Journal of Integrative Agriculture 2026, 25(3): 1286–1296

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Risk assessment and residue behavior of a novel pesticide cyetpyrafen and its metabolites from fresh tea leaves to tea infusion

Yue Hu[1, 2], Yating Ning[1, 2, 3], Yan Zhao[1, 4], Yaqi Wang[1, 2], Fengjian Luo[1, 3, 5], Li Zhou[1, 5], Xinzhong Zhang[1, 3, 5#]

[1] *Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China*
[2] *Graduate School of Chinese Academy of Agricultural Sciences, Beijing 100081, China*
[3] *Hangzhou Hope Agricultural Technology Co., Ltd., Hangzhou 310008, China*
[4] *College of Horticulture and Landscape, Tianjin Agricultural University, Tianjin 300384, China*
[5] *National Key Laboratory for Tea Plant Germplasm Innovation and Resource Utilization, Hangzhou 310008, China*

Highlights

- Residual dissipation of cyetpyrafen and its three metabolites was studied across tea cultivation, processing, and brewing.
- Cyetpyrafen dissipated rapidly ($t_{1/2}$=0.59 d) in the field, with M-309 identified as the major metabolite.
- The dietary intake risk of cyetpyrafen from tea consumption was negligible to human health.
- Cyetpyrafen and its main metabolites were predicted to be harmful to non-target organisms.

Abstract

China has limited acaricide options for tea plantations. Cyetpyrafen, a novel domestic acaricide with high efficacy, low toxicity and a negative temperature coefficient, offers an alternative for tea pest control; however, its residue fate in tea remains unclear. This study developed a method to simultaneously detect cyetpyrafen and its metabolites (M-309, M-325-1, and M-409-3) in different tea matrices to investigate their fate. Recoveries of compounds ranged from 73.4 to 106.2% with the relative standard deviations (RSDs) below 12.0%. During tea cultivation, the dissipation half-life of cyetpyrafen was 0.59 d, with M-309 as a major metabolite. The residues of cyetpyrafen and M-309 were affected by different processing stages, especially water loss and high temperatures during fixing, drying and withering. The total processing factors ranged from 1.39 to 1.71 for green tea and 1.48 to 2.28 for black tea (processed from fresh tea leaves sampled at 1, 5, and 7 d), respectively. The leaching rates of cyetpyrafen from green tea and black tea into tea infusions were 7.4 and 6%, respectively. The risk associated with cyetpyrafen intake from tea consumption was low, with risk quotient values below 100%. However, theoretical calculation indicated potential harm to non-target organisms from its metabolites. This research provides a reference for the safe and efficient use of cyetpyrafen in tea gardens.

Keywords: cyetpyrafen, leaching rate, tea, residue dissipation, processing

1. Introduction

Tea, *Camellia sinensis* (L.), one of the world's bestselling non-alcoholic drinks, is widely cultivated across the globe including China, India, Sri Lanka, and Japan. The appropriate tea planting environment is warm and humid, which also tends to cause numerous insects, mites, and diseases to infest tea trees. The serious mite pest that is found in almost every area where tea is grown, the red spider mite, *Oligonychus coffeae* (Nietner), causes a reduction of 35–45% in tea production (Shourove *et al.* 2024). Chemical pesticides are still the most prevalent control method; however, due to the common use of pesticides and large number of generations per year for mites, tea mites have developed resistance to many acaricides, including abamectin, fenpropathrin, fenazaquin, ethion and dicofol (Amsalingam *et al.* 2017; Roy 2019; Schmidt-Jeffris *et al.* 2021). Only a limited number of acaricides are officially approved in Chinese tea gardens, such as fenazaquin, matrine, mineral oil, and lime sulphur. There is an urgent need to identify optimal acaricides suitable for application in tea gardens. These acaricides should

Received 28 February 2025; Received in revised form 6 November 2025; Accepted 1 December 2025; Available online 6 January 2026
#Correspondence Xinzhong Zhang, E-mail: zxz.1982@163.com, zhangxinzhong@tricaas.com

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2026.01.001

exhibit efficacy against all life stages of the red spider mite, encompassing adult mites, juvenile mites, and eggs, and possess excellent quick-acting characteristics to ensure rapid control of the pest population. Moreover, it is crucial that these acaricides are environmentally benign and pose no harm to tea plants, and retain their acaricidal activity even under the low-temperature conditions prevalent in spring and autumn.

Cyetpyrafen (CAS No. 1253429-01-4, $C_{24}H_{31}N_3O_2$) is a new pyrazole acaricide with highly efficient acaricidal activity and broad acaricidal spectrum developed by Shenyang Sinochem Agrochemicals R&D Co., Ltd., China. Currently, 30% of cyetpyrafen suspension concentrate (SC) has been registered in China to control *Panonychus citri* McGregor, *Tetranychus urticae* and *Tetranychus turkestani* Ugarove&Nikolski in apple, citrus and strawberry (GB 2763-2021 2021). Moreover, research has shown that cyetpyrafen is an effective way to manage common mites including *Thrips hawaiiensis*, *Tetranychus cinnabarinus*, *Polyphagotarsonemus latus* (Banks), and *T. urticae* in tea, fruits (Luo and Bristow 2023; Jiang *et al.* 2024), vegetables (Ouyang *et al.* 2018; Tian *et al.* 2022), and field crops (Lin *et al.* 2021; Shang *et al.* 2022). Additionally, there have been reports indicating that cyetpyrafen can be used in conjunction with predatory mites in integrated pest management systems. Especially at low concentrations, cyetpyrafen can effectively suppress spider mites' population growth and increase the predatory ability of *Neoseiulus californicus* and *Phytoseiulus persimilis* Athias-Henriot (Ouyang *et al.* 2018; Zhao *et al.* 2023). These results demonstrate that cyetpyrafen exhibits remarkable fast-acting and long-term effects. As such, cyetpyrafen holds great potential to serve as an alternative pesticide in tea gardens; it can effectively control mites and contribute to alleviating the resistance issues associated with currently available pesticides, thereby providing a sustainable solution for pest management in tea growing areas.

After application in the field, pesticides may result in residues in agricultural products, environmental soil and water, and may even produce a variety of toxic transformation products (Miao *et al.* 2023). Both laboratory and field experiments have shown that cyetpyrafen can transform into some major metabolites, including M-309, M-409 and M-391. These metabolites have been predicted to pose a risk to non-target organisms (Li R N *et al.* 2022; Wang *et al.* 2023). However, current studies mainly focused on formulation studies and field efficacies of cyetpyrafen (Song *et al.* 2017; Chen *et al.* 2019; Sun *et al.* 2022). Residual degradations of cyetpyrafen in plants and the environment have only been reported for grapefruit, cotton, and strawberry (Gao 2015; Lai 2019; Liu *et al.* 2023), and there were almost no reports on its metabolites. Human exposure to pesticide residues primarily occurs through tea infusion. The actual impact of pesticides on the human body is contingent upon the degree to which pesticide residues are dissolved in the tea soup. Therefore, it is of utmost importance to conduct an in-depth investigation into the transformation process of cyetpyrafen from tea garden to cup.

In this work, residues of a novel acaricide, cyetpyrafen, along with its metabolites, were comprehensively analyzed across all stages of tea planting, processing and brewing to provide a reliable basis for the safe application of pesticides in tea gardens. Firstly, an effective analytical method was successfully established to detect cyetpyrafen and its three metabolites (M-309, M-325-1 and M-409-3) in multiple matrices, including fresh tea leaves, tea processing samples, and tea infusions. This method combined a modified QuEChERS extraction approach with ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for accurate quantification. Then, a comprehensive exploration was carried out on the degradation, metabolism, and risk assessment of cyetpyrafen throughout the process from tea plants to the cup. Overall, the study provides an indispensable analytical method and valuable scientific field data, contributing valuable insights for the rational application of cyetpyrafen in tea gardens, ultimately ensuring the drinking safety for consumers.

2. Materials and methods

2.1. Materials and reagents

Cyetpyrafen (CAS No. 1253429-01-4, $C_{24}H_{31}N_3O_2$, Z-2-(4-tert-butylphenyl)-2-cyano-1-(1-ethyl-3-methyl-1H-pyrazol-5-yl)eth-1-en-1-yl 2,2-dimethylpropanoate, purity of 99%) and its metabolites M-309 (CAS No. 1253428-85-1, $C_{19}H_{23}N_3O$, 4-(1,1-dimethylethyl)-α-[(1-ethyl-3-methyl-1H-pyrazol-5-yl)hydroxymethylene]-benzeneacetonitrile, purity of 96%), M-325-1 ($C_{19}H_{23}N_3O_2$, purity of 96%) and M-409-3 ($C_{24}H_{31}N_3O_3$, purity of 95%) were synthesized by our laboratory. Fig. 1 showed the chemical structures of cyetpyrafen and its three metabolites. A 30% cyetpyrafen suspension concentrate (SC) was supplied by Shenyang Sciencreat Chemicals Co., Ltd. (Shenyang, China). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were obtained from Merck KGaA (Darmstadt, Germany). HPLC-grade formic acid (FA, ≥99%), HPLC-grade ammonium acetate (AMA), ammonium hydroxide solution ($NH_3{\cdot}H_2O$), and multi-walled carbon nanotubes (MWCNTs, >95%) were supplied by Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Analytical-grade sodium chloride (NaCl), sodium acetate (NaAc), magnesium sulfate ($MgSO_4$), graphitized carbon black (GCB, 120–140 mesh), and primary secondary amine (PSA, 40–63 μm) were supplied by ANPEL Laboratory

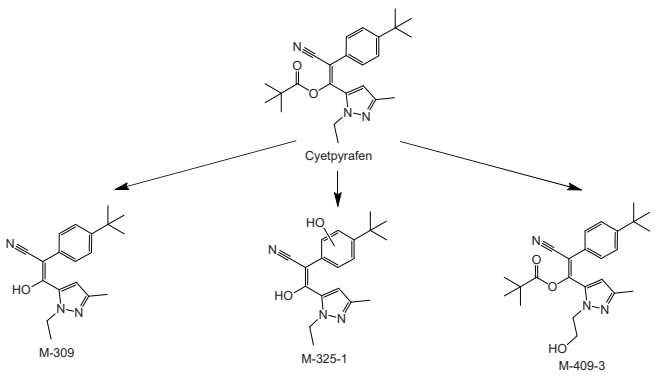


Fig. 1 The chemical structures of cyetpyrafen and its three metabolites.

Technologies Co., Ltd. (Shanghai, China). ZrO_2 (20–40 nm) and biochar (purity>90%) were supplied by Jiangsu Xianfeng Nanomaterials Technology Co., Ltd. (Jiangsu, China). Purified water (H_2O) was supplied by Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China).

2.2. UPLC-MS/MS conditions

The analysis of cyetpyrafen and its three metabolites was conducted using a Quattra Premier XE triple-quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) with electrospray ionization (ESI) source interfaced with the UPLC system. The MassLynx 4.1 software workstation was used for system control, data acquisition, and data analysis.

All samples were analyzed using a Lux® 3 μm Cellulose-2 (3 μm×150 mm×2 mm, Phenomenex, CA, USA) with a mobile phase consisting of ACN with 0.1% FA (solvent A) and H_2O with 0.05% FA (solvent B). The flow rate was set at 0.3 mL min^{-1}. The gradient chromatographic program was set as follows: 0–1.0 min, 30–70% A; 1.0–1.5 min, 70–90% A; 1.5–2.0 min, 90–100% A; 2.0–5.5 min, 100%; 5.5–6.0 min, 100–30% A. The total analysis time was 8.5 min, with an additional 2.5 min column equilibration period. The column temperature was maintained at 40°C, while the injection volume was 5 μL. The residues of cyetpyrafen and its three metabolites were determined by positive ESI mode with multiple reactions monitoring (MRM) as shown in Table 1. The capillary voltage was set at 3.5 kV, with the source temperature and the desolvation set at 150°C and 350°C, respectively. Argon (Ar, 99.995%) was used as the collision gas, while nitrogen (N_2, 99.95%) was used as the cone and desolvation gas at rates of 50 and 700 L h^{-1}, respectively.

2.3. Field experiments and processing

In August 2024, the field experiment was conducted in Shengzhou, Zhejiang, China (29.71°N, 120.81°E), following the sampling methods outlined in the Guidelines on Pesticide Residue Trials in China (NY/T 788-2018 2018). A total of 5.0 kg of fresh tea leaves (comprising one bud and two leaves) before spraying was selected as blank samples, and subsequently processed to both black and green tea, using the tea cultivar 'Zhongcha 111'. A dosage of 0.75 g.ai per 100 m^2 for 30% cyetpyrafen SC (Currently, cyetpyrafen is not registered for use in tea gardens in China, so, the recommended dosage from its existing registration on citrus crops was applied in this study.) was sprayed for both dissipation trials and final residue trials. A total of 2.0 kg of fresh tea leaves was randomly sampled at 2 h, 1, 2, 3, 5, 7, 10, 14, and 21 d after cyetpyrafen application for dissipation research. The fresh tea leaves were picked at 1, 5, 7, 10, and 14 d after application and then processed to green and black tea for final residue research. Black tea processing involved the steps of withering, rolling, fermentation, and drying, while green tea processing stages included spreading, fixing, rolling, and drying (Li *et al.* 2021). All the samples were stored at –20°C before analysis.

2.4. Sample preparation, extraction and purification

Firstly, 5.0 g of fresh tea leaves (2.0 g of green or black tea) was weighed and placed into the 50 mL centrifuge tube. Subsequently, 2.0 mL H_2O was added to the tube, and the mixture was fully vortexed and stood for 10 min. Following this, 20.0 mL of ACN was added to the mixture, followed by vigorous shaking for 5 min and sonication for an additional 10 min. To facilitate phase separation, 1.0 g of NaCl was added and shaken for 5 min before centrifugation at 6,000 r min^{-1} for 5 min. After centrifugation, 6.0 mL of the supernatant (9.0 mL for black tea and green tea) was put into the 15 mL centrifuge tube containing different adsorbents (60 mg MWCNTs, 100 mg $MgSO_4$, and 40 mg PSA for fresh tea leaves; 90 mg MWCNTs, 150 mg $MgSO_4$, and 60 mg PSA for black and green tea). The mixture was vortexed completely for 1 min and centrifuged again at 6,000 r min^{-1} for 5 min. After clean-up, 4.0 mL (8.0 mL for black and green tea) of supernatant was placed into the 100 mL flask and evaporated to dryness at 35°C. Then, the residue was subsequently dissolved by 1.0 mL ACN assisted by ultrasonication. An additional freezing step at –20°C for 1 h was performed before centrifuging at 10,000 r min^{-1} for 2 min for black and green tea samples. Lastly, the solutions were filtered through a 0.22 μm membrane for analysis.

For tea infusion, the procedure was performed in accordance with Methodology for Sensory Evaluation of Tea (GB/T 23776-2018 2018). Specifically, 3.0 g of black or green tea was steeped in 150 mL of boiling water at a ratio of 1:50. The infusion was then filtered, which was brewed a total of three times. After that, 20.0 mL of infusions were taken into the 50 mL tube and 20.0 mL of ACN was added. The mixture was shaken for 5 min to ensure thorough mixing. Following this, 7.0 g of NaCl was added and shaken for 5 min before centrifuging at 6,000 r min^{-1} for 5 min. After centrifugation, 15.0 mL of the supernatant was transferred into the 100 mL flask, using a rotary evaporator at 35°C to remove the

Table 1 The optimized UPLC-MS/MS conditions for cyetpyrafen and its three metabolites

Compounds	Molecular formula	Retention time (min)	Parent ions (*m*/*z*)	Daughter ions (*m*/*z*)[1)]	Cone voltage (V)	Collision energy (eV)
Cyetpyrafen	$C_{24}H_{31}N_3O_2$	3.2	394.25	310*	40	25
				254.1		30
M-325-1	$C_{19}H_{23}N_3O_2$	2.7	326.18	270.1	35	20
				82*		40
M-309	$C_{19}H_{23}N_3O$	3.0	310.19	254.1	30	20
				109*		30
M-409-3	$C_{24}H_{31}N_3O_3$	3.1	410.24	326*	25	25
				252.1		25

[1)] * Value represents quantitative ion transition.

solvents. Then, the residue was dissolved by 1.5 mL ACN assisted by ultrasonication. The samples were transferred to the 2 mL tube containing various adsorbents, including 30 mg MWCNTs, 50 mg $MgSO_4$, and 20 mg PSA. The mixture was vortexed thoroughly for 1 min and centrifuged at 12,000 r min^{-1} for 5 min. Finally, the solvents were filtered through a 0.22 μm membrane for analysis.

2.5. Method validation

To assess the suitability of the analytical method for determining cyetpyrafen and three metabolites in tea samples, various parameters including linearity, limits of detection (LODs), matrix effects (MEs), limits of quantification (LOQs), accuracy and precision were evaluated. Standard solutions containing different concentrations ranging from 0.001 to 2.0 mg L^{-1} (0.001, 0.002, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, and 2.0 mg L^{-1}) were prepared in the solvent ACN and different blank tea matrices outlined in Section 2.4. These were analyzed in triplicate for each concentration to obtain different linear relationships. The MEs represent the extent of matrix interference for the analyte during analysis, assessed by the deviation of the slope ratio (matrix-matched standard curve/solvent standard curve) from 1, expressed as a percentage. An ME>0 indicates matrix enhancement, while an ME<0 indicates matrix suppression (Ning *et al.* 2025). Spiked recoveries were used to assess the accuracy and precision of the method for cyetpyrafen and its three metabolites. The spiked levels for fresh tea leaves were 0.005, 0.01, 0.10, and 1.00 mg kg^{-1} (0.001, 0.01, 0.10, and 1.00 mg kg^{-1} for cyetpyrafen), while green and black tea samples were spiked at levels of 0.005, 0.025, 0.25, and 2.50 mg kg^{-1}. The spiked concentration levels were 0.0002, 0.001, 0.01, and 0.1 mg L^{-1} in green and black tea infusion. Each spiked level was tested in five replicates to verify recovery and relative standard deviations (RSDs). The LODs were calculated using the lowest spiking levels with a signal-to-noise (S/N) ratio of 3. The lowest spiking levels of cyetpyrafen and its metabolites were used to confirm the LOQs, with the method ensuring that recoveries were between 70 and 120% and RSDs ≤20% (SANTE/11312/2021).

2.6. Statistical analysis

The residue dissipation kinetics of cyetpyrafen in tea plants were calculated by eq. (1) and its half-lives in tea leaves were calculated by eq. (2):

$$C_t=C_{0t}^{-kt} \quad (1)$$

$$t_{1/2}=\ln 2/k \quad (2)$$

where C_0 represents initial residue (mg kg^{-1}); C_t represents residue (mg kg^{-1}) at interval t (d) after cyetpyrafen application; k is dissipation rate constant; $t_{1/2}$ is dissipation half-life.

The variation in residues of compounds before and after tea processing of black and green tea was quantified using processing factor (*PF*), calculated by eq. (3):

$$PF=R_{after}/R_{before} \quad (3)$$

where R_{before} and R_{after} represent the residue levels (mg kg^{-1}) of cyetpyrafen (or its three metabolites) before and after tea processing step, respectively.

The leaching rate (*LR*) is defined as the proportion of compounds that migrated from tea into tea infusions, calculated by eq. (4):

$$LR\ (\%)=R_i/R_t\times 100\% \quad (4)$$

where R_i represents residues in tea infusions, while R_t refers to the total residue concentration following the brewing process.

The chronic risk (RQc) calculated by eq. (5) and acute risk (RQa) calculated by eq. (6) were used to assess the risk related to consuming cyetpyrafen residues in tea. RQ<1 denotes an acceptable risk, but RQ≥1 denotes an unacceptable level for pesticide residues exposed to humans:

$$RQc\ (\%)=\frac{FI\ (kg)\times STMR\ (mg\ kg^{-1})\times LR}{bw\ (kg)\times ADI\ (mg\ kg^{-1})}\times 100 \quad (5)$$

$$RQa\ (\%)=\frac{FI\ (kg)\times HR\ (mg\ kg^{-1})\times LR}{bw\ (kg)\times ARfD\ (mg\ kg^{-1})}\times 100 \quad (6)$$

where STMR stands for median residue (STMR) and HR stands for highest residue (HR) observed in experiments, while FI stands for food intake (FI), ADI stands for acceptable daily intake, ARfD for acute reference dose, and bw for average adult human body weight (63 kg).

3. Results and discussion

3.1. Optimization of UPLC-MS/MS conditions

The ESI in positive ionization mode was utilized to identify cyetpyrafen and its three metabolites, since these four chemicals generated better fragmentation patterns and greater precursor ion signal intensities compared to the negative ionization mode. The protonated molecule ions $[M+H]^+$ of cyetpyrafen, M-325-1, M-309, and M-409-3 at *m/z* 394.25, 326,18, 310.19, and 410.24 were respectively confirmed to be precursor ions in ESI positive mode, and further induced dissociation at different collision energies (5–50 V). The optimal cone voltages, collision energies, and other experimental parameters for cyetpyrafen and its three metabolites were provided in Table 1. Both ACN and MeOH with various additions of FA and AMA were tested as organic mobile phases to achieve acceptable chromatographic separation. Finally, a mobile phase system consisting of ACN containing 0.1% FA as phase A and H_2O containing 0.05% FA as phase B was selected, with gradient elution applied. This combination yielded the most effective chromatographic separation results, such as the reduction of peak tailing.

3.2. Optimization of clean-up procedure

Selection of sample extraction Tea is a complex matrix containing abundant pigments, polyphenols, caffeine, and other ingredients, which makes the analysis of pesticide residues extremely challenging (Li *et al.* 2021). Studies have shown that adding H_2O into the extraction process can effectively improve the recoveries of pesticides from tea leaves (Cajka *et al.* 2012; Hou *et al.* 2013; Zhang *et al.* 2014; Li H X *et al.* 2022b). The pH of the solvent directly affects the extraction of residual compounds in the sample, especially the metabolites (Li H X *et al.* 2022b; Ning *et al.* 2025). Thus, the recoveries of cyetpyrafen and its three metabolites in tea leaves during soaking were evaluated using 10 mL of H_2O containing different concentrations of NaAc (1, 2, and 5%) or FA (1, 2, and 5%), with the results presented in Fig. 2-A.

The four target compounds exhibited recoveries of over 80% across different pH values of H_2O, and the RSDs were below 20%. H_2O was selected as the soaking solvent to establish a green and economical method. On this basis, the amount of H_2O used for soaking fresh tea leaves, black tea, and green tea was optimized due to their different moisture contents. The findings in Fig. 2-B indicated that the addition of water significantly enhanced the recoveries. No significant difference was observed between the extraction effects of 2 and 10 mL of H_2O. Therefore, 2 mL of H_2O was ultimately selected as the soaking solvent for fresh tea leaves, black tea, and green tea, from an economic and environmentally friendly perspective.

Cyetpyrafen is a polar compound that is easy to ionize, making it suitable for extraction with ACN, a commonly used organic solvent in the QuEChERS method. Earlier research on cyetpyrafen has demonstrated that ACN extraction produces superior results (Wang *et al.* 2023). As a result, the recoveries of cyetpyrafen and its metabolites were compared using 20 mL of ACN containing different concentrations of $NH_3{\cdot}H_2O$ (1 and 2%) or FA (1 and 2%), as shown in Fig. 3. In ACN with $NH_3{\cdot}H_2O$, the recoveries of cyetpyrafen and the metabolite M-409-3 failed to meet the requirements. Pure ACN was the optimal extraction solvent, as the recoveries of all compounds satisfied the require criteria. Finally, 20 mL of ACN and 2 mL of H_2O were selected as the extraction and soaking solvents, respectively, for the analysis of cyetpyrafen and its three metabolites in tea.

Selection of purification Tea is a complex matrix compared with other agricultural products. Thus, different adsorbents (MWCNTs, biochar, GCB, PSA, ZrO_2 and $MgSO_4$) at a series of dosages (5, 10, 20, 30, 40, 50, and 100 mg) were tested to evaluate their purification performance, with the results shown in Fig. 4. Firstly, MWCNTs, biochar, and GCB are widely employed as effective adsorbents for the removal of pigments, hydrophobic substances, and other impurities due to their strong adsorption capacities (Hou *et al.* 2014; Zhou *et al.* 2020). Compared with MWCNTs and GCB, biochar adsorbed most impurities in the matrix more effectively; however, it also exhibited stronger adsorption of the target compounds, resulting in the recoveries that failed to meet the analytical requirements. After purification, GCB was less effective than MWCNTs in decolorizing the sample solution. Therefore, 30 mg MWCNTs was selected to eliminate pigments and other impurities in tea leaves. Furthermore, compared with mixed adsorbent formulations, a single adsorbent exhibit limited efficacy against co-extracted impurities. ZrO_2, characterized by high stability and inertness, was used to adsorb lipids and heavy metals. PSA was utilized to remove polar compounds including fatty acids, while $MgSO_4$ was used to eliminate water. Thus, different dosages of PSA, $MgSO_4$, and ZrO_2 were tested to compare their purification effects. In summary, a mixture of 30 mg MWCNTs, 20 mg PSA, and 50 mg $MgSO_4$ was used to purify 1.5 mL of the extracted tea sample. Based on previous research (Ning *et al.* 2025), low-temperature freezing for 1 h was additionally applied to remove precipitates in the processed tea sample.

3.3. Method validation

Linearities and MEs After optimizing the conditions, the analytical method validation was carried out by assessing parameters including linearities, LODs, MEs, recoveries, RSDs, and LOQs. Table 2 presents linear equations and correlation coefficients (R^2) for cyetpyrafen and its three metabolites, with concentrations ranging from 0.002 (0.001) to 2.0 mg L^{-1} across different matrices. These results indicated that cyetpyrafen and its metabolites exhibited excellent linearities with R^2 above 0.9952. As matrix interference is known to significantly interfere with the analytical process and affect the accuracy of the results, the MEs of cyetpyrafen and its three metabolites in multiple matrices range from –14 to –81%, as shown in Table 2. Notably, ME suppression of these four compounds was observed in different matrices, and it was more pronounced in tea leaves, black tea, and green tea compared to tea infusions. Thus, the residues of cyetpyrafen and its three metabolites should be accurately quantified using an external matrix-matched calibration curve.

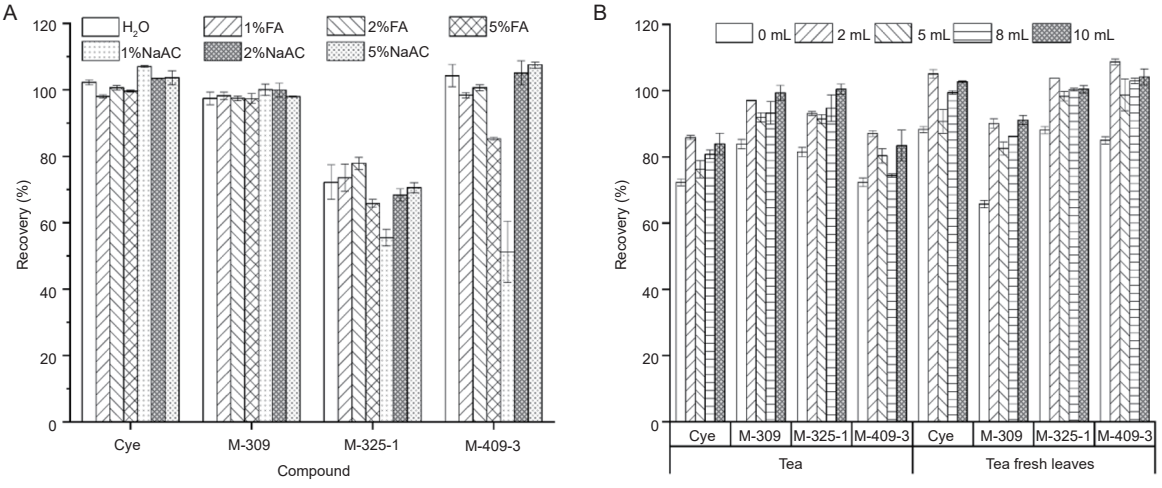


Fig. 2 The recoveries of cyetpyrafen and its three metabolites with various soaking solvents (A), and various volumes of H_2O (B) for tea and fresh leaves.

Recovery and precision According to the method in Section 2 “Materials and methods”, recoveries and RSDs were determined to verify the analytical method by spiking cyetpyrafen and its metabolites into various blank samples at low, medium, and high concentration levels, with five replicates for each level. The mean recoveries of cyetpyrafen and its three metabolites (M-325-1, M-309, and M-409-3) were in the range of 73.4–106.2%, with RSDs below than 9.1% in fresh tea leaves. The mean recoveries ranged from 80.4 to 99.4%, with RSDs less than 8.8% in both black tea and green tea. For tea infusions, the mean recoveries were in the range of 74.9–103.3%, with RSDs below 12.0%. All these results are presented in Table 2. The lowest spiking concentration was used to define the LOQs. The LOQs for cyetpyrafen and its three metabolites were 0.005 mg kg^{-1} in fresh tea leaves, black tea, and green tea (except for cyetpyrafen in fresh tea leaves, which had an LOQ of 0.001 mg kg^{-1}), and 0.2 μg L^{-1} in tea infusions. Based on a S/N ratio of 3, the LODs of cyetpyrafen and its three metabolites were 0.002 mg kg^{-1} in fresh tea leaves, black tea, and green tea (except for cyetpyrafen in fresh tea leaves, which had an LOD of 0.0004 mg kg^{-1}), and 0.1 μg L^{-1} in tea infusions. These results demonstrated that the developed method can be used to analyze cyetpyrafen and its metabolites in various tea matrices and real samples with satisfactory sensitivity, recovery, and precision. As shown in Table 3, the LOQs obtained by our method outperformed those of many previously published methods, enabling the simultaneous detection of major metabolites in complicated matrices.

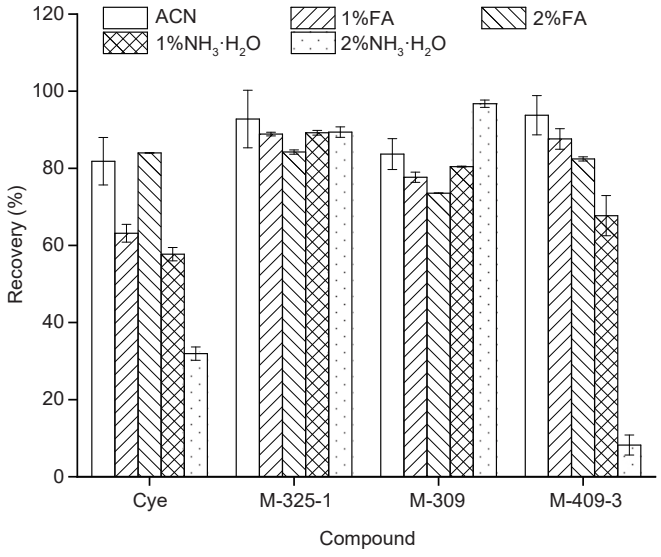


Fig. 3 The recoveries of cyetpyrafen and its metabolites using various extraction solvents.

3.4. Dissipation behavior of cyetpyrafen on tea leaves

The residues of cyetpyrafen and its three metabolites in tea plants over time under field trial conditions are presented in Fig. 5 and Appendix A. After applying 30% cyetpyrafen SC at a dosage of 0.75 g.ai per 100 m^2, the initial residue for cyetpyrafen on tea leaves was 6.31 mg kg^{-1} at 2 h. Within 2 d, the residue dissipated by 90.2%. The degradation dynamics of cyetpyrafen under field conditions matched first-order kinetics, and the dissipation rate was described by the equation C=6.4776e$^{-1.1717t}$ with R^2=0.9552 and the half-life ($t_{1/2}$) was 0.59 d. The half-life is shorter than 2.2 d reported for orange fruits and peels (Wang *et al.* 2023), 1.5–7.0 d for cotton and 3.0–12.0 d for planting soil (Gao 2015), indicating that cyetpyrafen is a readily degradable pesticide. Crop variety and experimental conditions, such as growing season and climate, may contribute to the variation in half-life across different crops. Growth dilution and sunlight photolysis have also been proposed as significant factors influencing pesticide dissipation (Li *et al.* 2021; Zhang *et al.* 2021). After cyetpyrafen application, only M-309 and M-409-3

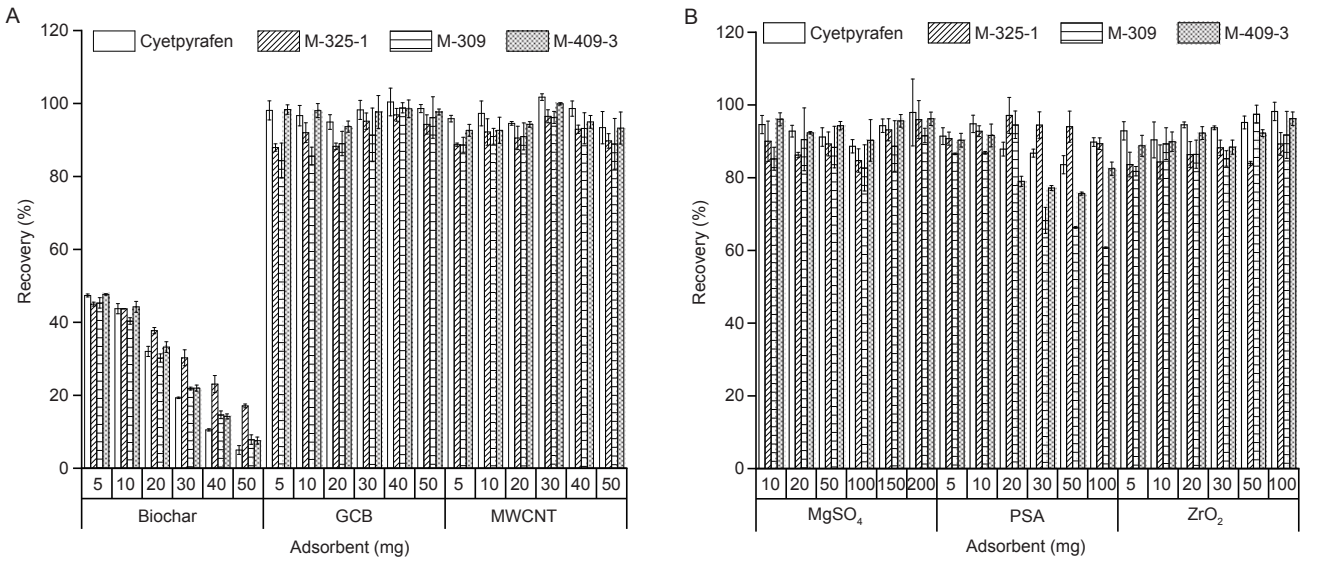


Fig. 4 The recoveries of cyetpyrafen and its three metabolites using various adsorbents: A, biochar, GCB (graphitized carbon black) and MWCNTs (multi-walled carbon nanotubess); B, $MgSO_4$, PSA (primary secondary amine) and ZrO_2.

Table 2 The linear ranges, regression equations, correlation coefficients (R^2), matrix effects (MEs), limits of quantitation (LOQs) and limits of detection (LODs) for cyetpyrafen and three metabolites in various sample matrices

Compounds	Matrices[1)]	Linear range (mg L^{-1})	Regression equation	R^2	MEs (%)	LOQs (mg kg^{-1})	LODs (mg kg^{-1})	Average recoveries±SD (%, n=5)[2)]				Relative standard deviations (RSDs, %)[2)]			
								C_1	C_2	C_3	C_4	C_1	C_2	C_3	C_4
Cyetpyrafen	Solvent	0.001–2	y=2,023,133x+33,997	0.999,2	–			–	–	–	–	–	–	–	–
	Fresh tea leaves		y=745,161x+16,752	0.998,1	–63	0.001	0.000,4	89.4±8.2	106.2±8.6	91.7±7.7	90.2±5.4	9.1	8.1	8.4	6.0
	Green tea		y=392,197x+3,427	0.999,4	–81	0.005	0.002	89.6±5.3	92.9±3.5	97.0±3.4	95.9±4.8	5.9	3.8	3.5	5.0
	Black tea		y=439,214x+2,530	0.999,8	–78	0.005	0.002	93.9±3.1	93.4±8.3	93.6±3.3	90.6±3.8	3.3	8.8	3.5	4.2
	Green tea infusion		y=1,000,860x+18,063	0.999,1	–51	0.000,2	0.000,1	82.4±8.6	88.0±5.3	93.7±3.4	96.8±11.5	10.5	6.0	3.6	11.8
	Black tea infusion		y=1,643,592x+30,547	0.999,0	–19	0.000,2	0.000,1	91.9±9.1	94.9±3.1	103.3±5.4	101.2±9.2	9.9	3.3	5.2	9.1
M-325-1	Solvent	0.002–2	y=46,1049x+4,231	0.999,0	–			–	–	–	–	–	–	–	–
	Fresh tea leaves		y=203,330x+156	1.000,0	–56	0.005	0.002	104.6±8.0	103.0±6.6	87.9±3.9	85.9±3.7	7.6	6.6	4.4	4.4
	Green tea		y=142,091x–161	1.000,0	–69	0.005	0.002	83.4±7.2	90.9±4.6	87.9±3.8	91.4±6.6	8.6	5.1	4.3	7.3
	Black tea		y=115,890x–1,204	0.999,5	–75	0.005	0.002	94.5±7.3	88.7±5.0	87.8±1.7	91.6±3.3	7.7	5.6	2.0	3.6
	Green tea infusion		y=249,838x+684	0.999,8	–46	0.000,2	0.000,1	74.9±4.7	83.2±4.9	91.7±9.1	90.4±6.8	6.3	5.8	9.9	7.5
	Black tea infusion		y=279,672x+2,960	0.998,8	–39	0.000,2	0.000,1	90.3±6.3	89.8±4.0	91.1±8.5	89.4±8.3	7.0	4.5	9.4	9.3
M-309	Solvent	0.002–2	y=679,369x+5,639	0.999,7	–			–	–	–	–	–	–	–	–
	Fresh tea leaves		y=265,034x+2,683	0.999,1	–61	0.005	0.002	84.8±5.8	83.6±4.3	73.4±2.4	73.6±3.1	6.8	5.1	3.3	4.3
	Green tea		y=217,199x+1,492	0.999,7	–68	0.005	0.002	85.7±3.1	83.3±6.6	80.4±1.8	82.7±2.8	3.6	7.9	2.2	3.4
	Black tea		y=155,056x–960	0.999,6	–77	0.005	0.002	99.4±7.0	90.7±3.1	97.9±2.9	96.2±5.0	7.1	3.4	3.0	5.2
	Green tea infusion		y=384,817x+2,681	0.999,8	–43	0.000,2	0.000,1	78.0±6.1	82.0±5.3	90.1±3.4	83.5±3.6	7.9	6.4	3.8	4.3
	Black tea infusion		y=533,333x+7,353	0.999,0	–21	0.000,2	0.000,1	90.2±9.9	86.0±4.3	95.4±5.9	91.6±9.4	11.0	5.0	6.2	10.2
M-409-3	Solvent	0.002–2	y=533,044x+4,169	0.999,0	–			–	–	–	–	–	–	–	–
	Fresh tea leaves		y=289,590x+4,125	0.999,3	–46	0.005	0.002	95.0±6.8	102.0±4.5	91.3±4.2	90.1±5.2	7.2	4.5	4.6	5.7
	Green tea		y=160,156x+1,699	0.999,5	–70	0.005	0.002	89.4±7.4	89.6±5.9	88.9±1.9	89.7±2.9	8.2	6.6	2.2	3.2
	Black tea		y=148,907x–409	0.999,9	–72	0.005	0.002	95.0±7.2	94.7±3.6	90.3±3.3	92.1±3.9	7.6	3.8	3.6	4.3
	Green tea infusion		y=246,456x+4,428	0.998,2	–54	0.000,2	0.000,1	81.7±8.5	85.6±3.6	90.8±3.9	97.6±11.8	10.4	4.2	4.3	12.0
	Black tea infusion		y=458,723x+11,630	0.995,2	–14	0.000,2	0.000,1	85.6±7.7	95.5±4.0	97.9±5.4	102.6±8.2	9.0	4.1	5.5	8.0

[1)] Fresh tea leaves: 0.005, 0.01, 0.1 and 1 mg kg^{-1} (cyetpyrafen was 0.001, 0.01, 0.1 and 1 mg kg^{-1}); green tea, black tea: 0.005, 0.025, 0.25 and 2.5 mg kg^{-1}; green tea infusion, black tea infusion: 0.0002, 0.001, 0.01, 0.1 mg L^{-1}.

[2)] C_1, C_2, C_3 and C_4 represented the spiked concentration, respectively.

were detected in fresh tea leaves. Hydroponic experiments on lettuce and rice have revealed that the metabolite M-325-1 is found only in the lettuce system, while M-309 and M-409-3 are identified as primary metabolites in both lettuce and rice systems (Li R N *et al.* 2022). M-309, the major metabolite of cyetpyrafen, was rapidly formed in the open field after cyetpyrafen spraying on tea plants, and over the subsequent 3 d, it degraded rapidly, with more than 90% of the residue dissipating.

3.5. Residue transmission of cyetpyrafen during tea processing

Residue transmission in green tea processing Tea leaves undergo specific procedures for processing into black tea and green tea, and the loss of water during processing may lead to the enrichment of pesticide residues (Gupta and Shanker 2009). To accurately evaluate the degradation of cyetpyrafen and its metabolites at each processing step, the residue levels were expressed on a dry weight basis. This was calculated by dividing the measured concentrations on a fresh weight basis by the percentage of dry matter. Fig. 6 shows that cyetpyrafen and M-309 residues exhibited slight variations across different stages of green tea and black tea processing. Detailed data on the residues and PFs of cyetpyrafen and its metabolites throughout the green tea and black tea processing stages can be found in Appendices B and C.

Fresh tea leaves were picked at 1, 5, 7 and 10 d after spraying. The residue levels of cyetpyrafen ranged from 0.006–1.51 mg kg^{-1} in fresh tea leaves to 0.007–1.63 mg kg^{-1} after spreading, 0.003–1.27 mg kg^{-1} after fixing, 0.002–1.24 mg kg^{-1} after rolling, and finally 0.001–2.58 mg kg^{-1} after drying. The PFs of cyetpyrafen were 1.08–1.22, 0.43–0.78, 0.67–1.22, and 0.50–2.08 for spreading, fixing, rolling, and drying, respectively. The main metabolite M-309 was detected only on d 1 and 5, the metabolite M-409-3 was below the LOQs during processing,

Table 3 Comparison of current analytical methods for the residues of cyetpyrafen and its metabolites in different agricultural crops[1)]

Compounds	Samples	Extraction solvent	Detection method	Mobile phases	LODs (μg kg^{-1})	LOQs (μg kg^{-1})	Reference
Cyetpyrafen	Cotton, soil	MeCN	LC-MS/MS	MeOH and H_2O with 0.05% FA	–	10	Gao (2015)
Cyetpyrafen	*Citrus grandis*	MeCN	HPLC-MS/MS	MeOH and H_2O with 0.1% FA	0.06	0.2	Lai (2019)
Cyetpyrafen	Goji berries	MeCN	UPLC-MS/MS	MeOH and H_2O with 0.1% FA	–	10	Niu *et al.* (2022)
Cyetpyrafen	–	–	qNMR	–	–	11	Lang *et al.* (2022)
Cyetpyrafen, M-309 and M-391	13 matrices (e.g., plants and soils)	MeCN	UHPLC-MS/MS	MeCN and H_2O with 0.2% FA	–	5–10	Wang *et al.* (2023)
Cyetpyrafen	Strawberry	MeCN	UPLC-MS/MS	MeOH and 5 mmol L^{-1} AMA	–	4	Liu *et al.* (2023)
Cyetpyrafen, M-309, M-325-1 and M-409-3	Fresh tea leaves, green tea, black tea, and tea infusion	MeCN and H_2O (QuEChERS)	UPLC-MS/MS	MeCN with 0.1% FA and H_2O with 0.05% FA	0.1–2	0.2–5	Our method

[1)] LODs, limits of detection; LOQs, limits of quantitation.

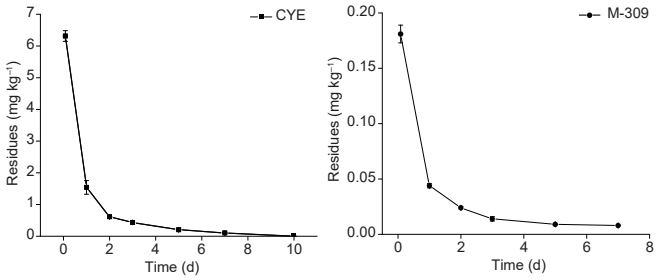


Fig. 5 The residues of cyetpyrafen (CYE) and M-309 in the growth of fresh tea leaves.

and the metabolite M-325-1 was not detected at all. The PFs of M-309 were 0.83–1.04 for spreading, 0.50–1.04 for fixing, 0.67–1.00 for rolling, and 3.40–7.59 for drying.

After accounting for moisture content, the residues of cyetpyrafen and its metabolites at various processing steps of green tea can be found in Appendix D. The residues of cyetpyrafen showed a general decreasing trend from fresh tea leaves to the final green tea, decreasing from 0.021–6.34 mg kg^{-1} in fresh tea leaves to 0.001–2.65 mg kg^{-1} in the final green tea, with the TPFs in the range of 0.05–0.42 on a dry weight basis. The PFs of cyetpyrafen during various stages were as follows: 0.91–0.98 for spreading, 0.32–0.69 for fixing, 0.64–1.15 for rolling, and 0.25–0.82 for drying. As for the metabolite M-309, its residue showed a fluctuating trend, from 0.048–0.20 mg kg^{-1} in fresh tea leaves, to 0.031–0.17 mg kg^{-1} after spreading, 0.013–0.16 mg kg^{-1} after fixing, 0.012–0.16 mg kg^{-1} after rolling, and 0.017–0.26 mg kg^{-1} after drying. The corresponding PFs were 0.65–0.88, 0.43–0.92, 0.63–0.94, and 1.39, respectively. The results demonstrated that the high temperature environment during fixing and drying caused cyetpyrafen to degrade rapidly, which has been proven in earlier research to be a crucial stage in pesticide degradation (Sood *et al*. 2004; Paramasivam *et al.* 2017; Dai *et al.* 2020; Zhong *et al.* 2022). Moreover, high temperature promoted the formation of the metabolite M-309. The residue of M-309 was found to decrease during the rolling stage, probably due to the inactivation of enzymes caused by the high-temperature and high-humidity conditions in fixing. This is consistent with the results of degradation studies of pyriproxyfen, chlorfluazuron, and flonicamid (Li *et al.* 2021; Li H X *et al.* 2022). Based on the PFs on a dry weight basis, the tea processing steps that most significantly influenced the residue level of cyetpyrafen were fixing and drying, while drying and rolling showed the greatest impact on its metabolite M-309.

Residue transmission in black tea processing The residues of cyetpyrafen during different processing steps on d 1, 5, 7, and 10 were as follows: 0.007–1.54 mg kg^{-1} for withering, 0.008–1.52 mg kg^{-1} for rolling, 0.007–1.39 mg kg^{-1} for fermentation, and 0.010–2.24 mg kg^{-1} for drying. Correspondingly, the PFs of cyetpyrafen during processing step were 1.02–1.29, 0.99–1.14, 0.88–0.96, and 1.43–1.86, respectively. The overall TPFs from fresh tea leaves to the drying stage ranged from 1.48 to 2.28. Similar to green tea processing, the main metabolite M-309 was detected only on d 1, 5, and 7. The PFs of M-309 were 0.75–1.06, 0.67–0.80, 1.17–1.25, and 4.57–6.14 for each step, respectively.

After correcting for moisture content, the residues of cyetpyrafen showed a general decreasing trend from fresh tea leaves to black tea, declining from 0.021–6.34 mg kg^{-1} in fresh tea leaves to 0.010–2.27 mg kg^{-1} in the final black tea; the TPFs varied from 0.36 to 0.65 on a dry weight basis. The PFs of cyetpyrafen during the processing steps of black tea were 0.70–0.88, 0.92–1.05, 0.93–0.98, and 0.59–0.75, respectively. The residues of M-309 showed a trend of decreasing first and then increasing, from 0.048–0.20 mg kg^{-1} in fresh tea leaves to 0.016–0.14 mg kg^{-1} after withering, 0.014–0.11 mg kg^{-1} after rolling, then increasing to 0.017–0.14 mg kg^{-1} after fermentation, and finally to 0.019–0.31 mg kg^{-1} after drying. The PFs of M-309 during these different steps were 0.48–0.73, 0.63–0.75, 1.17–1.27, and 1.93–2.27, respectively. In terms of the PFs, drying and withering are key steps for the degradation of cyetpyrafen in black tea. The loss of cyetpyrafen was related to the duration of withering and the evaporation of water caused by this process (Dai *et al.* 2020; Zhong *et al.* 2022). After 12 h of withering, black tea can degrade 30% of cyetpyrafen, in comparison to green tea which undergoes 4 h of spreading. Due to the different

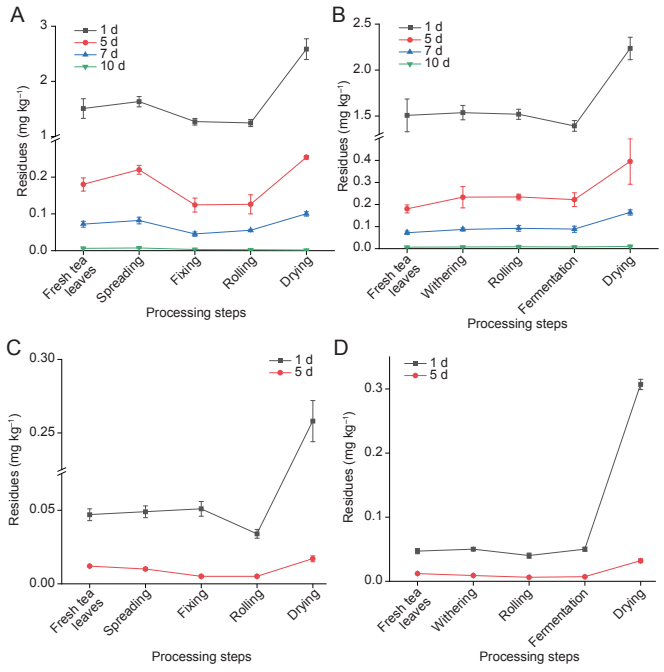


Fig. 6 The residual degradation of cyetpyrafen (A, B) and M-309 (C, D) during green tea (A, C) and black tea (B, D) processing.

key processes of black tea and green tea production, cyetpyrafen and M-309 exhibited different residue behaviors. In green tea processing, the high temperature and high humidity during the fixing step inactivates enzymes, which may rapidly reduce the metabolic process of cyetpyrafen in the subsequent rolling step (Guo *et al.* 2023). Regarding fermentation, the crucial stage of black tea processing, the warm, humid environment may trigger microbial activity on pesticides, which was also mentioned in studies on sulfoxaflor and cycloxaprid (Liu *et al.* 2020, 2022). The drying step notably reduced cyetpyrafen residues while increasing M-309 levels, because cyetpyrafen forms metabolites in a high-temperature environment. Compared to green tea, the variations in temperature and humidity during black tea processing may give rise to distinct degradation mechanisms. This difference warrants further investigation to elucidate the influence of processing methods on pesticide degradation across different tea types.

3.6. Leaching rates of cyetpyrafen in tea for human consumption

Currently, the common route of human exposure to tea is through drinking, making it essential to investigate pesticide residues in tea infusions. The transfer of pesticide residues from tea into tea infusion is influenced by factors like water solubility and the octanol-water partition coefficient (Kow). This research examined the residues and transfer rates of cyetpyrafen and its metabolites from tea into tea infusion under three different brewing times. Additionally, the residues in spent tea were assessed. The detailed data were presented in Appendices E and F.

Cyetpyrafen was detected in infusions prepared from the first-day samples of black tea and green tea. The residues in green tea infusion varied from 0.058 to 0.063 mg kg^{-1} with a total leaching rate (TLR) of 7.4%, while those of black tea infusion ranged from 0.010 to 0.059 mg kg^{-1} with a TLR of 6.0%. This may be due to cyetpyrafen being difficult to dissolve in water, with a water solubility of 1.05 mg L^{-1} (25°C, predicted by T.E.S.T., version 5.1.2) and a Kow of 1.6 (Li R N *et al.* 2022). This indicates that cyetpyrafen has relatively low solubility in tea infusion, given that its leaching rate is much lower than that of highly water-soluble pesticides such as flonicamid, which has an average leaching rate of over 90% in tea infusion (Li *et al.* 2021). Metabolites M-309 and M-409-3 were detected in both black tea infusions and green tea infusions at various intervals, while the metabolite M-325-1 was not detected at all. M-309, the major metabolite, was detected in three rounds of tea infusions on the first day, with residues varying from 0.13 to 0.18 mg kg^{-1} and a TLR of 63.4%. This metabolite was also detected in green tea infusions on d 5, 7, and 10, with residues ranging from 0.011 to 0.022 mg kg^{-1}. In black tea infusions on the first day, M-309 residues ranged from 0.030 to 0.049 mg kg^{-1}, with a TLR of 40.6%. The residue of the metabolite M-409-3 was 0.009 mg kg^{-1} in tea infusions on the first day. Notably, the residues and leaching rates of the metabolite M-309 in tea infusions were higher than those of cyetpyrafen. This could be attributed to the higher water solubility of the metabolite M-309 compared to its parent compound cyetpyrafen.

3.7. Dietary intake risk assessment of cyetpyrafen for tea drinking

Based on GB/T 2763-2021 National Food Safety Standard Maximum Residue Limits for Pesticides in Food, the ADI of cyetpyrafen was established at 0.1 mg (kg bw)$^{-1}$, while the MRL was temporarily set at 0.05 mg kg^{-1} for cottonseed and 0.1 mg kg^{-1} for tangerine, mandarin, orange, and apple. The ADI value was utilized for computation in this research since no specified ARfD was available. The RQc and RQa values of cyetpyrafen in tea following cyetpyrafen application were determined using the equations provided in Section 2.6. The results are presented in Table 4. On the first day after spraying, the RQc values of cyetpyrafen were 0.040% for green tea and 0.028% for black tea, while the RQa values of cyetpyrafen were 0.042% for green tea and 0.029% for black tea. Overall, these values were significantly below 100%, suggesting that the residues resulting from cyetpyrafen application on tea plants pose no harm to consumer health.

However, M-309 is the main metabolite of cyetpyrafen and its LR is higher than that of the parent compound cyetpyrafen, while its toxicological properties remain unidentified. The toxicities of cyetpyrafen and its metabolites were predicted using the Toxicity Estimation Software Tool (T.E.S.T., version 5.1.2), as summarized in Table 5. The toxicity levels of the metabolites to Fathead minnow, *Daphnia magna*, and rat were found to be comparable to those of the parent compound. Notably, M-409-3 was estimated to be more toxic to fish, which is consistent with the ecotoxicity profiles of cyetpyrafen and its metabolites forecasted by ECOSAR (Li R N *et al.* 2022). Therefore, further research is needed

Table 4 Dietary intake risk assessment of cyetpyrafen in black tea and green tea at the first day

Item[1)]	Green tea	Black tea
FI (kg)	0.013	0.013
STMR (mg kg^{-1})	2.6	2.24
LR (%)	7.4	6
bBw (kg)	63	63
ADI (mg kg^{-1} bw)	0.1	0.1
RQc (%)	0.040	0.028
HR (mg kg^{-1})	2.76	2.35
ARfD (mg kg^{-1} bw)	0.1	0.1
RQa (%)	0.042	0.029

[1)] FI, food intake; STMR, supervised trials median residue; LR, leaching rate; bw, average adult human body weight (63 kg); ADI, acceptable daily intake; RQc, chronic dietary intake risk; HR, highest residue; ARfD, acute reference dose; RQa, acute dietary intake risk.

Table 5 The water solubilities and acute toxicities of cyetpyrafen and its metabolites predicted by T.E.S.T.

Compounds	Water solubility (mg L^{-1})	Fathead minnow (96 h) LC_{50} (mg L^{-1})	*Daphnia magna* (48 h) LC_{50} (mg L^{-1})	Rat LD_{50} (mg kg^{-1})
Cyetpyrafen	1.05	0.19	0.11	897.07
M-309	3.82	1.35	0.93	641.59
M-325-1	5.47	1.32	0.62	674.76
M-409-3	1.8	0.0688	0.23	913.5

to determine whether the metabolite M-309 should be incorporated into risk assessment in the future.

4. Conclusion

This research established an effective UPLC-MS/MS analytical approach for the simultaneous determination of cyetpyrafen and its three metabolites (M-309, M-325-1 and M-409-3) in tea for the first time. This method demonstrated excellent sensitivity, linearity, accuracy, and precision, meeting the necessary standards for pesticide residues analysis in different matrices. Additionally, this method was further successfully applied to study the degradation of cyetpyrafen throughout the stages of tea cultivation, processing, and brewing. The findings showed that cyetpyrafen and M-309 can be detected from tea garden to tea cup. Cyetpyrafen is an easily degradable pesticide with the $t_{1/2}$ of 0.59 d. Notably, cyetpyrafen residues were considerably reduced during the processing of green tea and black tea, particularly during fixing, drying, and withering. Moreover, the LRs of cyetpyrafen in tea infusions were found to be low. Furthermore, dietary intake risk assessment indicated that the potential acute and chronic risk associated with cyetpyrafen are insignificant. Currently, pesticide resistance to chemical control methods in tea gardens is a serious problem. Cyetpyrafen, a new domestically developed pesticide, has the characteristics of rapid degradation and low residue in tea gardens. It can effectively address the shortage of effective acaricides in Chinese tea gardens, ensuring tea quality and safety. This study contributes valuable insights and provides a reference for the future application of cyetpyrafen in tea gardens.

Acknowledgements

This work was financially supported by the National Key Research and Development Program of China (2022YFD1600803), the National Natural Science Foundation of China (31772077), the Yunnan Provincial Tea Industry Technology Innovation Center, China (202505AK340010) and the Chinese Academy of Agricultural Sciences for Agricultural Science and Technology Innovation Program of Tea Research Institute (CAAS-ASTIP-2021-TRI).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2026.01.001

References

Amsalingam R, Gajjeraman P, Sam N, Rahman V J, Azariah B. 2017. Mechanism of fenpropathrin resistance in red spider mite, *Oligonychus coffeae* (Acarina:Tetranychidae), infesting tea [*Camellia sinensis* L. (O. Kuntze)]. *Applied Biochemistry and Biotechnology*, **181**, 548–561.

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. *Analytica Chimica Acta*, **743**, 51–60.

Chen J C, Gong Y J, Shi P, Wang Z H, Cao L J, Wang P, Wei S J. 2019. Field-evolved resistance and cross-resistance of the two-spotted spider mite, *Tetranychus urticae*, to bifenazate, cyenopyrafen and SYP-9625. *Experimental and Applied Acarology*, **77**, 545–554.

Dai J X, Jiang C L, Gao G W, Zhu L, Chai Y F, Chen H P, Liu X. 2020. Dissipation pattern and safety evaluation of cartap and its metabolites during tea planting, tea manufacturing and brewing. *Food Chemistry*, **314**, 126165.

Gao Y. 2015. Residue analysis of pyridaben in cucumber, B-azolemiteacrylic in cotton and their planting soil. MSc thesis, Shandong University, China. (in Chinese)

GB 2763–2021. 2021. National food Safety Standard-Maximum Residue Limits for Pesticides in Food. Standards of the People's Republic of China. China Agriculture Press, Beijing. (in Chinese)

GB/T 23776–2018. 2018. Methodology for Sensory Evaluation of Tea. Standards of the People's Republic of China. China Agriculture Press, Beijing. (in Chinese)

Guo M M, Sun H Z, Wang X R, Yu J W, Luo F J, Zhang X Z, Yang M, Li Z Q, Chen Z M, Zhou L. 2023. Residue behavior and risk assessment of afidopyropen and its metabolite M440I007 in tea. *Food Chemistry*, **404**, 134413.

Gupta M, Shanker A. 2009. Fate of imidacloprid and acetamiprid residues during black tea manufacture and transfer into tea infusion. *Food Additives & Contaminants: Part A*, **26**, 157–163.

Hou R Y, Jiao W T, Qian X S, Wang X H, Xiao Y, Wan X C. 2013. Effective extraction method for determination of neonicotinoid residues in tea. *Journal of Agricultural and Food Chemistry*, **61**, 12565–12571.

Hou X, Lei S R, Qiu S T, Guo L A, Yi S G, Liu W. 2014. A multi-residue method for the determination of pesticides in tea using multi-walled carbon nanotubes as a dispersive solid phase extraction absorbent. *Food Chemistry*, **153**, 121–129.

Jiang L, Wang H, Qiao K, Wu C. 2024. Increasing cyetpyrafen spray volume and ozone spray improves the control effects against two-spotted spider mite (*Tetranychus urticae*) in strawberries. *Plants-Basel*, **13**, 1792.

Lai T C. 2019. SYP-9625 residue in *Citrus grandis* 'Guanxi miyou' tested by HPLC-MS/MS and bagging effect on residue. *Journal of Agriculture*, **9**, 63–66. (in Chinese)

Lang J, Yang H B, Yu H B. 2022. Determination of absolute content of cyetpyrafen by qNMR analysis. *International Journal of Food Science and Agriculture*, **6**, 149–153.

Li H X, Zhong Q, Wang M, Luo F J, Wang X R, Zhou L, Zhang X Z. 2022. Residue degradation, transfer and risk assessment of pyriproxyfen and its metabolites from tea garden to cup by ultra performance liquid chromatography tandem mass spectrometry. *Journal of the Science of Food and Agriculture*, **102**, 3983–3993.

Li H X, Zhong Q, Wang X R, Luo F J, Zhou L, Chen Z M, Zhang X Z. 2022. Simultaneous quantitative determination of residues of pyriproxyfen and its metabolites in tea and tea infusion using ultra-performance liquid chromatography-tandem mass spectrometry. *Agronomy*, **12**, 1829.

Li H X, Zhong Q, Wang X R, Luo F J, Zhou L, Sun H Z, Yang M, Lou Z Y, Chen Z M, Zhang X Z. 2021. The degradation and metabolism of chlorfluazuron and flonicamid in tea: A risk assessment from tea garden to cup. *Science of the Total Environment*, **754**, 142070.

Li R N, Wang S J, Chang J H, Pan X L, Dong F S, Li Z Y, Zheng Y Q, Li Y B. 2022. Insight into the uptake and metabolism of a new insecticide cyetpyrafen in plants. *Environment International*, **169**, 107522.

Lin T, Zeng Z H, Chen Y X, You Y, Hu J F, Yang F H, Wei H. 2021. Compatibility of six reduced-risk insecticides with *Orius strigicollis* (Heteroptera: Anthocoridae) predators for controlling *Thrips hawaiiensis* (Thysanoptera: Thripidae) pests. *Ecotoxicology and Environmental Safety*, **226**, 112812.

Liu C, Yu X K, Xin Y, Zhang L. 2023. Determination of residues of the new acaricide acetazodimefon and pyrimilate in strawberry. *Journal of Zhejiang Agricultural Sciences*, **64**, 1781–1784. (in Chinese)

Liu H C, Jiang M M, Li Q W. 2020. Determination of neonicotinoid sulfoxaflor residues and stereoselective degradation in Pu-erh tea and Black tea by liquid chromatography-high-resolution mass spectrometry. *Journal of Food Processing and Preservation*, **44**, e14589.

Liu H C, Tian H, Jiang M M, Lin T, Li J, Huang X Z. 2022. Enantioselectivity and residue analysis of cycloxaprid and its metabolite in the pile and fermentation processing of Puer tea by ultraperformance liquid chromatography-high-resolution mass spectrometry. *Food Science & Nutrition*, **10**, 3043–3051.

Luo C, Bristow J T. 2023. Composition useful for killing acarids, preventing and controlling animal pests of Acarina, spider mites on stone fruits, citrus, cotton or fruit trees, comprises alkylphenyl sulfide derivative, etoxazole, cyetpyrafen and cyenopyrafen. WO2023065237(A1), 2023-05-14. (in Chinese)

Miao S W, Wei Y, Pan Y, Wang Y F, Wei X L. 2023. Detection methods, migration patterns, and health effects of pesticide residues in tea. *Comprehensive Reviews in Food Science and Food Safety*, **22**, 2945–2976.

Ning Y T, Hu Y, Luo F J, Wang X Y, Zhou L, Han B J, Chen L Z, Zhang X Z. 2025. Residue analysis and risk assessment of cyflumetofen and its three metabolites from fresh tea leaf to tea infusion through purification with new nano adsorbents and determination by ultra-performance liquid chromatography-tandem mass spectrometry. *Microchemical Journal*, **208**, 112470.

Niu Y, Chen X, Yang J, Wu Y, Zhao Z D, Liu X. 2022. Study on the detection of cyetpyrafen in goji berries (*Lycium bararum* L.). *Storage and Process*, **9**, 71–76. (in Chinese)

NY/T 788-2018. 2018. Guideline for the Testing of Pesticide Residues in Crops. Standards of the Ministry of Agriculture and Rural Affairs of the People's Republic of China. China Agriculture Press, China. (in Chinese)

Ouyang J, Tian Y, Jiang C, Yang Q, Wang H, Li Q. 2018. Laboratory assays on the effects of a novel acaricide, SYP-9625 on *Tetranychus cinnabarinus* (Boisduval) and its natural enemy, *Neoseiulus californicus* (McGregor). *PLoS ONE*, **13**, e199269.

Paramasivam M, Deepa M, Selvi C, Chandrasekaran S. 2017. Dissipation kinetics of beta-cyfluthrin and imidacloprid in tea and their transfer from processed tea to infusion. *Ecotoxicology and Environmental Safety*, **144**, 531–536.

Roy S. 2019. Detection and biochemical characterization of acaricide resistance in field populations of tea red spider mite, *Oligonychus coffeae* (Acari: Tetranychidae), in Assam tea plantation of India. *International Journal of Acarology*, **45**, 470–476.

SANTE/11312/2021.Version 2. Implemented by 01.01.2024. Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. [2024-12-10]. https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE-11312_2021-V2.pdf

Schmidt-Jeffris R, Snipes Z, Bergeron P. 2021. Acaricide efficacy and resistance in South Carolina tomato populations of two-spotted spider mite. *The Florida Entomologist*, **104**, 1–8.

Shang S Q, Chang Y, Li W Z, Qing W C, Cheng N P. 2022. Effects of B-azolemiteacrylic on life-history traits and demographic parameters of two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). *Experimental and Applied Acarology*, **86**, 61–71.

Shourove J H, Meem F C, Chowdhury R S, Eti S A, Samaddar M. 2024. Biocontrol agents and their potential use as nano biopesticides to control the tea red spider mite (*Oligonychus coffeae*): A comprehensive review. *Heliyon*, **10**, e34605.

Song Y Q, Feng C, Liu S W, Zhang J L, Li B. 2017. Bioactivity and application research of the new acaricide SYP-9625. *Agrochemicals*, **56**, 628–631. (in Chinese)

Sood C, Jaggi S, Kumar V, Ravindranath S D, Shanker A. 2004. How manufacturing processes affect the level of pesticide residues in tea. *Journal of the Science of Food and Agriculture*, **84**, 2123–2127.

Sun J Y, Li C J, Jiang J Q, Song C G, Wang C, Feng K Y, Wei P, He L. 2022. Cross resistance, inheritance and fitness advantage of cyetpyrafen resistance in two-spotted spider mite, *Tetranychus urticae*. *Pesticide Biochemistry and Physiology*, **183**, 105062.

Tian T, Zhang Y J, Wang S. 2022. Screening of highly effective pesticides against *Polyphagotarsonemus latus*. *China Vegetables*, **6**, 84–88. (in Chinese)

Wang S J, Li R N, Dong F S, Zheng Y Q, Li Y B. 2023. Determination of a novel pesticide cyetpyrafen and its two main metabolites in crops, soils and water. *Food Chemistry*, **400**, 134049.

Zhang X Z, Cui X, Wang X R, Zhong Q, Zhou L, Luo F J, Wang F, Wu L C, Chen Z M. 2021. Residue dissipation, transfer and safety evaluation of picoxystrobin during tea growing and brewing. *Journal of the Science of Food and Agriculture*, **101**, 194–204.

Zhang X Z, Luo F J, Lou Z Y, Lu M L, Chen Z M. 2014. Simultaneous and enantioselective determination of *cis*-epoxiconazole and indoxacarb residues in various teas, tea infusion and soil samples by chiral high performance liquid chromatography coupled with tandem quadrupole-time-of-flight mass spectrometry. *Journal of Chromatography A*, **1359**, 212–223

Zhao S, Zhao Q Y, Dai X Y, Lv B, Wang R J, Yin Z J, Zhang F, Liu Y, Su L, Chen H, Zheng L, Li H M, Xie L X, Zhai Y F. 2023. Control of two-spotted spider mite, *Tetranychus urticae*, on strawberry by integrating with cyetpyrafen and *Phytoseiulus persimilis*. *CABI Agriculture and Bioscience*, **4**, 54.

Zhong Q, Li H X, Wang M, Luo F J, Wang X R, Yan H F, Cang T, Zhou L, Chen Z M, Zhang X Z. 2022. Enantioselectivity of indoxacarb during the growing, processing, and brewing of tea: Degradation, metabolites, and toxicities. *Science of the Total Environment*, **823**, 153763.

Zhou Y, Cao S R, Xi C X, Li X L, Zhang L, Wang G M, Chen Z Q. 2020. A three-dimension magnetic bio-char composite-based quick, easy, cheap, effective, rugged and safe method for multi-pesticides analysis of vegetables. *Journal of Chromatography A*, **1615**, 460770.

Executive Editor-in-Chief Qiang Wang
Managing Editor Lingyun Weng