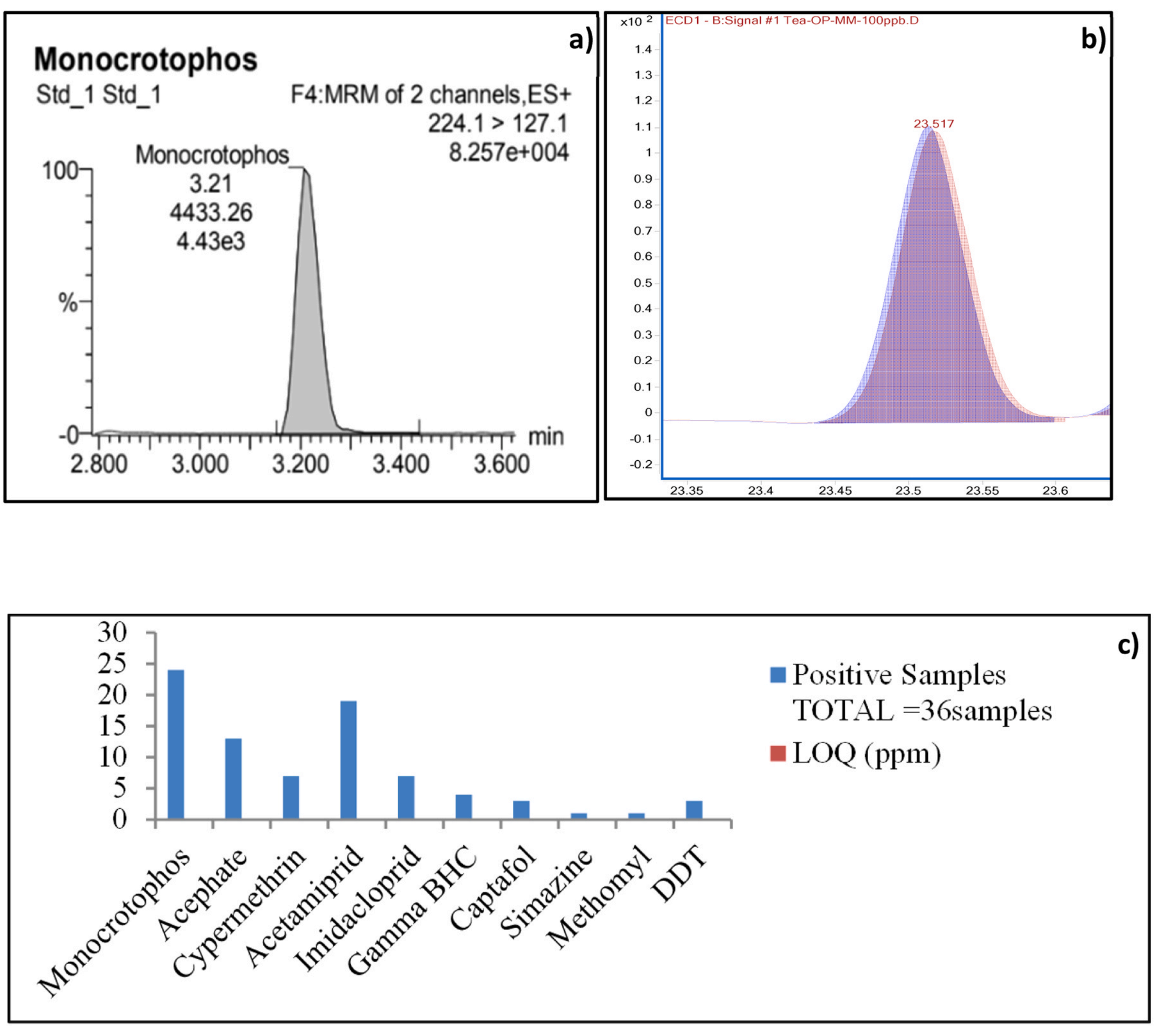


Fig. 3. a) Chromatograms of neat standard monocrotophos (1 μg/kg in LC-MS/MS); b) Neat & matrix matched standard of cypermethrin (100 μg/kg in GC-ECD); c) Comparison of detected pesticides with respect to LOQ 0.01 mg/kg for LC and 0.1 mg/kg for GC.

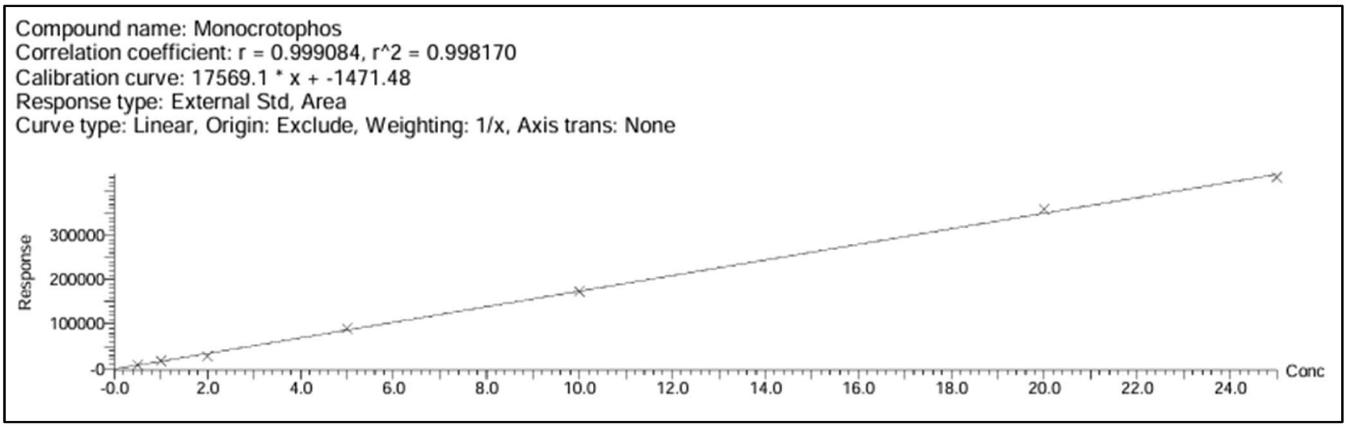


Fig. 4. Calibration curve of monocrotophos in LC-MS/MS.

Table 5
Pesticide residue levels in tea samples with detection ranges and risk assessment values.

Pesticide	No. of Positive samples (%)	Concentration Range (mg/kg)	MRL FSSAI	LOQ (mg/kg)	Lower bound (LB) (mg/kg)	Upper bound (UB) (mg/kg)	Mean Value
Acephate	10 (27.8 %)	0.010–0.067	0.01	0.001	0.00612	0.01417	6.12E−03*/ 1.42E−02#
Monocrotophos	23 (63.8 %)	0.013–0.127	0.01	0.001	0.02650	0.03100	2.65E−02*/ 3.10E−02#
Acetamiprid	17 (47.2 %)	0.010–0.1477	0.01	0.001	0.02000	0.24166	2.00E−02*/ 2.42E−01#
Imidacloprid	8 (22.22 %)	0.0100–0.0251	0.01	0.001	0.00000	0.01000	0.00E+ 00*/ 1.00E−02#
Dinotefuran	2 (5.56 %)	0.01	0.01	0.001	0.00000	0.01000	0.00E+ 00*/ 1.00E−02#
Simazine	1 (2.78 %)	0.122	0.01	0.001	0.00338	0.01310	3.38E−03*/ 1.31E−02#
Methomyl	1 (5.56 %)	0.01	0.01	0.001	0.00000	0.01000	0.00E+ 00*/ 1.00E−02#
Cypermethrin[€]	8 (22.22 %)	0.025–0.130	0.01	0.01	0.01136	0.01969	1.14E−02*/ 1.97E−02#
4,4'-DDT	2 (5.56 %)	0.010–0.086	0.05	0.01	0.00411	1.84800	4.11E−03*/ 1.85E+ 00#
Captafol	5 (13.89 %)	0.056–0.121	0.01	0.01	0.01072	0.11966	1.07E−02*/ 1.20E−01#
Gamma BHC	3 (8.33 %)	0.046 – 0.317	0.01	0.01	0.01231	0.02147	1.23E−02*/ 2.15E−02#

#UB: Upper bound; *LB: Lower bound; Source: (FSSAI, 2018); (CODEX, 2022); [€]Calculated as sum of isomers.

threshold indicates non-compliance with national food safety standards and may pose potential health risks to consumers. According to the Codex Alimentarius Commission, monocrotophos and cypermethrin have MRLs of 0.01 mg/kg, respectively. The higher frequency of insecticide detection compared to fungicides likely reflects the greater vulnerability of tea plantations to insect pest infestations. This pattern is consistent with previous studies highlighting the widespread use of organophosphates (OPs) and synthetic pyrethroids (SPs) in Indian tea plantations due to their cost-effectiveness and efficacy (Zhu et al., 2014; Yadav et al., 2017). Residue monitoring in South Indian tea has revealed frequent detection of compounds such as dicofol, ethion, fenvalerate, fenpropathrin, and quinalphos (Seenivasan and Muraleedharan, 2011), Surveys conducted in North India indicated a predominance of organochlorine (OC) and organophosphate (OP) pesticides, whereas synthetic pyrethroids were frequently undetected (Jaggi et al., 2001). The presence of multiple residues in individual samples may be attributed to the use of pesticide mixtures for broader pest and disease control, resistance management, and reduced application frequency (Zheng et al., 2022). The divergence between Indian and Codex Alimentarius Commission Maximum Residue Limits (MRLs) for pesticides in tea samples demands a careful examination, particularly when residues exceed Indian standards but remain within Codex parameters (Tripathy et al., 2023). This situation presents a multifaceted dilemma, touching upon regulatory strictness, consumer protection, and international trade implications (Zikankuba et al., 2019). A complete evaluation should investigate whether India should consider revising its MRLs, taking into account factors such as dietary exposure assessments, risk management strategies, and international trade agreements (Zhang, 2025). It is crucial to determine if Codex MRLs are more lenient due to outdated scientific data or broader concerns of global agricultural practices (Handford et al., 2015). A thorough study into the potential health hazards posed by trace amounts of pesticide residues and their degradation products is also essential, given the paramount importance of ensuring food safety (Gilbert-López et al., 2009). The observed differences between Indian and Codex MRLs for certain pesticides exceeded Indian MRLs but remained within Codex limits, suggesting a difference in safety standards. These differences may be due to varying risk assessment methods, consumption patterns, agricultural practices, and trade significances among countries. Codex being international regulation, their MRLs, often aim to help trading easy while ensuring safety, but may not reflect country-specific dietary exposures. The fact that Indian MRLs are stricter in some cases could reflect a precautionary approach based on local consumption data. However, this also poses challenges for exporters and supervisory bodies in terms of agreement and implementation. Therefore, these findings underscore the need for regular reassessment and possible coordination of Indian MRLs with international standards, while ensuring that local risk factors are not compromised.

These findings emphasise the need for rigorous residue monitoring, adherence to national MRLs, and coordination with international food safety standards to ensure consumer protection.

3.4. Consumer risk assessment

3.4.1. Dietary intake

The dietary exposure to pesticide residues through black tea consumption was obtained from Global Environment Monitoring System (GEMS) food cluster diets, Group G05 (WHO/GEMS/FOODS, 2003), by taking a daily intake of 0.00129 kg of tea and standard body weights of 60 kg for adults and 25 kg for children (NIN, 2010). We acknowledge that tea consumption patterns vary considerably across regions, socio-economic groups, and cultural practices, potentially influencing actual exposure estimates. In this study, the risk assessment is based on dry tea residue levels, rather than brewed tea, so actual exposure via infusion may be lower depending on transfer rates. Table 6 presents the dietary exposure and hazard quotient (HQ) based risk assessment of pesticide residues detected in tea for both adults and children. The exposure values, calculated using lower bound (LB) and upper bound (UB), are compared against the acceptable daily intake (ADI) set for each pesticide. The LB scenario assumed all non-detected residues were zero, reflecting a best-case exposure estimate. The UB scenario assumed non-detected residues were present at the LOQ, representing a worst-case exposure. This dual approach provides a more comprehensive view of potential consumer risk. For most pesticides, the HQ values for both adults and children remain well below 1, indicating no significant health risk. However, relatively higher HQs were observed for Monocrotophos and 4,4′-DDT, especially in children under the upper bound scenario, suggesting a potential health concern if exposure persists at these levels. Overall, the risk is low but not negligible, emphasising the need for continuous monitoring and stricter residue control in tea. Among the detected residues, acetamiprid exhibited the highest estimated dietary exposure, with upper bound (UB) values reaching 5.20×10^{-6} mg/kg bw/day for both adults and children, followed by cypermethrin (3.66×10^{-6} mg/kg bw/day in children), and captafol (7.14×10^{-6} mg/kg bw/day in children). While monocrotophos did not show the highest exposure, it still presented a notable UB exposure of 6.67×10^{-7} mg/kg bw/day in adults and 1.60×10^{-6} mg/kg bw/day in children. Pesticides such as methomyl, simazine, dinotefuran, and imidacloprid exhibited comparatively lower exposures, mostly in the 10^{-7} or 10^{-6} range. Overall, all estimated exposures were well below their respective Acceptable Daily Intakes (ADIs), indicating that chronic exposure through black tea consumption is unlikely to pose significant health risks under the current consumption patterns.

3.4.2. Risk through Hazard Quotient (HQ)

Chronic dietary risk was further evaluated using the Hazard Quotient (HQ), calculated as the ratio of estimated exposure to the ADI (FAO/WHO JMPR, 2020). Since values under the lower bound (LB) scenario were consistently lower, only the upper bound (UB) scenario is discussed for conservative risk estimation. The highest HQ values among frequently detected pesticides were observed for monocrotophos, with 1.11×10^{-3} for adults and 2.67×10^{-3} for children, remaining well below the critical threshold of 1.0. Other notable HQs included simazine (3.38×10^{-5} in children), captafol (1.71×10^{-5} in children), and acetamiprid (1.25×10^{-5} in children). Cypermethrin, while exhibiting high residue concentrations, showed comparatively low HQs (1.76×10^{-7} in adults and 8.80×10^{-6} in children) due to its higher ADI (0.02 mg/kg bw/day). Although 4,4′-DDT and gamma-BHC showed unusually elevated HQs 9.52×10^{-3} and 2.22×10^{-4}, respectively, for children these may reflect isolated or outlier contamination events, as these compounds are largely banned and rarely detected. All evaluated HQs remained well below 1, reinforcing that the chronic dietary intake of these pesticide residues from black tea does not pose a significant health concern. These findings align with earlier studies where HQs below unity have been associated with safe consumption levels (Yao et al., 2020; Sharma et al., 2022; Lin et al., 2022).

3.4.3. Cumulative risk through Hazard Index (HI)

The cumulative dietary risk from simultaneous exposure to multiple pesticides was estimated using the Hazard Index (HI), calculated as the sum of individual HQs. The HI values under the LB scenario were 0.00097 for adults and 0.00256 for children, whereas under the UB scenario, values increased slightly to 0.00125 in adults and 0.01260 in children. As all HI values were well below 1.0, these results suggest that cumulative exposure to multiple pesticide residues in tea samples is within the acceptable risk limit and previous studies reported in the past also correlated the safe consumption of a products with the HQs of the pesticides present in them (Sharma et al., 2021; Tripathy et al., 2021). Therefore, regular consumption of black tea under typical dietary conditions does not pose any significant cumulative health hazard. This study is among the first comprehensive cumulative risk assessments of FSSAI regulated pesticides in Indian tea and underscores the necessity for routine surveillance and regulatory enforcement to ensure consumer safety. It is important to note that the dietary risk assessment in this study was limited to 78 pesticides, selected based on their usage patterns, regulatory importance, and detection frequency in previous surveillance studies, particularly in the tea matrix. However, the potential presence of additional, unmonitored pesticide residues cannot be ruled out. As such, the reported HI values represent the risk associated only with the monitored compounds, and the overall exposure may be underestimated. Future investigations using an expanded list of analytes or non-targeted screening methods could offer a more comprehensive assessment of cumulative risk.

Table 6
Exposure and Hazard Quotient based risk analysis of detected pesticides in tea.

	Pesticide	ADI (mg/kg bw/day)	Exposure (Adult) (mg/kg bw/day)	Exposure (Children) (mg/kg bw/day)	HQ (Adult)	HQ (Children)
1	Monocrotophos	0–0.0006	5.70E−07*/6.67E−07#	1.37E−6*/1.60E−06 #	9.50E−04*/1.11E−03#	2.28E−03*/2.67E−03#
2	Acephate	0.03	1.32E−07*/3.04E−07#	3.17E−07*/7.29E−07#	4.40E−06*/1.01E−05#	1.06E−05*/2.43E−05#
3	Acetamiprid	0.07	4.30E−07*/5.20E−06#	4.30E−07*/5.20E−06#	4.30E−07*/5.20E−06#	1.03E−06*/1.25E−05#
4	Imidacloprid	0.06	0.00E+ 00*/ 2.15E−07#	0.00E+ 00*/5.16E−07#	0.00E+ 00*/3.58E−06#	0.00E+ 00*/8.60E−06#
5	Dinotefuran	0.06	0.00E+ 0*/ 2.15E−07#	0.00E+ 00*/5.16E−07#	0.00E+ 00*/3.58E−06#	0.00E+ 00*/8.60E−06#
6	Simazine	0.02	7.26E−08*/2.82E−07#	1.94E−07*/6.75E−07#	3.63E−06*/1.41E−05#	9.69E−06*/3.38E−05#
7	Methomyl	0.02	0.00E+ 00*/2.15E−07#	0.00E+ 00*/1.08E−05#	0.00E+ 00*/5.16E−07#	0.00E+ 00*/2.58E−05#
8	Cypermethrin	0.02	2.44E−07*/7.32E−08#	1.22E−05*/3.66E−06#	5.06E−07*/1.76E−07#	2.53E−05*/8.80E−06#
9	4,4’-DDT	0.01	8.84E−08*/3.97E−05#	8.84E−06*/3.97E−03#	2.12E−07*/9.52E−05#	2.12E−05*/9.52E−03#
10	Captafol	0.01	2.31E−07*/7.14E−08#	2.31E−05*/7.14E−06#	5.53E−07*/1.71E−07#	5.53E−05*/1.71E−05#
11	Gamma BHC	0–0.005	2.65E−07*/4.62E−07#	5.29E−05*/9.24E−05#	6.35E−07*/1.11E−06#	1.27E−04*/2.22E−04#

#UB: Upper bound; *LB: Lower bound; $ADI: Acceptable Daily Intake, Source: (FAO/WHO JMPR, 2020),(EFSA, 2021); (European Commission Database, 2024).

3.4.4. Transfer and mitigation of pesticide residues

Although method development and multi-residue detection in tea is crucial for regulatory compliance, understanding the transfer of these residues into tea infusions provides a more accurate estimate of actual consumer exposure. Also, evaluating mitigation strategies during processing or preparation further supports the development of effective food safety recommendations. The transfer of pesticide residues from dry tea leaves into tea infusion is a crucial factor in estimating consumer exposure and varies with the chemical nature of the pesticides. Previous studies have reported high transfer rates up to 103.6 % for neonicotinoids and organophosphates, while pyrethroids such as cypermethrin typically exhibit lower transfer efficiencies (Wang et al., 2019; Heshmati et al., 2021).

In the current study, frequently detected compounds such as monocrotophos, acephate, acetamiprid, imidacloprid, and cypermethrin belong to pesticide classes that are either systemic or water-soluble, suggesting the potential for their transfer into tea infusions and subsequent consumer exposure. Monocrotophos and acephate were detected most commonly among the detected pesticides in the tea samples, even though these are restricted or banned in several countries due to their neurotoxic effects and environmental persistence. In India, monocrotophos has been officially banned, with the Central Insecticides Board and Registration Committee listing it under banned pesticides for agriculture as per S.O. 1482(E) and subsequent updates. Importantly, in March 2024, FSSAI issued a directive (Direction No. QA/3/2021/FSSAI-Part 3) mandating all notified laboratories to test for 20 banned pesticides in tea, reinforcing stricter monitoring following long-standing concerns about residue levels. Despite these regulatory efforts, the detection of these substances in our samples may point to continued use of outdated pesticide stock, off-label application, or a lack of awareness among farmers. Many tea plantations in India are located in rural and hilly areas where growers may not have regular access to updated regulations or training in sustainable alternatives such as integrated pest management. Since tea leaves are not washed after harvest, any pesticide applied during cultivation can persist through processing and remain in the final product. In this study, two samples were labelled as organic and carried certification marks; notably, no pesticide residues were detected in these, indicating accordance with organic farming standards. While detailed metadata on farming practices and source locations was limited, primary observations suggested that locally sold or unbranded teas had a higher occurrence of banned or restricted pesticide residues compared to certified or branded samples. These findings highlight the need for improved farmer outreach, regulatory implementation, and traceability systems to strengthen food safety and consumer assurance in Indian tea. However, mitigation measures like rinsing tea leaves prior to infusion can significantly reduce residue levels. For instance, (Gao et al., 2019) reported up to 59 % reduction in residue concentration through simple rinsing. To reduce residue-related health risks, the use of plant-based biopesticides containing compounds like terpenoids, alkaloids, and phenolics is recommended. These eco-friendly alternatives can be integrated into Integrated Pest Management (IPM) systems to maintain effective crop protection while minimising chemical pesticide use (Gonzalez-Coloma et al., 2013; Raveau et al., 2020). In this study, the presence of multiple residues in black tea highlights the importance of adopting sustainable farming practices and strengthening food safety monitoring, so that residue levels remain low and consumers can enjoy safer products, as also reflected in the risk assessment findings.

4. Conclusion

Tea is a major agricultural commodity in India with significant domestic consumption and export value, making the monitoring of pesticide residues and their risk assessment crucial. The present study found a higher detection frequency of insecticides than fungicides, reflecting the greater susceptibility of tea plants to insect pest infestations. A robust and validated modified QuEChERS method was developed for the simultaneous detection of multiple pesticide residues in Indian black tea. By understanding the complex nature of the tea matrix through sample hydration and enhanced clean-up using C18 and GCB, the method achieved better recovery and reduced matrix interference. This reliable analytical approach serves as a vital tool for ensuring food safety and protecting consumer health. Dietary risk assessments indicated that pesticide residues detected in the black tea samples do not pose any significant health risk to Indian consumers, with all hazard quotients and hazard indices well below threshold levels. The dataset captures mainstream consumer tea products collected from major tea-producing regions, ensuring that the findings are broadly representative of the Indian black tea market. Although residues in tea are generally within safe limits, prolonged or excessive consumption may pose health risks, underscoring the need for regular monitoring and regulatory compliance. The differences between Indian and Codex MRLs underscore the need for harmonized pesticide standards. India's stricter limits for some pesticides may impact tea exports, highlighting the importance of aligning national regulations with Codex standards to support trade and global food safety compliance. These findings support the enforcement of pesticide regulations and encourage the safe and judicious use of pesticides in tea cultivation. Continued surveillance and stringent regulatory actions will help India maintain its status as a safe and consumer-friendly tea producer and exporter. Approaches such as the use of plant-derived biopesticides rich in terpenoids, alkaloids, and phenolics offer promising, eco-friendly alternatives like biopesticide formulations that reduce health risks while maintaining effective pest management in tea cultivation. Based on the findings, we recommend strengthening residue monitoring, promoting farmer education on safe pesticide use, and implementing traceability systems to enhance food safety and consumer protection. Future efforts should focus on establishing periodic nationwide surveillance programs, promoting farmer training on integrated pest management (IPM) practices, and enhancing consumer awareness regarding safe tea consumption. Such initiatives would support both domestic food safety assurance and international trade compliance.

CRediT authorship contribution statement

Mithilaa Selvaraj: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Mathen Mathew:** Validation, Software, Methodology, Data curation. **Sindhu R. Nambiar:** Supervision, Funding acquisition, Conceptualization.

Ethics declaration

All experiments involving hazardous substances were conducted in accordance with institutional safety protocols and standard laboratory practices.

Ethics statement

Not applicable – this study did not involve human participants or animal experiments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors acknowledge the Council of Scientific and Industrial Research (CSIR), Ministry of Science and Technology, Government of India, New Delhi, for financial support under the Special Projects Scheme (CSP024004). The authors also sincerely acknowledge the facilities provided by the Director, CSIR-CFTRI, Mysuru, Karnataka (CSIR-CFTRI Manuscript Communication Number: PMC/2025–26/74).

Data availability

Data will be made available on request.

References

Agilent Technologies, 2014. Optimizing sample preparation for LC/MS/MS of pesticide residues in herbal teas using the QuEChERS approach (Application Note No. 5991-3728EN). https://www.agilent.com/cs/library/applications/5991-3728EN.pdf.

Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J., 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J. AOAC Int. 86 (2), 412–431.

Cajka, T., Sandy, C., Bachanova, V., Drabova, L., Kalachova, K., Pulkrabova, J., Hajslova, J., 2012. Streamlining sample preparation and gas chromatography-tandem mass spectrometry analysis of multiple pesticide residues in tea. Anal. Chim. Acta 743, 51–60. https://doi.org/10.1016/j.aca.2012.06.051.

Chen, H., Gao, G., Chai, Y., Ma, G., Hao, Z., Wang, C., Liu, X., Lu, C., 2017. Multiresidue method for the rapid determination of pesticide residues in tea using ultra performance liquid chromatography orbitrap high resolution mass spectrometry and in-syringe dispersive solid phase extraction. ACS Omega 2 (9), 5917–5927. https://doi.org/10.1021/acsomega.7b00863.

CODEX (Codex Alimentarius Commission). (2022). *DT 1114 – Tea, green, black (black, fermented and dried).* ⟨https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/commodities-detail/en/?c_id=101⟩. (Accessed on April 18, 2022).

EFSA (European Food Safety Authority), 2021. The 2019 European Union report on pesticide residues in food. Eur. Food Saf. Auth. J. 19 (4), 6491. https://doi.org/10.2903/j.efsa.2021.6491.

EURACHEM/CITAC, 2012. In: Ellison, S.L.R., Williams, A. (Eds.), In Guide Quantifying Uncertainty in Analytical Measurement, 3rd Ed., pp. 1–141.

European Commission, 2024. EU Pesticide Residue MRLs Database. Directorate General for Health and Food Safety. ⟨https://food.ec.europa.eu/plants/pesticides/eu-pesticides-database_en⟩.

FAO (Food and Agricultural Organisation) of the United Nations. (1999). Recommended methods of sampling for the determination of pesticide residues for compliance with MRLs CAC/GL 33–1999. ⟨https://www.fao.org/faolex/results/details/en/c/LEX-FAOC035778/⟩ (Accessed on October 5, 2020).

FAO & WHO. (2020). *Pesticide residues in food – 2019: Evaluations – Part I – Residues* (FAO/WHO Joint Meeting on Pesticide Residues (JMPR)). FAO. ⟨https://www.fao.org/3/ca7352en/ca7352en.pdf⟩.

FAO (Food and Agricultural Organisation) of the United Nations. (2020). Codex Pesticides Residues in Food Online Database. ⟨http://www.fao.org/fao⟩⟨–whocodexalimentarius/codex–texts/dbs/pestres/en/⟩ (Accessed on November 5, 2020).

FSSAI (Food Safety and Standards Authority of India). (2018). *Gazette Notification on Food Safety and Standards (Contaminants, Toxins and Residues) Amendment Regulation related to MRL of pesticide. The Gazette of India: Extraordinary,* [Part III—Section 4], pp. 1–51. https://fssai.gov.in/home/fss-legislation/notifications/gazette-notification.html/ (Accessed on April 13, 2022).

Food Safety and Standards Authority of India (FSSAI). (2024, March 4). *Direction to all notified laboratories regarding testing of pesticide in tea samples* (Direction No. QA/3/2021/FSSAI-Part3). ⟨https://www.fssai.gov.in/upload/advisories/2024/03/65e6f53962cfbDirection_04.03.2024.pdf⟩.

Gao, W., Yan, M., Xiao, Y., Lv, Y., Peng, C., Wan, X., Hou, R., 2019. Rinsing Tea before Brewing Decreases Pesticide Residues in Tea Infusion. J. Agric. Food Chem. 67 (19), 5384–5393. https://doi.org/10.1021/acs.jafc.8b04908.

Gad Alla, S.A., Loutfy, N.M., Shendy, A.H., Ahmed, M.T., 2015. Hazard index, a tool for a long term risk assessment of pesticide residues in some commodities, a pilot study. Regulatory Toxicol. Pharmacol. RTP 73 (3), 985–991. https://doi.org/10.1016/j.yrtph.2015.09.016.

Gilbert-López, B., García-Reyes, J.F., Molina-Díaz, A., 2009. Sample treatment and determination of pesticide residues in fatty vegetable matrices: a review. Talanta 79 (2), 109–128. https://doi.org/10.1016/j.talanta.2009.04.022.

Gonzalez-Coloma, A., Reina, M., Sáenz, C., Lacret, R., 2013. Natural product-based biopesticides for insect control. Phytochem. Rev. 12 (1), 227–244. https://doi.org/10.1007/s11101-012-9263-7.

Handford, C.E., Elliott, C.T., Campbell, K., 2015. A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. Integr. Environ. Assess. Manag. 11 (4), 525–536. https://doi.org/10.1002/ieam.1635.

Heshmati, A., Mehri, F., Mousavi Khaneghah, A., 2021. Simultaneous multi-determination of pesticide residues in black tea leaves and infusion: a risk assessment study. Environ. Sci. Pollut. Res. Int. 28 (11), 13725–13735. https://doi.org/10.1007/s11356-020-11658-3.

Huang, Y., Shi, T., Luo, X., Xiong, H., Min, F., Chen, Y., Nie, S., Xie, M., 2019. Determination of multi-pesticide residues in green tea with a modified QuEChERS protocol coupled to HPLC-MS/MS. Food Chem. 275, 255–264. https://doi.org/10.1016/j.foodchem.2018.09.094.

ISO/IEC 17025. (2017). *General requirements for the competence of testing and calibration laboratories.* International Organization for Standardization (ISO)/International Electrotechnical Committee (IEC), Geneva. ⟨https://www.iso.org/standard/66912.html⟩.

Jaga, K., Dharmani, C., 2006. Ocular toxicity from pesticide exposure: A recent review. Environ. Health Prev. Med. 11 (3), 102–107. https://doi.org/10.1265/ehpm.11.102.

Jaggi, S., Sood, C., Kumar, V., Ravindranath, S.D., Shanker, A., 2001. Leaching of pesticides in tea brew. J. Agric. Food Chem. 49 (11), 5479–5483. https://doi.org/10.1021/jf010436d.

JMPR (Joint FAO/WHO Meeting on Pesticide Residues). (2020). *Inventory of evaluations performed by the JMPR.* ⟨https://apps.who.int/pesticide⟩⟨–residues–jmpr–database/⟩ (Accessed on August 15, 2020).

Karthika, C., Muraleedharan, N.N., 2009. Contribution of leaf growth on the disappearance of fungicides used on tea under South Indian agroclimatic conditions. J. Zhejiang Univ. Sci. B 10 (6), 422–426. https://doi.org/10.1631/jzus.B0920026.

Lin, T., Chen, X.L., Guo, J., Li, M.X., Tang, Y.F., Li, M.X., Li, Y.G., Cheng, L., Liu, H.C., 2022. Simultaneous Determination and Health Risk Assessment of Four High Detection Rate Pesticide Residues in Pu'er Tea from Yunnan, China. Molecules (Basel Switz.) 27 (3), 1053. https://doi.org/10.3390/molecules27031053.

Lozowicka, B., Kaczynski, P., Paritova, C.A., Kuzembekova, G.B., Abzhalieva, A.B., Sarsembayeva, N.B., Alihan, K., 2014. Pesticide residues in grain from Kazakhstan and potential health risks associated with exposure to detected pesticides. Food Chem. Toxicol. Int. J. Publ. Brit. Ind. Biol. Res. Assoc. 64, 238–248. https://doi.org/10.1016/j.fct.2013.11.038.

Lu, E.H., Huang, S.Z., Yu, T.H., Chiang, S.Y., Wu, K.Y., 2020. Systematic probabilistic risk assessment of pesticide residues in tea leaves. Chemosphere 247. https://doi.org/10.1016/j.chemosphere.2019.125692.

Ly, T.K., Ho, T.D., Behra, P., Nhu-Trang, T.T., 2020. Determination of 400 pesticide residues in green tea leaves by UPLC-MS/MS and GC-MS/MS combined with QuEChERS extraction and mixed-mode SPE clean-up method. Food Chem. 326, 126928. https://doi.org/10.1016/j.foodchem.2020.126928.

National Institute of Nutrition (NIN – ICMR) (2010). *Dietary guidelines for Indians: A manual.* Hyderabad: NIN–ICMR.

Paya, P., Anastassiades, M., Mack, D., Sigalova, I., Tasdelen, B., Oliva, J., Barba, A., 2007. Analysis of pesticide residues using the QuEChERS pesticide extraction method in combination with gas and liquid chromatography and tandem mass spectrometric detection. Anal. Bioanal. Chem. 389 (6), 1697–1714. https://doi.org/10.1007/s00216-007-1610-7.

Raveau, R., Fontaine, J., Lounès-Hadj Sahraoui, A., 2020. Essential oils as potential alternative biocontrol products against plant pathogens and weeds: a review. Foods 9 (3), 365. https://doi.org/10.3390/foods9030365.

Roy, S., Handique, G., Muraleedharan, N., Dashora, K., Roy, S.M., Mukhopadhyay, A., Babu, A., 2016. Use of plant extracts for tea pest management in India. Appl. Microbiol. Biotechnol. 100 (11), 4831–4844. https://doi.org/10.1007/s00253-016-7522-8.

SANTE (2022). *Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed.* Document No. SANTE/11312/2021. European Commission. pp. 1-43. (Accessed on May 9, 2022).

Sharma, A., Gupta, M., Shanker, A., 2008. Fenvalerate residue level and dissipation in tea and in its infusion. Food Addit. Contam. Part A Chem. Anal. Control Expo. risk Assess. 25 (1), 97–104. https://doi.org/10.1080/02652030701518080.

Sharma, K.K., Tripathy, V., Mohapatra, S., Matadha, N.Y., Pathan, A.R.K., Sharma, B.N., Dubey, J.K., Katna, S., George, T., Tayade, A., Sharma, K., Gupta, R., Walia, S., 2021. Dissipation kinetics and consumer risk assessment of novaluron + lambda-cyhalothrin co-formulation in cabbage. Ecotoxicol. Environ. Saf. 208, 111494. https://doi.org/10.1016/j.ecoenv.2020.111494.

Sharma, K.K., Tripathy, V., Sharma, K., Gupta, R., Yadav, R., Devi, S., Walia, S., 2022. Long-term monitoring of 155 multi-class pesticide residues in Indian vegetables and their risk assessment for consumer safety. Food Chem. 373 (Pt B), 131518. https://doi.org/10.1016/j.foodchem.2021.131518.

Seenivasan, S., Muraleedharan, N., 2011. Survey on the pesticide residues in tea in south India. Environ. Monit. Assess. 176 (1-4), 365–371. https://doi.org/10.1007/s10661-010-1589-y.

Singh, A.P., Balayan, S., Hooda, V., Sarin, R.K., Chauhan, N., 2020. Nano-interface driven electrochemical sensor for pesticides detection based on the acetylcholinesterase enzyme inhibition. Int. J. Biol. Macromol. 164, 3943–3952. https://doi.org/10.1016/j.ijbiomac.2020.08.215.

Tea Board of India, 2021. Annual report 2020–21. ⟨https://www.teaboard.gov.in⟩.

Tripathy, V., Sharma, K.K., George, T., Patil, C.S., Saindane, Y.S., Mohapatra, S., Siddamallaiah, L., Pathan, A.R.K., Yadav, A.K., Sharma, K., Yadav, R., Gupta, R., Walia, S., 2021. Dissipation kinetics and risk assessment of iprovalicarb + propineb fungicide in tomato under different agroclimates. Environ. Sci. Pollut. Res. Int. 28 (24), 31909–31919. https://doi.org/10.1007/s11356-021-12919-5.

Tripathy, V., Sharma, K.K., Gupta, R., Yadav, R., Devi, S., Sharma, K., Singh, G., Kalra, S., Aggarwal, A., Tandekar, K., Verma, A., Walia, S., 2023. Simultaneous monitoring and dietary risk assessment of 386 pesticides in market samples of black tea. Food Chem. 420, 136103. https://doi.org/10.1016/j.foodchem.2023.136103.

Wang, L., Luo, B., Huang, W., 2019. Transfer rates of pesticide residues from tea to infusion and the effect of rinsing. J. Agric. Food Chem. 67 (30), 8427–8434. https://doi.org/10.1021/acs.jafc.9b02254.

WHO (World Health Organization). (2018). *Generic risk assessment model for insecticides used for larviciding and mollusciciding, second edition,* ISBN 978-92-4-151504-7. ⟨https://apps.who.int/iris/bitstream/handle/10665/276706/9789241515047-eng.pdf?ua=1⟩.

World Health Organization, 2003. GEMS/ Food regional diets: regional per capita consumption of raw and semi-processed agricultural commodities / prepared by the Global Environment Monitoring System/Food Contamination Monitoring and

Assessment Programme (‼GEMS/ Food)‼. Rev. ed. World Health Organization. 〈https://iris.who.int/handle/10665/42833〉.
Wu, Y., An, Q., Li, D., Kang, L., Zhou, C., Zhang, J., Pan, C., 2022. Multi-residue analytical method development and risk assessment of 56 pesticides and their metabolites in tea by chromatography tandem mass spectroscopy. Food Chem. 375. https://doi.org/10.1016/j.foodchem.2021.131819.
Yadav, S., Rai, S., Srivastava, A.K., Panchal, S., Patel, D.K., Sharma, V.P., Jain, S., Srivastava, L.P., 2017. Determination of pesticide and phthalate residues in tea by QuEChERS method and their fate in processing. Environ. Sci. Pollut. Res. Int. 24 (3), 3074–3083. https://doi.org/10.1007/s11356-016-7673-2.
Yao, Q., Yan, S.A., Li, J., Huang, M., Lin, Q., 2020. Health risk assessment of 42 pesticide residues in Tieguanyin tea from Fujian, China. Drug Chem. Toxicol. 45 (2), 932–939. https://doi.org/10.1080/01480545.2020.1802476.
Zheng, K., Lin, R., Liu, X., Wu, X., Chen, R., Yang, M., 2022. Multiresidue pesticide analysis in tea using GC–MS/MS to determine 12 pesticide residues (GB 2763-2021). Molecules 27 (23), 8419. https://doi.org/10.3390/molecules27238419.
Zhu, P., Miao, H., Du, J., Zou, J.H., Zhang, G.W., Zhao, Y.F., Wu, Y.N., 2014. Organochlorine pesticides and pyrethroids in Chinese tea by screening and confirmatory detection using GC-NCI-MS and GC-MS/MS. J. Agric. Food Chem. 62 (29), 7092–7100. https://doi.org/10.1021/jf5012424.
Zikankuba, V.L., Mwanyika, G., Ntwenya, J.E., James, A., 2019. Pesticide regulations and their malpractice implications on food and environment safety. Cogent Food Agric. 5 (1). https://doi.org/10.1080/23311932.2019.1601544.
Zhang, S., 2025. Maximum residue limits and agricultural trade: evidence from China. Sustainability 17 (8), 3435. https://doi.org/10.3390/su17083435.
Zhao, Y., Li, Y., Xie, H., Ma, Y., Wang, J., Liu, X., 2022. Optimization of QuEChERS sample preparation method for pesticide residue analysis in tea using GC–MS/MS based on GB 2763–2021. Molecules 27 (23), 8419. https://doi.org/10.3390/molecules27238419.