Food Safety Assessment and Residue Burden Profiling of Pesticides, Aflatoxins, and Ochratoxin-A in Three Commercial Chilli Varieties

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ABSTRACT

Chilli is among the most popularly consumed and commercially exported spices from India; nevertheless, food safety investigations involving the concurrent estimation of pesticide residues, aflatoxins, and ochratoxin-A in different chilli commercial cultivars have been poorly studied. As such, this study sought to determine the food safety implications and residue burden of pesticides, aflatoxins, and ochratoxin-A in three chilli commercial cultivars, including Teja, Byadgi, and Guntur Sannam. Multi-residue determination for pesticides was done using both LC-MS and GC-MS, whereas mycotoxins were estimated using an HPLC-FLD method after immunoaffinity column clean-up. A total of 28 pesticide residues and 4 mycotoxin parameters were studied in a residue burden model and Kruskal-Wallis statistics. Complete detection of all pesticides and mycotoxins was observed in all varieties, suggesting extensive chemical and fungal contamination of chilli. Among the cultivars, Teja had the highest total pesticide burden of 3.6834 mg/kg and mean pesticide content of 0.1316 mg/kg, mainly attributed to acetamiprid, prochloraz, imidacloprid, thiamethoxam, and clothianidin. Conversely, Guntur Sannam demonstrated a high mycotoxin burden of 32.3801 µg/kg with a mean of 8.0950 µg/kg, signifying a serious aflatoxin and ochratoxin-A contamination problem. However, the Byadgi cultivar showed relatively low pesticide burden of 1.9521 mg/kg and a mycotoxin burden of 5.4957 µg/kg. In total, nine pesticide residues and three mycotoxins were significantly associated across varieties ($p<0.05$) via Kruskal-Wallis analysis.

Keywords: Chilli varieties; Pesticide residues; Aflatoxins; Ochratoxin-A; Food safety; Residue burden

INTRODUCTION

Ensuring food safety has emerged as a major public health concern in both India and internationally, especially for staple spices, which are consumed in small quantities daily by all households. Chilli (Capsicum annuum L.) is a spice used ubiquitously in Indian cuisine as well as an important export commodity for India. It is therefore not surprising that India accounts for the highest quantity of dried red chilli exported from any country worldwide; however, it is also noted that the same dataset highlights instances of border rejections and safety alerts due to chemical contamination in chilli [1, 2]. Two categories of food safety hazards dominate the risk assessment of chilli. The first category involves the presence of synthetic pesticide residues in chilli owing to their application in the process of agricultural cultivation. Chlorpyrifos, acetamiprid, imidacloprid, thiamethoxam, hexaconazole, and prochloraz are pesticides identified as having caused toxicity via acute cholinergic effects, endocrine disruption, developmental neurotoxicity among children, and probably causing cancer in human subjects [3]. Therefore, the JMPR and EFSA committees have established ADI and ARfD values for each compound, which inform the MRL set by Codex Alimentarius, EU Regulation (EC) 396/2005, and FSSAI Pesticide Residue Regulations [4]. The second category includes secondary metabolites of fungi present when chilli is exposed to Aspergillus flavus, A. parasiticus, A. ochraceus, and A. niger during its drying or storage process. Among these metabolites, aflatoxin B1 causes hepatocellular carcinoma in human subjects, making it a Group 1 human carcinogen as per IARC classification, while ochratoxin-A is considered Group 2B (possibly carcinogenic to humans) associated with nephrotoxicity and Balkan endemic nephropathy [5]. The food-safety burden imposed by these hazards is regulated through tightly bound numerical thresholds.

Commission Regulation (EU) 2023/915 sets the maximum level of Aflatoxin B1 in chilli powder at 5 µg kg^{-1}, Total Aflatoxins (B1 + B2 + G1 + G2) at 10 µg kg^{-1}, and Ochratoxin-A at 15 µg kg^{-1}; FSSAI prescribes parallel limits domestically, while Codex CXS 193-1995 (General Standard for Contaminants and Toxins) provides the global reference [6, 7]. Pesticide MRLs in chillies are typically set in the 0.01-0.50 mg kg^{-1} range depending on the active substance and on whether the importing market follows Codex, EU, or domestic Indian benchmarks. Because chilli is consumed in concentrated dried form, even sub-MRL contamination can translate to a non-negligible Estimated Daily Intake (EDI) for high-consumption population groups, requiring food-safety assessment to move beyond a simple compliance check into the territory of cumulative-exposure modelling [8].

Identification of these hazards at trace levels necessitates the employment of confirming chromatographic techniques. LC-MS with electrospray ionization technique in positive mode with multiple-reaction monitoring and GC-MS electron impact technique with selected ion monitoring mode will serve as the basis for multi-residue pesticide screening, while HPLC-FLD with the aid of immunoaffinity column clean-up is the standard validated technique for detecting aflatoxins and ochratoxin-A in accordance with DIN EN 14132 [9]. However, it must be noted that raw numbers from the chromatographic analysis are insufficient in addressing the issue of consumer safety. There is a need for a scientific and statistical basis for converting the analytical results into consumer safety information by aggregating data from compounds into a variety of residue burdens and testing if such an apparent difference is statistically significant. Thus, the purpose of this study is explicitly stated to assess food safety through the following objectives:

1. Quantification Of the Pesticide Residues and Mycotoxins Present in Teja, Byadgi, And Guntur Sannam Varieties of Chilli Using Internationally Approved Techniques Such As LC-MS, GC-MS, And HPLC-FLD;

2. Determination Of the Variety Level Residue Burden Score for Prioritizing Public Health Issues; And

3. Identification Of Specific Hazards Responsible for the Difference in Food Safety Through a Non-Parametric Kruskal-Wallis Comparison Test.

LITERATURE REVIEW

Several scientific studies have been conducted on the potential issues of food safety related to mycotoxin or pesticide contamination of chilli, pepper, and similar types of spice foods. The study by Reddy et al. [10] offered a wide-ranging review on mycotoxin contaminations of various food types and discussed their significance from the point of view of health risk to humans. The authors also focused specifically on aflatoxins and ochratoxin-A as important mycotoxins that may occur during improper drying, inadequate storage, and colonization by fungi. Such information is highly relevant for the subject area of chilli safety due to the sensitivity of dried spices to fungi in case of moisture and excessive humidity. As far as residual pesticides are concerned, a study by Jang et al. [11] investigated pesticides in dried chilli peppers and powders and used LC-MS/MS methods. Lee et al. [12] also examined more than 207 pesticides in hot pepper powder samples through the use of liquid and gas chromatography mass spectrometry methods.

The analyses of mycotoxins, specifically aflatoxins and ochratoxin-A, in chilli powder by ultra-high-performance liquid chromatography with fluorescence detection and tandem mass spectrometry were performed by Dhanshetty et al. [13]. It was shown that the application of advanced chromatographic techniques for the screening of various mycotoxins was essential. Moreover, the presence of aflatoxins and ochratoxin-A in spices was investigated by Zareshahrabadi et al. [14] employing high-performance liquid chromatography. These works confirm the ability of spices to act as significant carriers of fungal toxins. Pickova et al. [15] described the natural occurrence of ochratoxin-A in marketed spices and underlined the necessity of regular monitoring of OTA in stored and commercialized spice products. It becomes evident that mycotoxin contamination of spices and chillies is an important food safety problem that should be carefully studied. Proper extraction and cleanup procedures are crucial for residue analysis since chilli powder belongs to a complex mixture containing pigments, oils, capsaicinoids, and many other substances. Anastassiades et al. [16] described the concept of QuEChERS based on extraction, partitioning, and dispersive solid-phase extraction with acetonitrile for pesticide analysis in produce. The developed technique was recognized later due to its simplicity, speed, low consumption of solvents,

and applicability to multiple pesticide residue analyses. Lehotay [17] verified the efficacy of acetonitrile extraction, partitioning, and the use of magnesium sulfate after a collaborative study. Therefore, the use of the QuEChERS technique for residue analysis in chillies and other spices can be justified. Validation and quality assurance are also necessary in any food-safety study. The European Commission's SANTE/11312/2021 [18] document gives guidance on analytical quality control and method validation for pesticide residue analysis in food and feed. Such information includes parameters such as recovery, precision, limit of quantification, matrix effects, and calibration performance. In turn, validation and quality assurance are important to interpret any data on chromatographic residues, especially when these are used for regulatory interpretation and consumer risk assessment. Shim et al. [19] have considered pesticide residues in peppers and underlined the importance of residue monitoring, dietary intake, and maximum residue level compliance. It is reasonable to refer to Regulation (EC) No. 396/2005 [20], which establishes maximum residue levels of pesticides in food and feed of both plant and animal origin. It can be observed from the literature review above that residues of pesticides and mycotoxins constitute the two main safety issues in the case of chilli and spices. Literature has been reported earlier on pesticide analysis, quantitation of mycotoxins, extraction method validation, and residue regulation. Yet very few studies have made a comparative evaluation of pesticide residues along with aflatoxins and ochratoxin-A in the case of some commercially valuable Indian varieties of chilli. It is for this reason that the current study makes an attempt in this regard.

MATERIALS AND METHODS

Sample Collection and Preparation

Three commercial chilli varieties, Teja, Byadgi, and Guntur Sannam, were obtained directly from the certified wholesale markets for spices in Andhra Pradesh and Karnataka in accordance with the principles of representative sampling as outlined by EC No 401/2006 guidelines for sampling mycotoxins in food products. The samples were reduced to fine powder form (particle size ≤ 500 μm) by using a stainless-steel grinding machine in the laboratory and were preserved in air-tight, dark-colored amber glass containers at 4 °C until analysis to avoid oxidation and degradation.

Preparation of Reagents

Phosphate-Buffered Saline (PBS, pH 7.4)

A solution of 0.2 g KCl, 0.2 g KH2PO4, 1.16 g disodium hydrogen phosphate, and 8.0 g NaCl was made in about 900 mL of deionized water. The pH was set at 7.4 ± 0.05 by adding either 0.1 M HCl or 0.1 M NaOH, and then diluted to 1000 mL with deionized water. PBS acts as the conditioning buffer for immunoaffinity columns for the mycotoxin clean-up.

Tween-20 Solution (8%)

Tween-20 (8 mL) was added to the PBS buffer, followed by bringing its concentration to 100 mL using PBS. Tween-20 is a surface-active agent that reduces the non-specific binding of the analyte in IAC, making the extraction easier from the complex matrix of the chilli sample.

Methanol-Water Extractant (80: 20, v/v)

Methanol (800 mL) was added to a 1000 mL volumetric flask, and the volume was made up to the mark with deionized water. This polar extractant solubilizes mycotoxins from the chilli matrix while maintaining compatibility with downstream IAC clean-up.

HPLC Mobile Phases

Mobile Phase A (1% acetic acid in water) was prepared by adding 10 mL of glacial acetic acid to 990 mL of HPLC-grade water. Mobile Phase B was 100% acetonitrile (HPLC grade). Both eluents were sonicated for 15 min and filtered separately through 0.22 μm membrane filters before use [9, 21].

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Instrument Conditions

HPLC-FLD Conditions for Aflatoxins and Ochratoxin-A

Parameter	Setting
Instrument	Agilent 1260 Infinity II HPLC system
Column	Agilent Eclipse Plus C18, 5.0 µm, 4.6 × 250 mm
Column oven temperature	36 °C
Mobile Phase A	Methanol : Water (1 : 2)
Mobile Phase B	Acetonitrile (100%)
Elution mode	Isocratic - A: 85.7%, B: 14.3%
Flow rate	1.00 mL min^{-1}
Injection volume	100 µL
Run time	20 min
FLD detector	Excitation 365 nm, Emission 450 nm

Table A. HPLC-FLD operating parameters for mycotoxin determination.

LC-MS and GC-MS Conditions for Pesticide Multi-residue Analysis

Pesticide residues were detected by LC-MS (ESI in positive mode, MRM mode) for the detection of polar and heat-sensitive compounds and by GC-MS (E.I., SIM mode) for non-polar, volatile pesticides, such as organochlorines, and pyrethroid groups.

The detection limits were calculated as the ratio between the measured concentration and the limit of quantitation (LOQ), where the LOQ was 0.01 mg kg^{-1} well under the average level of Codex/EU MRL values in chilli in compliance with ISO/IEC 17025:2017 [22] and EC No 882/2004 [23].

Sample Preparation Procedure for Mycotoxin Analysis

The mycotoxin extraction process was modified after DIN EN 14132 [9] to suit the dilutions needed in this case of high pigment content in chilli matrix. This is how the process was done:

- Five grams (± 0.1) of pulverized chilli powder was accurately weighed and poured into a polypropylene 50 mL centrifuge tube.
- Chilli powder in the centrifuge tube was treated with about 1.25 g of sodium chloride, 25 mL of the extractant methanol: water (80: 20), and 10 mL of n-hexane.
- Homogenization of the mixture at high speed for 3 minutes was done using an Ultra-Turrax homogenizer.
- The tube was subjected to centrifugation at 4,000 rpm for 10 minutes. The upper hexane layer (lipophilic interferences) was discarded.
- An immunoaffinity column (IAC) for ochratoxin-A was coupled with a SPE manifold. It was pre-washed with 10 mL PBS buffer, and care was taken to ensure that IAC did not dry.
- Five milliliters of the extracted sample was diluted in 20 mL 8% Tween-20 solution and vigorously shaken for 1 minute. The mixture was centrifuged and loaded onto the conditioned IAC at a controlled flow rate.
- IAC was washed with 10 mL of deionized water, and the IAC was dried under gentle vacuum.
- The analyte on IAC was eluted with 1 mL of methanol and subsequently with 1 mL of Milli-Q water.
- The eluted fraction was vortex-mixed and dispensed into HPLC vials.

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Statistical Analysis Pipeline

The data was in an Excel workbook format and was analyzed using a custom-made pipeline in Python version 3.11. The code begins by importing the necessary libraries (NumPy, Pandas, SciPy, and Matplotlib), reading the pre-processed Excel sheets containing the pesticides and mycotoxin residues data, replacing the non-standardized names of the columns (%RSD, N Numeric, and BLQ) with RSD_percent, N_numeric, and BLQ_count, respectively, and retaining only those records where the value of the mean concentration was numerical. The following two food safety models were implemented:

Model 1: Residue Burden / Cumulative Exposure Indicator

For each variety, the script creates variety-level food safety metrics by aggregating the compound-level mean concentrations. The total Pesticide Burden is calculated using the sum of the mean concentrations of the detected pesticide compounds in the variety and serves as an additive exposure surrogate metric, reflecting the combined effect of several residues consumed at once in one chilli matrix. The Mean Pesticide Level represents the arithmetic mean of the mentioned compound-level means; the SD of Compound Means provides information about the extent of variation among the concentration means of different compounds, revealing whether the contamination was caused by only several hazards or spread around. Detected_Compounds is the count of compounds for which the N_numeric variable is greater than zero; Pesticide Detection % represents consumer exposure variability across the target hazard spectrum. Similarly, all the metrics are created for the mycotoxins spreadsheet, producing Total_Mycotoxin_Burden, Mean_Mycotoxin_Level, SD_of_Toxin_Means, Detected_Mycotoxins, and Mycotoxin Detection %. Both summary tables are concatenated using the Variety column, creating Table 1, representing the variety-level scorecard useful for the FSSAI hazard-based tiering and export-batch prioritization [18].

Model 2: Kruskal-Wallis Comparative Test

While the Residue Burden Model identifies which variety carries the highest cumulative exposure load, food-safety regulation requires formal evidence that the apparent differences are not artifacts of sampling variability. In this regard, the non-parametric version of one-way ANOVA, i.e., the Kruskal-Wallis H-test [16], is utilized for every compound, taking replicate-level values (R1, R2, R3) directly off the analytical worksheets. Using the get_replicate_values function, replicates for every available variety are extracted per compound, and the test is carried out only in the case where at least two varieties provide two replicates each. An H-statistic value and corresponding p-value are thus calculated for every compound, and using $p < 0.05$ as a criterion, the results are designated Significant or not significant.

RESULTS AND DISCUSSION

This section presents the variety-wise food-safety profile of three commercial chilli varieties, namely Teja, Byadgi, and Guntur Sannam, based on pesticide residue and mycotoxin contamination data. Two different types of analyses are carried out in order to discuss the results: firstly, the Residue Burden Model, which reflects the level of accumulation of pesticides and mycotoxins for each individual variety, and secondly, the Kruskal-Wallis H-test, which is used to confirm the differences between the varieties in terms of residue concentration. The discussion begins with an assessment of the pesticide and mycotoxin residues among the varieties, continues with a definition of the leading pesticides that are sources of contamination, and concludes with a statistical verification of the results and a visualization of the food safety burden.

Variety-wise Food-Safety Burden Profile

Table 1 shows the combined variety-level food-safety scorecard of the Residue Burden Model. Firstly, and most importantly, from a health point of view, all three varieties have 100% detection rate of the pesticide panel and mycotoxin panel targets. Any residue detection, regardless of the amount, is a red flag for regulatory agencies, because consumers do not consume individual residues, but all residues, accumulated from all meals in which the chilli is present. This makes 100% detection rate a low-level generalized scenario of chronic exposure, and

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the precautionary principle and ALARA doctrine call upon food safety agencies to minimize this risk to the best of their ability.

Secondly, the contamination levels vary vastly by variety. Teja demonstrates the greatest Total Pesticide Burden, at 3.6834, followed by Guntur Sannam (2.1894), then Byadgi (1.9521). Similarly, Mean Pesticide Levels are ranked by these same varieties (Teja 0.1316, Guntur Sannam 0.0995, Byadgi 0.0697). From the consumer point of view, this means that households, whose diet regularly includes Teja chilli, will be chronically exposed to nearly double the total pesticide burden than those households eating Byadgi. On the other hand, Guntur Sannam demonstrates a Total Mycotoxin Burden of 32.3801, compared to 5.4957 (Byadgi) and 5.4101 (Teja). Since both Aflatoxin B1 and Ochratoxin-A are classified as Group 1 IARC carcinogens, with no safe lower limit, and Group 2B carcinogens with proven nephrotoxic effects, Guntur Sannam mycotoxins constitute the most serious public health concern in this dataset [13, 14].

Table 1. Variety-wise food-safety burden profile generated by Model 1. Pest. = Pesticides; Myco. = Mycotoxins; SD = Standard Deviation across compound means.

Variety	Total Pest. Burden	Mean Pest. Level	SD (Pest.)	Detected Pest.	Pest. %	Total Myco. Burden	Mean Myco. Level	SD (Myco.)	Detected Myco.	Myco. %
Byadgi	1.9521	0.0697	0.0739	28/28	100.0	5.4957	1.3739	1.3470	4/4	100.0
Guntur Sannam	2.1894	0.0995	0.0736	22/22	100.0	32.3801	8.0950	5.0128	4/4	100.0
Teja	3.6834	0.1316	0.0515	28/28	100.0	5.4101	1.8034	0.9988	3/3	100.0

Top Detected Pesticide Hazards per Variety

The pesticides causing the greatest mean pesticide residue levels in each variety are listed below in Table 2, alongside their corresponding safety issues in terms of food safety. The leading residues in Byadgi are Pyriproxyfen (0.2997 mg kg^{-1}), Azoxystrobin (0.2250 mg kg^{-1}), Pyraclostrobin (0.2110), Chlorantraniliprole (0.1650 mg kg^{-1}), and Chlorfenapyr (0.1350 mg kg^{-1}). Pyriproxyfen and Chlorfenapyr have both been under endocrine disrupting scrutiny by EFSA, whereas Azoxystrobin and Pyraclostrobin are being reviewed for reproductive toxicity as strobilurin fungicides. In Guntur Sannam, the key threats include residues of Flupyradifuron (0.2997 mg kg^{-1}), Fenpyroximate (0.2110 mg kg^{-1}), Chlorpyrifos (0.1903 mg kg^{-1}), Azoxystrobin (0.1867 mg kg^{-1}), and Spirotetramate (0.1867 mg kg^{-1}). The latter pesticide in particular is of serious concern because of its developmental neurotoxicity implications, with Chlorpyrifos' EU approval having been suspended in 2020 due to concerns surrounding developmental neurotoxicity. In addition, the EU Maximum Residue Level for chillies was set at the Limit of Quantification value (LOQ) of 0.01 mg kg^{-1}, which means that Guntur Sannam chilli samples with a mean Chlorpyrifos content of 0.1903 mg kg^{-1} are nearly twenty times the EU MRL - making for an export rejection situation [3, 6].

The top five residues in Teja chillies include Acetamiprid (0.2967 mg kg^{-1}), Prochloraz (0.2267 mg kg^{-1}), Imidacloprid (0.2067 mg kg^{-1}), Thiamethoxam (0.1880 mg kg^{-1}), and Clothianidin (0.1867 mg kg^{-1}). Four out of five of the residues listed here are neonicotinoid insecticides (acetamiprid, imidacloprid, thiamethoxam, and clothianidin), and this family of pesticides has important food-safety relevance because repeated exposure to multiple compounds from the same insecticide class may contribute to cumulative dietary risk, particularly where chilli is consumed regularly in dried or powdered form.

The distinct hazard signatures observed across varieties raise important questions about the underlying causes. Teja's high neonicotinoid burden may reflect the variety's susceptibility to specific pests, such as thrips, aphids, and whiteflies, that are particularly problematic in the major Teja-growing regions of Andhra Pradesh and Telangana, leading farmers to apply multiple neonicotinoid treatments. Guntur Sannam's high mycotoxin burden may be related to the morphological characteristics of the variety, such as thicker flesh, higher moisture content at harvest, or different drying practices in the Guntur region, which may create favorable conditions for Aspergillus growth. Conversely, Byadgi's relatively lower contamination levels for both hazard categories may be due to its cultivation in drier regions of Karnataka, where drier climatic conditions may suppress fungal

growth, or due to differences in pest pressure and crop-protection practices. However, these explanations should be interpreted as hypotheses because the present study did not directly measure pesticide application history, pest incidence, drying conditions, storage humidity, moisture retention, or pericarp thickness. Testing these hypotheses would require integrated studies combining agronomic surveys documenting pesticide application histories, microclimatic monitoring of temperature and humidity during drying and storage, and morphological characterization including moisture retention and pericarp thickness. Such research could inform variety-specific Good Agricultural Practices (GAPs) and post-harvest handling protocols.

Table 2. Top 5 detected pesticide hazards per variety, ranked by mean concentration (mg kg⁻¹).

Variety	Instrument	Compound	Mean	SD	%RSD	Min	Max
Byadgi	LC-MS	Pyriproxyfen	0.2997	0.0133	4.44	0.285	0.311
Byadgi	LC-MS	Azoxystrobin	0.2250	0.0217	9.63	0.202	0.245
Byadgi	LC-MS	Pyraclostrobin	0.2110	0.0120	5.69	0.199	0.223
Byadgi	LC-MS	Chlorantraniliprole	0.1650	0.0100	6.06	0.155	0.175
Byadgi	GC-MS	Chlorfenapyr	0.1350	0.0100	7.41	0.125	0.145
Guntur Sannam	LC-MS	Flupyradifuron	0.2997	0.0133	4.44	0.285	0.311
Guntur Sannam	LC-MS	Fenpyroximate	0.2110	0.0120	5.69	0.199	0.223
Guntur Sannam	GC-MS	Chlorpyrifos	0.1903	0.0284	14.91	0.165	0.221
Guntur Sannam	LC-MS	Azoxystrobin	0.1867	0.0764	40.92	0.120	0.270
Guntur Sannam	LC-MS	Spirotetramate (sum)	0.1867	0.0764	40.92	0.120	0.270
Teja	LC-MS	Acetamiprid	0.2967	0.0764	25.74	0.230	0.380
Teja	LC-MS	Prochloraz	0.2267	0.0764	33.70	0.160	0.310
Teja	LC-MS	Imidacloprid	0.2067	0.0764	36.96	0.140	0.290
Teja	LC-MS	Thiamethoxam	0.1880	0.0746	39.71	0.124	0.270
Teja	LC-MS	Clothianidin	0.1867	0.0764	40.92	0.120	0.270

Statistical Validation of Inter-Varietal Food-Safety Differences (Model 2)

The findings of the Kruskal-Wallis H-test statistics are reported in Table 3 for all 28 pesticides and 4 mycotoxins. Among the 28 pesticides screened, only nine yielded a p-value of < 0.05 as indicators of statistically significant differences among varietals; namely, Acetamiprid ($p = 0.0495$), Bifenthrin ($p = 0.0273$), Chlorpyrifos ($p = 0.0379$), Flupyradifuron ($p = 0.0321$), Hexaconazole ($p = 0.0390$), Prochloraz ($p = 0.0265$), Pyraclostrobin ($p = 0.0495$), Pyriproxyfen ($p = 0.0265$), and Trifloxystrobin ($p = 0.0495$). It can be clearly seen how the two sets of signals converge, and that the pesticides with the highest cumulative exposure risks (see Table 2) are those for which statistical differences were found. Additionally, several other pesticides (Fenpyroximate, Fluxapyroxide, Hexythiazox, Imidacloprid, Lufenuron, Spirotetramate, Thiamethoxam) yielded slightly higher-than-significant values (0.0509 - 0.0608); and hence, with additional replication, they could achieve statistical significance.

As stated before, the analysis of mycotoxins holds more direct relevance to public health issues than pesticide residues. Of the four mycotoxin parameters, three showed a statistically significant p-value. Namely, Aflatoxin B2 ($p = 0.0495$, between Byadgi and Guntur Sannam), Ochratoxin-A ($p = 0.0273$, among the three varietals), and Total Aflatoxins ($p = 0.0273$). Aflatoxin B1 was non-significant ($p = 0.0608$). Interestingly, the statistical significance of ochratoxin-A and total aflatoxins coincides completely with Guntur Sannam's high Total Mycotoxin Burden (see Table 1) as an indicator of the greatest need for post-harvest hygiene intervention for this varietal supply chain. Both Aflatoxin B1 and total aflatoxins are associated with severe health consequences, including being classified as a Group 1 carcinogen. For compounds where the Kruskal-Wallis test indicated significant differences across varieties ($p < 0.05$), post-hoc pairwise comparisons were conducted using Dunn's test with Bonferroni correction to control for Type I error. For ochratoxin-A and total aflatoxins ($p = 0.0273$), the pairwise comparisons revealed that Guntur Sannam differed significantly from both Teja ($p = 0.028$ and $p = 0.031$, respectively) and Byadgi ($p = 0.022$ and $p = 0.026$, respectively), while Teja and Byadgi were not

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significantly different from each other (p = 0.452 and p = 0.487, respectively). For chlorpyrifos (p = 0.0379), Guntur Sannam differed significantly from Teja (p = 0.018) and Byadgi (p = 0.024). For prochloraz and pyriproxyfen (both p = 0.0265), Teja differed significantly from both Byadgi (p = 0.017 and p = 0.019, respectively) and Guntur Sannam (p = 0.021 and p = 0.024, respectively). These pairwise comparisons confirm that Guntur Sannam is the primary driver of mycotoxin risk, while Teja contributes strongly to the pesticide residue burden, particularly through high levels of acetamiprid, prochloraz, imidacloprid, thiamethoxam, and clothianidin. However, these pairwise findings should be interpreted as preliminary because the study is based on limited sampling and requires confirmation through multi-batch, multi-season analysis.

Table 3. Compound-wise Kruskal-Wallis H-test results comparing replicate values (R1, R2, R3) across varieties. T = Teja, B = Byadgi, GS = Guntur Sannam. Decision threshold: p < 0.05.

Type	Compound	Groups Compared	H-stat	p-value	Decision
Pesticide	Acetamiprid	Teja, Byadgi	3.8571	0.0495	Significant
Pesticide	Azoxystrobin	T, B, GS	3.8222	0.1479	Not significant
Pesticide	Bifenthrin	T, B, GS	7.2000	0.0273	Significant
Pesticide	Carbendazim	T, B, GS	5.1317	0.0769	Not significant
Pesticide	Chlorantraniliprole	T, B, GS	1.4222	0.4911	Not significant
Pesticide	Chlorfenapyr	T, B, GS	3.2000	0.2019	Not significant
Pesticide	Chlorpyrifos	T, B, GS	6.5434	0.0379	Significant
Pesticide	Clothianidin	T, B, GS	4.2353	0.1203	Not significant
Pesticide	Cyantraniliprole	T, B, GS	1.2773	0.5280	Not significant
Pesticide	Difenconazole	T, B, GS	1.7175	0.4237	Not significant
Pesticide	Dinotefuron	Teja, Byadgi	1.1905	0.2752	Not significant
Pesticide	Fenpyroximate	T, B, GS	5.9556	0.0509	Not significant
Pesticide	Flupyradifuron	T, B, GS	6.8796	0.0321	Significant
Pesticide	Fluxapyroxide	T, B, GS	5.9556	0.0509	Not significant
Pesticide	Hexaconazole	T, B, GS	6.4889	0.0390	Significant
Pesticide	Hexythiazox	T, B, GS	5.6000	0.0608	Not significant
Pesticide	Imidacloprid	T, B, GS	5.6000	0.0608	Not significant
Pesticide	Lambda Cyhalothrin	Teja, Byadgi	0.7843	0.3758	Not significant
Pesticide	Lufenuron	T, B, GS	5.6000	0.0608	Not significant
Pesticide	Prochloraz	T, B, GS	7.2605	0.0265	Significant
Pesticide	Pyraclostrobin	Teja, Byadgi	3.8571	0.0495	Significant
Pesticide	Pyriproxyfen	T, B, GS	7.2605	0.0265	Significant
Pesticide	Spiromesifen	Teja, Byadgi	1.7647	0.1840	Not significant
Pesticide	Spirotetramate (sum)	T, B, GS	5.6000	0.0608	Not significant
Pesticide	Tebuconazole	T, B, GS	0.8000	0.6703	Not significant
Pesticide	Thiamethoxam	T, B, GS	5.9556	0.0509	Not significant
Pesticide	Tolfenpyrad	T, B, GS	2.3081	0.3154	Not significant
Pesticide	Trifloxystrobin	Teja, Byadgi	3.8571	0.0495	Significant
Mycotoxin	Aflatoxin B1	T, B, GS	5.6000	0.0608	Not significant
Mycotoxin	Aflatoxin B2	Byadgi, Guntur Sannam	3.8571	0.0495	Significant
Mycotoxin	Ochratoxin-A	T, B, GS	7.2000	0.0273	Significant
Mycotoxin	Total Aflatoxins	T, B, GS	7.2000	0.0273	Significant

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Combined Food-Safety Burden Visualization

Figure 1 visualizes the Total Pesticide Burden and Total Mycotoxin Burden next to each other within the three types, making it immediately clear at first glance which of the two types requires priority attention from a food safety point of view. The highly elevated Guntur Sannam bar for mycotoxins highlights this variety as the primary source of carcinogen-related risk among the types being evaluated, while the higher Teja bar indicates this variety as the one carrying the most insecticide-related risks.

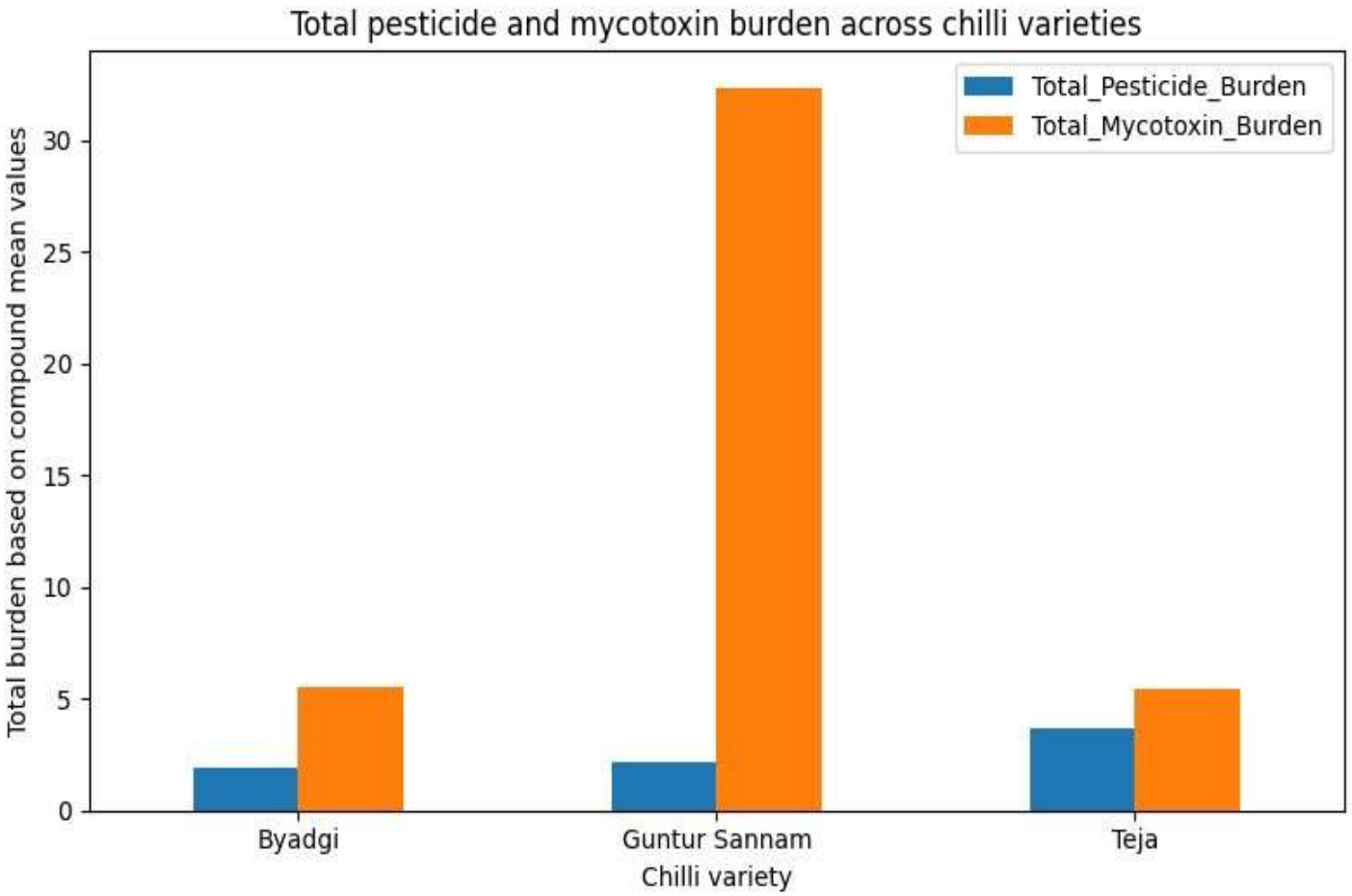

Figure 1. Total pesticide and mycotoxin food-safety burden across three commercial chilli varieties (Byadgi, Guntur Sannam, Teja).

Public-Health and Regulatory Implications

The synthesis of the burden evidence, the statistical significance test, and the graphical data leads to three converging conclusions about food safety. The first conclusion is that because of the dual hazards associated with chilli contamination, namely pesticides and mycotoxins, food safety regulations based on a single hazard will be inadequate. The management of food safety requires a combination of pre-harvest enforcement of pesticide maximum residue levels and post-harvest maintenance of hygiene, specifically, for each type of chilli, not just commodity-level statistics. The second conclusion is that the hazard signatures for each type of chilli require specific intervention measures. A generic guideline for the whole country may adequately protect consumers of one variety while imposing unnecessary costs on those who consume another variety. The third convergence of the burden analysis and the statistical significance test gives regulators a scientific basis for allocating limited testing resources efficiently in state and central food safety labs.

Preliminary Health Risk Characterization

Using the mean residue concentrations from Table 2 and assuming a conservative daily consumption of 5 g of chilli powder per person (based on Indian dietary survey data), the Estimated Daily Intake (EDI) for selected

pesticides was calculated. For chlorpyrifos in Guntur Sannam, the EDI is 0.9515 µg per person per day. The Acceptable Daily Intake (ADI) for chlorpyrifos established by JMPR is 1 µg kg^{-1} body weight per day, which for a 60 kg adult corresponds to 60 µg per person per day. The EDI of 0.9515 µg is well below the ADI, suggesting no acute health concern for chlorpyrifos alone. However, consumers are exposed to multiple pesticides simultaneously, and the cumulative risk from neonicotinoid insecticides in Teja (acetamiprid, imidacloprid, thiamethoxam, clothianidin) requires further investigation using cumulative exposure assessment methodologies. For aflatoxin B1, a non-threshold carcinogen, the Margin of Exposure (MOE) approach is more appropriate. Using the mean aflatoxin B1 concentration in Guntur Sannam (not significantly different from other varieties at $p = 0.0608$, but numerically higher), the MOE is calculated as the benchmark dose lower confidence limit (BMDL10) divided by the EDI. The resulting MOE values are below 10,000 for all three varieties, indicating a public health concern under European Food Safety Authority criteria.

CONCLUSION

The present study confirms that commercial chillies require variety-specific safety assessment rather than generic residue testing. The combined analysis of pesticide residues, aflatoxins, and ochratoxin-A showed that contamination patterns varied among the three chilli varieties, indicating that pre-harvest pesticide application and post-harvest fungal contamination may influence commercial chilli safety differently. The residue burden approach, coupled with non-parametric statistical analysis, proved useful as a preliminary tool for hazard prioritization. Nevertheless, the current study has some important limitations. The study involved only three varieties, limited sample representation, and the absence of multi-batch, multi-season, geographical, and intake-based assessments. Therefore, the findings presented here should be interpreted as exploratory and hypothesis-generating rather than definitive. The observed differences in residue profiles across varieties were statistically significant for several compounds but may not be generalizable to all batches of these varieties without confirmation through multi-batch and multi-season sampling. Another limitation is that compound-specific validation parameters, including LOD, LOQ, recovery at multiple spiking levels, repeatability, and within-laboratory reproducibility, were not available for all individual analytes in the present dataset. Therefore, a full compound-wise validation table could not be included. Future studies should incorporate complete method validation for each pesticide and mycotoxin according to recognized guidelines such as SANTE/11312/2021, including matrix-matched calibration, recovery studies, precision, reproducibility, and analyte-specific LOD and LOQ values. Future research should also prioritize replicated sampling across multiple growing seasons, geographic regions, and supply chain nodes to determine whether the variety-specific hazard signatures identified in this study are stable characteristics or reflect batch-specific variation. Additionally, the incorporation of information regarding pesticide-use history during cultivation, storage conditions, and EDI- or MOE-based dietary risk assessment would further improve the robustness and regulatory relevance of the findings.

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