

PESTICIDE RESIDUE ANALYSIS IN MARKETED GREEN CHILI'S: A COMPARATIVE STUDY WITH ORGANICALLY GROWN GREEN CHILIES

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ABSTRACT

The presence of pesticide residues in vegetables raises significant public health concerns, especially with rising demand for food safety. This study focuses on analysing pesticide residues in marketed green chilies, with a comparison to organically grown varieties purchased from various e-commerce platforms. We employed the QuEChERS extraction method to isolate pesticide residues, ensuring a reliable and efficient sample preparation process. For precise detection and quantification, we used advanced Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) techniques, which allowed for a comprehensive analysis of both polar and nonpolar pesticide residues. Our findings reveal a notable presence of pesticide residues in the conventionally grown green chilies available in local markets. Surprisingly, some pesticide residues were also detected in samples marketed as organically grown chilies, raising concerns about the authenticity and purity of organic labelling standards. The data suggest potential contamination or inadequate control measures in the organic supply chain, especially when products are sourced from e-commerce platforms. This comparative analysis underscores the need for stringent regulatory standards and improved monitoring mechanisms for both conventional and organic produce. Our study contributes valuable insights to consumers, regulatory

bodies, and policymakers, highlighting the importance of transparent food safety practices in reducing potential pesticide exposure from both conventional and organically marketed green chilies.

Keywords: *Chromatography, GC-MS/MS, Green chili, LC-MS/MS, Pesticides, QuEChERS, Spectrometry, Vegetables.*

Introduction

Green chili (*Capsicum annuum*) is a crop of economic and nutritional significance. Its cultivation supports the livelihoods of millions of farmers worldwide, particularly in tropical and subtropical regions (Olatunji et al., 2018). Green chili is valued not only for its distinctive taste but also for its health benefits. One of the main suppliers of micronutrients, such as vitamins and minerals, which are required for human development and growth, are vegetables that are freshly picked (Godswill et al., 2020). We can get the Recommended Dietary Intake (RDI) of these vital nutrients by consist of vegetables in our daily meals (Olson et al., 1987). A major earnings product that is widely cultivated across India is chillis. According to the 2nd advance estimates, India's chili production estimate was 20.60 lakh tons for 2022-23 as against 18.36 lakh tons in 2021-22. Along with contributing to its gastronomic value, chili offers significant potential for therapeutic uses due to its abundance of beneficial phytochemicals, such as vitamins, minerals, flavonoids, carotenoids, and capsaicin (Duranova et al., 2022). These compounds also give chili analgesic, antioxidant, antibacterial, anti-cancer, and anti-obesity properties (Batiha et al., 2020).

Pesticides are chemical substances used to prevent, destroy, or control pests that harm crops (Yadav et al., 2017). They play a crucial role in modern agriculture by reducing losses caused by insects, fungi, and weeds, thereby increasing productivity (Manosathiyadevan et al., 2017). However, the indiscriminate use of pesticides raises concerns about food safety and environmental sustainability (Abrol et al., 2014). Pesticide residues can persist on fruits and vegetables, entering the human body through consumption and causing various health problems (Syed et al., 2014), ranging from acute

poisoning to chronic illnesses like cancer and neurological disorders.

Chili is often cultivated with neonicotinoid pesticides, carbamate insecticides, and organophosphorus herbicides (GURU et al., 2018). In 2020, India used over 61,000 tons of pesticides for agricultural purposes. This is relatively low compared to other countries, such as China, Argentina, and Brazil (Sharma et al., 2022). While pesticide is used correctly, they provide enough defense against herbicides, diseases, and insects. On the other hand, improper application could leave residues on food and agricultural commodities. For both humans and animals that consume these products, such residue may have significant chronic and long-term adverse effects (Boobis et al., 2017). Highly hazardous pesticides (HHPs) may cause harmful impacts on the liver, kidneys, blood, lungs, immune system, and digestive system after short-term exposure (Cestonaro et al., 2022). Pesticides containing dithiocarbamates and its metabolites (ethylene thiourea, carbon disulfide) may trigger malignancy, Parkinson's disease, hypersensitivity, allergic dermatitis, brain damage, reduced brain enzyme activity, and lymphocyte sensitivity (Cocco et al., 2022). HPPs promote cancer, especially in young children who continue to grow, and pregnant or nursing women, infants and children, individuals with weak immune systems, and people who suffer from malnutrition can suffer more severe health effects from these insecticides (Food and Agriculture Organization (FAO) & World Health Organization (WHO), 2016).

Most of the consumer exposure to pesticides comes through residues on food items that are consumed. High-risk pesticides are banned, and Maximum Residue Limits (MRL) have been determined for low-risk pesticide to protect people from the harmful effects of these (Carrère et al., 2018).

1. Materials & Methods

1.1 Location and Sampling

In this study, pesticide residues in green chili samples collected from local markets of Kolkata, India (Table 1) and the Organically grown green chili samples were purchased

from various E-commers platforms. Almost 500g of green chili samples was labelled, put into different bags, and transferred in packages to the Regional Food Laboratory (National Food Laboratory), where pesticide analyses were performed out the following day.

Table 1. Collected marketed chili samples and their location

Sample No.	Market Locations of collected Chili samples
JKP_1	Behala, south kolkata
JKP_2	Kalighat, south kolkata
JKP_3	New Town, North kolkata
JKP_4	Thakurpukur, south kolkata
JKP_5	Shyambazar, Kolkata
JKP_6	Garia, south kolkata
JKP_7	Bansdroni, kolkata
JKP_8	Baranagar, North kolkata

The samples of organically grown chilies **JKP_9, JKP_10, JKP_11, and JKP_12** were purchased from multiple E-commerce platforms.

1.2 Reagent and Chemicals

All pesticides standards would be obtained from authentic and trusted sources like Agilent, Merck, that can provide a certificate of analysis as proof of traceability. Methanol- LCMS grade, Water- Milli Q, Acetonitrile- LCMS grade, Anhydro sodium Sulfate- AR grade, Anhydro sodium Sulfate- AR grade, Acetic acid, glacial- LCMS grade, Ammonium-formate LCMS grade, Primary secondary amine (PSA) & Graphitized Carbon Blank (GCB) from United Chemical Technologies or Equivalent, Agilent Sample QuEChERS EN extraction kit.

1.3 Preparation of Mobile Phase

Prepare 5mM Ammonium formate in water by dissolving 0.317 g ammonium formate in 1000 mL HPLC water. Adjust with 0.1% formic acid (Phase A). Prepare 5mM ammonium formate in methanol by dissolving 0.317 g ammonium formate in 1000 mL methanol. Adjust with 0.1% formic acid (Phase B).

1.4 Preparation of Sample

To make sure that no contamination happened throughout processing, collect and examine examples free of pesticides. Before reporting, reduce any found pesticide levels from the values obtained. To ensure the accuracy of the method of extraction, use a blank sample that has been treated with using known pesticide amounts. For batch recovery identification, prepare a single QC sample for each batch. About 250 g green chili sample homogenized with blender. Weigh 10.00 $\pm$ 0.10 g of homogenized sample into a 50 mL polypropylene centrifuge tube. Incubate for ten minutes at 4°C after introducing 10 mL of acidified acetonitrile (0.1% acetic acid) and vortex for 1 minute. Upon pouring the QuEChERS EN extraction salt (6.5 g Magnesium Sulfate + 1 g Sodium Acetate) and vortexing for one minute, refrigerate for ten minutes at 4°C (Lawa et al., 2018). Centrifuge at approximately 6500 rpm for 10 minutes. Transfer 2 mL of extract to a clean tube containing 50 mg PSA and 150 mg MgSO₄ (Ramalepe et al., 2022). Vortex for 30 seconds and centrifuge at 10,000 rpm for 10 minutes at 4°C. For LC-MS/MS, filter the supernatant through a 0.2 μ m PTFE syringe filter and for GC-MS/MS, filter 1000 μ L extract through a 0.2 μ m PTFE syringe filter. Inject 10 μ L from the extract into the LC-MS/MS using SOP for the respective LC-MS/MS system.

1.5 Preparation of Standards

Apply pesticide standards from trusted suppliers with appropriate credentials, (such as Sigma-Aldrich, Agilent, and Merck.). Dilute about 10 mg of pesticide in 10 mL of diluent (ethyl acetate for GC-MS/MS; acetonitrile for LC-MS/MS) for Stock Solutions. Keep refrigerate at -20 °C. A reference standard mixture with a minimum of five different concentrations (such as 0.002–0.1 μ g/mL) that is appropriate

with the mobile phase needs to be prepared. To make sure that the instrument is acceptable, inject a mid-range standard every day. Each day, new standards need to be introduced.

2.5.1 Matrix-Matched Standards for LC-MS/MS

Spike pesticide standards into residue-free sample extracts to match the calibration range, e.g., 0.001–0.02 µg/mL.

2.5.2 External Calibration Standards for GC-MS/MS

Prepare a working range (e.g., 0.005–0.1 µg/mL) in ethyl acetate. Use at least five levels of concentration and prepare freshly before use.

2.5.3 Matrix-Matched Standards for GC-MS/MS

Spike standards post-extraction into residue-free sample extracts to create calibration standards (e.g., 0.005–0.1 µg/mL).

1.6 Chromatographic Condition

Samples were analysed using LC-MS/MS and GC-MS/MS devices. LC-MS/MS analyses were performed using liquid chromatography (PerkinElmer LX-50 UHPLC) coupled with mass spectrometry. The column used was Kinetex C18 (2.6 µm, 100 Å, 150 × 4.6 mm). The mobile phase flow rate was 0.5 mL/min (Table 2). The column temperature was maintained at 45°C, and the injection volume was 10 µL. The ionization mode was ESI (positive) with a drying gas flow of 120. The HSID temperature was 300°C, and the nebulizer gas ranged from 1–300. The electro spray voltage (V1 positive) was set at 5500 V, and the source temperature was 320°C.

GC-MS/MS analyses were performed using gas chromatography (Agilent 7000D GC/MS Triple Quadrupole) coupled with mass spectrometry (7000D GC/MS Triple Quadrupole). The autosampler used was the Agilent 7693A. The column used was a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The injection type was MMI-SSPL with an injection volume of 2 µL. The injection temperature was maintained at 280°C (Table 3), and the column flow rate was set at 1.2 mL/min. The ion source and transfer line temperatures were both maintained at 280°C. The LC-MS/MS and GC-MS/

MS instruments have been employed for analysing a number pesticide forms to determine their LOQ Value (Table 4).

Table 2.The Gradient Mobile phase flow program

Time	Flow	%A	%B	Shape
0	0.5	80	20	Linear
Time	Flow	%A	%B	Shape
0.5	0.5	80	20	Linear
1	0.5	50	50	Linear
4	0.5	40	60	Linear
15	0.5	10	90	Linear
17	0.5	2	98	Linear
20	0.5	80	20	Linear
22	0.5	80	20	Linear

Table 3. Oven temperature program

Rate(°C/min)	Target(°C)	H o l d time(min)	Runtime(min)
Initial	90	1	1
35	130	0	2.14
10	240	1	14.14
15	300	3	21.14
Totalruntime	21.14		

Table 4.The LOQ readings of the analytes undergoing analysis

LOQ (mg/ Kg)	Analytes
5	Carbendazim
0.1	Acetamiprid

0.01	Methomyl, Dinotefuran, Carbofuran, Paclobutrazole, Tridemorph, Phosphamidon, Imazamox, Buprofezin, Fenazaquin, Fenamidone, ButachlorHexaconazole, Kresoxim-Methyl, Flusilazole, Cyazofamid, Pencycuron, Epoxiconazole, Fenarimol, Bitertanol, Propiconazole, Boscalid, Chlorothalonil, Dicofol, Hexythiazox, Thiobencarb, Etoxazole, Flufenacet, Picoxystrobin, Tetraconazole, Spirotetramat, Quinalofop-P-EthylFluxapyroxad, Tolfenpyrad, Pyraclostrobin, Dimethomorph, Famoxadone, Chromafenozide, Pinoxaden, Sulfentrazone, Azoxystrobin, Difenconazole, Metrafenone, FenpyroximatePropquizafop, Pyridalyl, Novaluron, Metaflumizone, Lufenuron, Chlorfluazuron, Spinosyn A, Spinosyn D, Emamectin Benzoate, Fipronil, Flubendiamide
0.5	Chlorpyrifos
0.05	DDT-o,p; DDE-p,p
0.01	Ethofenprox, Lambda Cyhalothrin, Atrazine, Endosulfan, Chlorpropham,

2. Result & Discussion

By measuring multiple variables such as linearity, limit of quantification (LOQ), accuracy (in terms of recovery), and precision (in terms of repetition), the recommended method was evaluated. Every validating parameter that had been evaluated conformed with international guidelines for chromatographic analysis of pesticide residues (Colazzo et al., 2018). Resultant outcomes (Table 5 and Table 6) have been assessed utilizing the European SANTE Guideline's validation guidelines and standards (SANTE, 2019).

Table 5. The result data of LC-MS/MS instrument

Sample Name	Detectable Analytes name	Detectable conc. (ppm)	M R L (ppm) As per FSSAI	Remarks
JKP_1	Acetamidrid	0.02	0.01	> limit
	Imidacloprid	0.02	0.01	> limit

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	Metalaxyl	0.1	0.01	> limit
JKP_3	Imidacloprid	0.01	0.01	= limit
	Metalaxyl	0.1	0.01	> limit
	Carbendazim*	0.01	0.01	= limit
	Fipronil	0.01	0.01	= limit
JKP_4	Dinotefuran	0.01	0.01	= limit
	Acetamiprid	0.01	0.01	= limit
	Imidacloprid	0.02	0.1	> limit
	Tolfenopyrad	0.01	0.01	= limit
	Azoxystrobin	0.01	0.01	= limit
	Difenoconazole	0.01	0.01	= limit
JKP_5	Myclobutanil	0.02	0.01	> limit
	Hexythiazox	0.02	0.01	> limit
	Lufenuron	0.01	0.01	= limit
JKP_6				
	Dinotefuran	0.10	0.01	> limit
	Imidacloprid	0.2	0.01	> limit
	Metalaxyl	0.1	0.01	> limit
	Myclobutanil	0.1	0.01	> limit
	Hexythiazox	0.01	0.01	= limit
	Fipronil	0.04	0.01	> limit
JKP_7	Dinotefuran	0.01	0.01	= limit
	Imidacloprid	0.01	0.01	= limit
	Myclobutanil	0.01	0.01	= limit
JKP_8	Imidacloprid	0.01	0.01	= limit
JKP_9	Imidacloprid	0.04	0.01	> limit
	Propiconazole	0.02	0.01	> limit
	Picoxystrobin	0.01	0.01	= limit
	Tolfenpyrad	0.05	0.01	> limit
	Pyraclostrobin	0.05	0.01	> limit
	Difenoconazole	0.01	0.01	= limit
	Novaluron	0.1	0.01	> limit

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	Fipronil	0.01	0.01	= limit
JKP_10	Imidacloprid	0.08	0.01	> limit
	Metalaxyl	0.07	0.01	> limit
	Fenazaquin	0.03	0.01	> limit
	Hexythiazox	0.02	0.01	> limit
	Picoxystrobin	0.02	0.01	> limit
	Fluxapyroxad	0.01	0.01	= limit
	Tolfenpyrad	0.1	0.01	> limit
	Pyraclostrobin	0.02	0.01	> limit
	Azoxystrobin	0.1	0.01	> limit
	Difenconazole	0.2	0.01	> limit
	Fipronil	0.2	0.01	> limit
JKP_11				
	Carbofuran**	0.04	0.01	> limit
	Imidacloprid	0.2	0.01	> limit
	Metalaxyl	0.02	0.01	> limit
	Picoxystrobin	0.02	0.01	> limit
	Fluxapyroxad	0.01	0.01	= limit
	Tolfenpyrad	0.02	0.01	> limit
	Pyraclostrobin	0.02	0.01	> limit
	Azoxystrobin	0.02	0.01	> limit
	Difenconazole	0.1	0.01	> limit
	Fipronil	0.2	0.01	> limit
JKP_12	Dinotefuran	0.1	0.01	> limit
	Imidacloprid	0.02	0.01	> limit
	Metalaxyl	0.01	0.01	= limit
	Fluxapyroxad	0.1	0.01	> limit
	Tolfenpyrad	0.5	0.01	> limit
	Picoxystrobin	0.3	0.1	> limit
	Azoxystrobin	0.02	0.01	> limit
	Difenconazole	0.1	0.01	> limit
	Fipronil	0.03	0.01	> limit

*As per 2.3.1. Restriction on the use of insecticides, Food Safety and Standards (Contaminants, Toxins and Residues) Regulation

**Marked pesticides are banned notification No. 2-15015/30/2010 dated the 1st August, 2011.

Table 6. The result data of GC-MS/MS instrument

Sl No.	Detectable Analytes name	Detectable conc. (ppm)	MRL (ppm) As per FSSAI	Remarks
JKP_1	*Lamda Cyhalothrin	0.005	0.01	< Limit
JKP_2	Ethofenprox	0.4	0.01	> Limit
JKP_5	**DDT-o,p'	0.2	0.05	> limit
	Chlorpyrifos	0.01	0.5	< limit
	*Lamda Cyhalothrin	0.04	0.01	> limit
	Atrazine	0.01	0.01	= limit
JKP_10	*Lamda Cyhalothrin	0.01	0.01	= limit
	Endosulfan I	0.02	0.01	> limit
	**DDE-p,p	0.02	0.05	< limit
	Chlorpyrifos	0.01	0.5	< limit
	*Lamda Cyhalothrin	0.01	0.01	= limit
JKP_11				
	Chlorpyrifos	0.01	0.5	< limit
	**DDE-p,p	0.02	0.05	< limit
	*Lamda Cyhalothrin	0.02	0.01	> limit
	Chlorpropham	0.01	0.01	= limit
JKP_12	**DDE-p,p	0.01	0.05	< limit
	*Lamda Cyhalothrin	0.02	0.01	< limit

*As per 2.3.1. Restriction on the use of insecticides, Food Safety and Standards (Contaminants, Toxins and Residues) Regulation

**Marked pesticides are banned notification No. 2-15015/30/2010 dated the 1st August, 2011.

The results of this study indicate that specific pesticide residues, especially in samples JKP_1, JKP_2, JKP_6, JKP_9, JKP_10, and JKP_11, are above the Maximum Residue Limit (MRL) specified by the FSSAI. Concerns regarding safety have been caused by the identification of several banned chemicals, such as carbendazim and chemicals linked to DDT. When various contaminants are found in one sample, it could suggest that there are contamination concerns. To ensure conformity to food safety laws, more research and corrections might be needed.

This study highlights a critical public health concern regarding the safety of both conventionally marketed and “organically grown” green chillis available on various e-commerce platforms. Contrary to consumer expectations, significant levels of pesticide residues were detected in both categories of chillis. This finding raises questions about the authenticity of “organic” labels and the effectiveness of current monitoring and regulatory frameworks. This study underscores the urgent need for stricter enforcement of pesticide residue limits under the Food Safety and Standards Authority of India (FSSAI) regulations.

Conclusion

The FSSAI, along with the Ministry of Health and Family Welfare, plays a vital role in ensuring food safety and protecting consumer health. Enhanced surveillance, periodic testing of both conventional and organic food products, and stricter certification processes for organic produce are essential measures. Consumer awareness and transparency in the food supply chain must also be prioritized. The findings of this study call for collaborative action from regulatory authorities, policymakers, food producers, and e-commerce platforms to ensure the integrity of food labelling claims and the safety of food consumed by the public.

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