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Comparative analysis of pesticide residue levels in brinjal (*Solanum melongena* L.) treated with drone and knapsack sprayers analyzed by LC-MS/MS

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Abstract
Indiscriminate insecticide use on vegetable crops results in pest resistance and harmful residues in produce, impacting human health and ecosystems. To reduce pesticide residues on brinjal crops, investigations were conducted utilizing drone sprayers for brinjal pest management during the rabi season of 2023-24 at the College of Horticulture, Venkataramannagudem, West Godavari district, Andhra Pradesh. Following the application of insecticides namely, Chlorantraniliprole, Flubendiamide, Thiacloprid, and Imidacloprid against major pests of brinjal via drone and knapsack spraying methodologies, the brinjal fruits were subsequently harvested and subjected to a comprehensive pesticide residue analysis at Food Quality Analysis Laboratory (PRFQAL) at the University of Agricultural Sciences, Raichur, Karnataka. The results indicated that Pesticide residue analysis of fruits drawn from 100% RDP through drone treated plots showed undetectable residues falling below the quantifiable limits except for thiacloprid which recorded 0.031 mg/kg while, the fruits harvested from 100% RDP through knapsack sprayed plots exhibited detectable pesticide residues: Flubendiamide at 0.036 mg/kg, Thiacloprid at 0.062 mg/kg, and Imidacloprid at 0.017 mg/kg but they remained below the MRLs established by the FSSAI.

Keywords: Pesticide residues, brinjal, drone sprayer, knapsack sprayer, LC-MS/MS and MRL

Introduction
The analysis of pesticide residues present in horticultural crops, with a particular emphasis on commonly consumed vegetables such as brinjal (*Solanum melongena* L.), holds paramount importance in the ongoing efforts to ensure food safety and protect consumer health from potential hazards. Brinjal is a vegetable that features prominently in various diets and is susceptible to a myriad of pest infestations, the necessity for pesticide applications in its cultivation becomes quite evident. It is imperative to engage in rigorous monitoring of pesticide residues, as excessive or improper utilization can lead to the accumulation of toxic substances within the food chain, thereby posing significant risks to both environmental ecosystems and human health as highlighted by various studies (Sharma *et al*., 2022; Gupta *et al*., 2022, Puvvala *et al*., 2020) [9, 2]. The evolution of analytical technologies, particularly the advent of liquid chromatography-tandem mass spectrometry (LC-MS/MS), has markedly enhanced the precision and sensitivity associated with the detection of pesticide residues, even at exceedingly low trace levels, thus enabling compliance with the maximum residue limits (MRLs) established by regulatory bodies such as the Food Safety and Standards Authority of India (FSSAI) (Thokchom *et al*., 2023; Kaur *et at*, 2022) [11, 4]. LC-MS/MS stands out as an exceptionally valuable tool in the analytical assessment of complex biological matrices, including brinjal, as it permits the simultaneous quantification of multiple pesticide residues, such as Chlorantraniliprole, Flubendiamide, Thiacloprid, and Imidacloprid, all while maintaining remarkable sensitivity and specificity (Kumar *et al*., 2020) [5]. This advanced methodology not only facilitates accurate quantification of pesticide residues but also plays a critical role in supporting compliance with regulatory standards, ultimately contributing to the promotion of safer agricultural practices and the enhancement of consumer protection.

The integration of such advanced analytical techniques into routine agricultural practices underscores the necessity for ongoing research and development aimed at mitigating the risks associated with pesticide usage. Furthermore, the commitment to ensuring food safety through rigorous monitoring and analysis of pesticide residues reflects a broader societal responsibility to safeguard public health and preserve environmental integrity. Therefore, it is essential to prioritize the implementation of these analytical methodologies in agricultural practices to uphold the standards of food safety and the health of consumers.

Materials and Methods

Pesticide residue analysis was conducted to detect levels of insecticides, including chlorantraniliprole, flubendiamide, thiacloprid, and imidacloprid, in brinjal fruits harvested from drone-sprayed, conventional-sprayed, and control plots. The experiment followed a t-test statistical model, consisting of five plots (200 m² each) with five different spraying treatments: T_1 (100% RDP with drone), T_2 (75% RDP with drone), T_3 (50% RDP with drone), T_4 (100% RDP with knapsack sprayer), and T_5 (untreated control with water spray). These treatments were applied sequentially at 15-day intervals during the 2023-24 rabi season at the College of Horticulture in Venkataramannagudem, West Godavari district, Andhra Pradesh.

Brinjal samples each of 3 kg were collected in a clean plastic cover from treatment plots sprayed through drone and conventional method separately. The samples were collected 10 days after the last spraying at harvest and stored at 8-10℃ before analysis. The collected samples were transported immediately in an ice box to the Pesticide Residue and Food Quality Analysis Laboratory (PRFQAL), University of Agriculture Sciences, Raichur, Karnataka for estimation of pesticide residues of different groups including neonicotinoid (imidacloprid and thiacloprid) and diamide (chlorantraniliprole and flubendiamide). The residues in brinjal samples were quantified using highly sensitive equipment like LC-MS/MS.

Steps involved in estimation of pesticide residues in brinjal samples using LC-MS/MS from plots sprayed with drone and conventional method

A. Sample preparation

The collected samples were made into an analyzable sample portion that represented the whole sample. The collected brinjal samples were homogenized by chopping, grinding, and blending to obtain a uniform particle size and stored at -20 ºC for further analysis.

B. Extraction

The collected sample was weighed 10±0.1 g of sample in a clean centrifuge tube of 50 mL by using an analytical balance. Distilled water of 5 mL was added followed by 10 mL of ethyl acetate and vortexed for 2-3 minutes properly.

C. Homogenization

The sample mixture was homogenized at 10000-12000 rpm for 2 min and added 5 g±0.1 g anhydrous sodium sulphate Na_2SO_4 to remove the moisture content and vortexed for 1-2 minutes.

D. High volume centrifugation

The sample mixture was centrifuged at 10000 rpm for 5 minutes at 10 ℃ to separate the organic layer.

E. Cleanup

After centrifugation, transferred 6-7 ml of supernatant into a 15 mL centrifuge tube containing 175 mg±0.1 mg primary secondary amine (PSA) and 1.05 g±0.1 g anhydrous magnesium sulphate ($MgSO_4$) to remove the pigments and others interfering co-extractives. Vortexed the sample for 1-2 minutes and centrifuged at 12000 rpm for 5 minutes at 10℃.

F. Evaporation

After clean-up 2 ml of the aliquot was transferred into the test tube, evaporated to near dryness in nitrogen evaporator at 35-40 ℃. Reconstituted the residue with 1.5 ml of methanol for LC-MS/MS analysis.

G. Filtration

The extract of 1.5 ml was then filtered to LC auto sampler vials through 0.22 µm PTFE membrane filter.

H. Sample injection

The sample was injected 2 µL of the filtrate to LC-MS/MS equipment for analysis with the below instrument conditions and time programme.

Table 1: Details of LC-MS/MS operating parameters

Parameter	LC-MS/MS
Model	SHIMADZU LC-MS 8040®
Column	Shimpack XR, ODS – C18, 2mm id x 150 mm
Flow rate	0.4 $mLmin^{-1}$
Heat block	400 ºC
Nebulizing gas flow	3 $Lmin^{-1}$
Source	ESI + ve probe
Dissolution temperature	250 ºC
Drying gas flow (N_2)	15 L min^{-1}
CID gas Argon	230 k pa
Injection volume	2 µL
Ion source temperature	-
MS interface	-
Detector temperature	-

Table 2: Time program of LC-MS/MS

Time (min)	Flow (mL/min)	Mobile phase	
		% A	% B
0.01	0.4	90	10
1.00	0.4	90	10
4.00	0.4	50	50
16.00	0.4	10	10
18.00	0.4	10	90
22.00	0.4	10	90
24.00	0.4	90	10
25.00	0.4	controller	stop

Method Validation

Before screening the samples with the developed LC-MS/MS method, recovery was performed in blank control matrices of brinjal at fortification level of 0.1 mg/kg for 04 pesticides (imidacloprid thiacloprid, chlorantraniliprole and flubendiamide). As per SANTE 2021 guidelines, the acceptable recovery is 70-120% with relative standard deviation (RSD) of ≤ 20%. The limit of quantification (LOQ) of the method was assessed at the lowest level of pesticide mixture (0.01 mg/kg) at which the analyte could able to meet the acceptable recovery of 70-120% and RSD of ≤ 20%. The samples were compared with the matrix-matched standard of brinjal with a minimum of 2 product ions for each of the pesticides. The retention time of each of pesticide are mentioned below,

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

Calculation

The weight of the sample and pesticide residue detected in brinjal samples were quantified using the mathematical formula

$$\text{Residue (mg/kg)} = \frac{a \times c \times e \times g}{b \times d \times f}$$

Where,

a-Sample peak area, b – Standard peak area, c-Concentration of standard (ppm), d- Weight of the sample (g), e- µL of standard injected, f- µL of sample injected, g-Final volume of the sample (mL)

$$\text{Weight of sample (g)} = \frac{\text{Sample weight (g)} \times \text{Aliquot taken (mL)}}{\text{Volume of extractant (mL)}}$$

Calculation: The recovery (%) and residues from the fortified sample were calculated by using the following formula.

$$\text{Recovery (\%)} = \frac{\text{Concentration of fortified sample (mg/kg)}}{\text{Concentration of analytical standard of pesticide}} \times 100$$

$$\text{Residue (mg/kg)} = \frac{\text{Peak area of sample} \times \text{Conc. of Std.} \times \mu\text{l std. injected}}{\text{Final volume of sample (1.5ml) x Peak area (standard)} \times \text{weight of the sample (g)} \times \mu\text{l sample injected}}$$

Fig 1: Chopping of brinjal fruits

Fig 2: Blending in grinder

Fig 3: Blended mixture

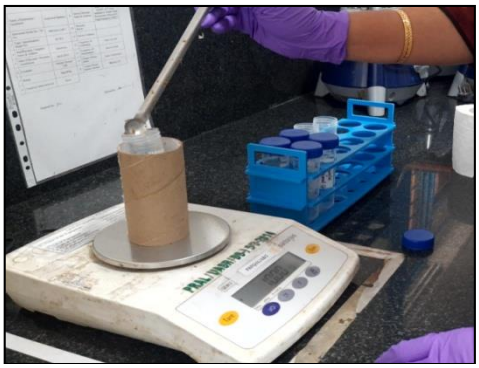

Fig 4: 10 gms of blended mixture

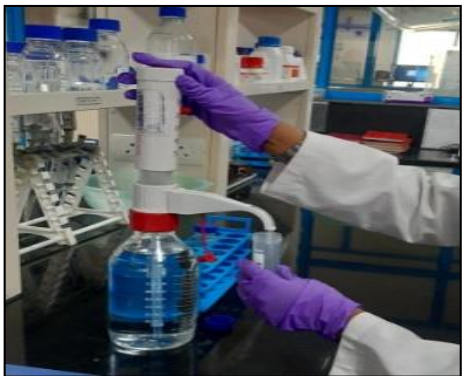

Fig 5: Addition of 10 ml ethyl acetate

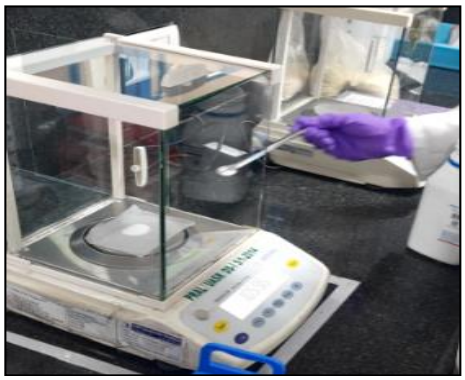

Fig 6: Weighing of 10 g anhydrous Na_2SO_4

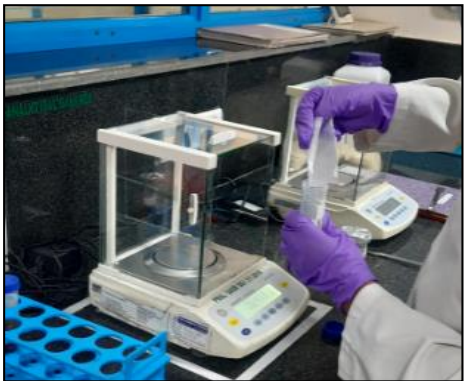

Fig 7: Addition of 10 g anhydrous Na_2SO_4

Fig 8: Homogenization

Fig 9: High volume centrifugation

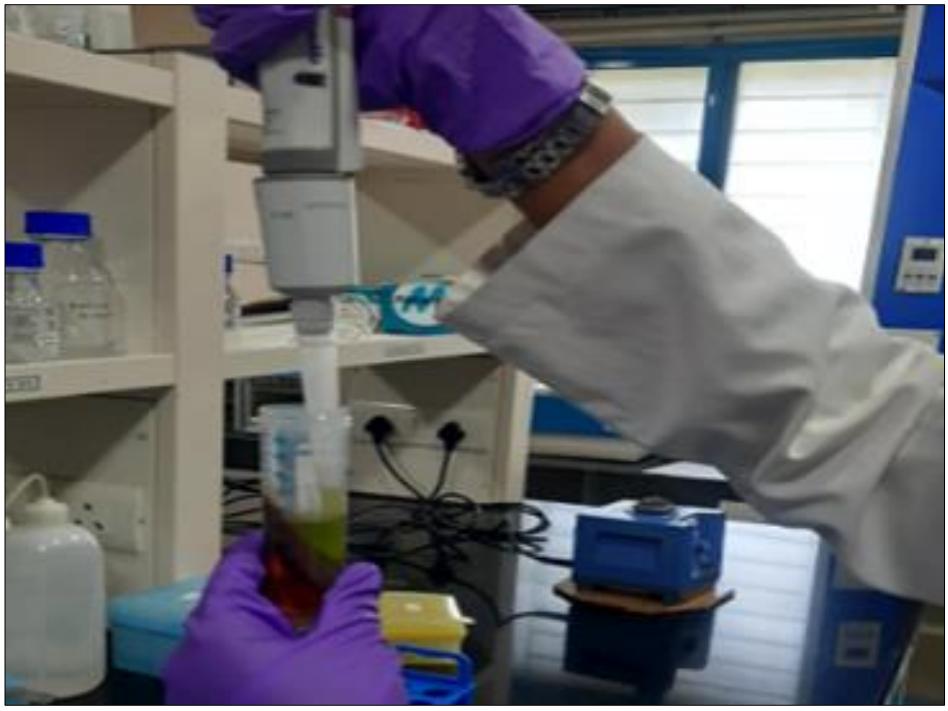

Fig 10: Transfer of 7 ml extract to 15 ml centrifuge tube containing 25 mg PSA and 150 mg $MgSO_4$

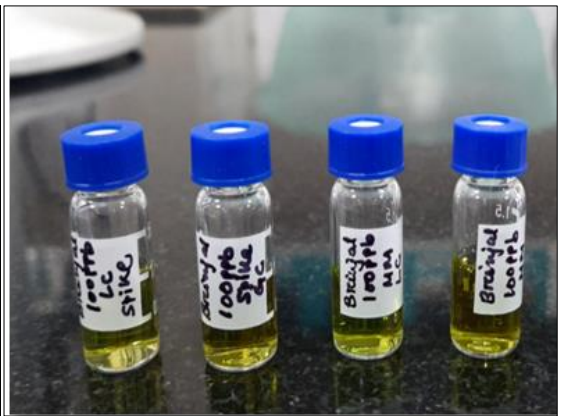

Fig 12: Evaporating to dryness using nitrogen concentrator **Fig 13:** Final 1.5 ml extract into LC auto sampler vials

Fig 14: 2 µL of the filtrate to LC-MS/MS equipment for analysis

Plate 3: Steps involved in estimation of pesticide residues in brinjal samples

Results and Discussion

Results of present investigation in table 1 and fig 1 suggested that insecticides when applied via drone sprayer utilizing a 100% RDP, the resultant residues of Chlorantraniliprole, Flubendiamide, and Imidacloprid present in the samples of brinjal fruits were not detectable through Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LCMS/MS) and were ultimately found below the quantifiable levels established by FSSAI. Conversely, it is noteworthy that brinjal fruits harvested after thiacloprid spray contained residues of 0. 031 mg/kg, which was much lower than the MRL of 0. 700 mg/kg.

In a contrasting scenario, when brinjal fruits were collected after being subjected to pesticide applications through

knapsack sprayer and subsequently analyzed for the presence of chemical residues, it was determined that specific residues were indeed detected in the brinjal fruits: Flubendiamide at a level of 0.036 mg/kg, thiacloprid at 0.062 mg/kg, and Imidacloprid at 0.017 mg/kg, all of which are critical data points for assessing the pesticide residues in brinjal fruits.

Surprisingly, these identified pesticide residue levels (mg/kg) were found to be significantly lower than the recommended Maximum Residue Limits (MRLs) in brinjal fruits when sprayed with knapsack sprayer as stipulated by the Food Safety and Standards Authority of India (FSSAI), which reinforces the notion of effective and safe pesticide application methods in brinjal crop pest management. However, it is important to note that despite the presence of other pesticide residues, chlorantraniliprole was not identifiable in the residue analysis in brinjal fruits when sprayed with knapsack sprayer and was, therefore, classified as being below the quantification levels established by the pesticide residue analysis protocols.

In the context of pesticide exposure and its associated toxicity to humans, especially concerning food crops and vegetables, residue concentrations that exceed the maximum residue limit (MRL) exert a direct influence on biological systems and serve as impediments to both export opportunities and local market consumption. Consequently, it was determined that the research on pesticide residue analysis proved beneficial that could be recommended for farmers post-application of a pesticide, thereby minimizing contamination in their final products and ensuring safety for human consumers. Therefore, the findings of the present investigation demonstrated that the consumption of brinjal fruit treated with chemicals chlorantraniliprole, flubendamide and thiacloprid after the 10th and 15th day of application at the recommended doses, utilizing both the drone and conventional spraying techniques presents no significant risk to human health, pollinators and natural enemies. These findings are consistent with findings of Sahoo *et al.* (2013) [7] who detected average initial deposits of thiacloprid on brinjal fruits of 1.05 mg/kg and it was 0.05 mg/kg after five days which was below the limit of quantification. In the succeeding year, Gupta *et al.* (2015) [3] affirmed that imidacloprid residues on brinjal, at a dosage of 20 g a.i. ha^{-1}, dissipated by 93.17% in 10 days, with a half-life of 2.65 days. In the same year, the average initial residues of 0.72 mg/kg after a single dose of 30 g a.i. ha^{-1} and 1.48 mg/kg after a double dose of 60 g a.i. ha^{-1}, with residues dissipating below detectable levels by the tenth day were validated by Vijayasree *et al.* (2015) [12].

The observation is further corroborated by the findings of Rani *et al.* (2019) [6] who reported GC-MS/MS method for quantifying chlorantraniliprole residues in brinjal, achieving 84% recovery and detection limits of 0.005 µg/mL. In the same time frame, dissipation of chlorantraniliprole residues on brinjal fruits was reported with residue deposits 0.19 mg kg^{-1} which reached below the maximum residue limit of 0.03 mg kg^{-1} after 7 days of spray by Sharma *et al.* (2019) [10]. Furthermore, Gandabhai *et al.* (2024) [1] stated initial deposits of chlorantraniliprole (5.097 mg/kg), thiamethoxam (4.886 mg/kg), and thiacloprid (3.332 mg/kg) and after 4 days, the degradation rates were noted as 97.97% for thiamethoxam, 96.97% for chlorantraniliprole, and 96.18% for thiacloprid. The current findings align with research findings of Shanmugam *et al.* (2024) [8], who explored insecticidal methods for controlling fall armyworm (FAW) in maize using UAV spraying and reported insecticide residues remained below detectable levels.

Table 1: Residue levels of insecticide in drone and conventional sprayed plots of brinjal

S. No	Name of the insecticide	Brinjal control sample	LOQ (mg/kg)	Recovery (%) at 0.1 mg/kg	Residues (mg/kg)		Retention time (min.)	FSSAI MRL value(mg/kg)
					Drone sprayed plots	Knapsack sprayed plots		
1.	Chlorantraniliprole	BQL	0.01	78.33%	BQL	BQL	10.598	0.600
2.	Flubendiamide	BQL	0.01	72.90%	BQL	0.036	13.568	0.100
3.	Thiacloprid	BQL	0.01	84.81%	0.031	0.062	6.416	0.700
4.	Imidacloprid	BQL	0.01	77.52%	BQL	0.017	5.545	0.200

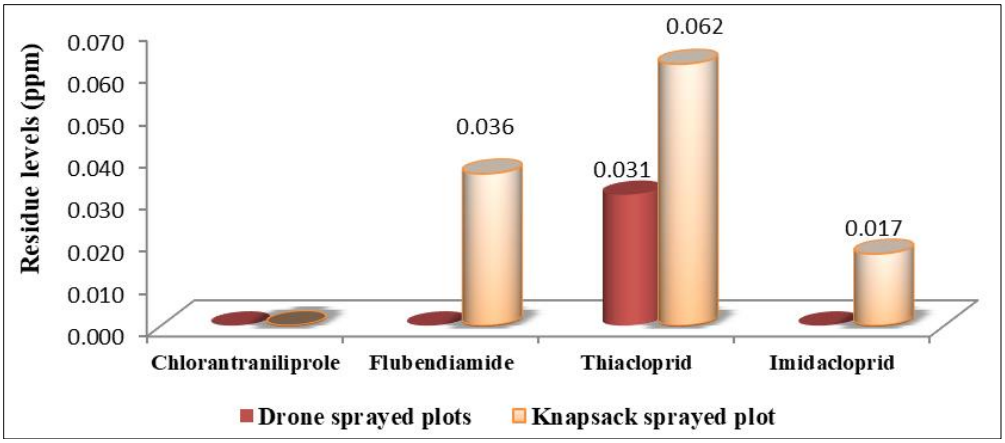

Fig 1: Residue levels of insecticide in drone and conventional sprayed plots of brinjal

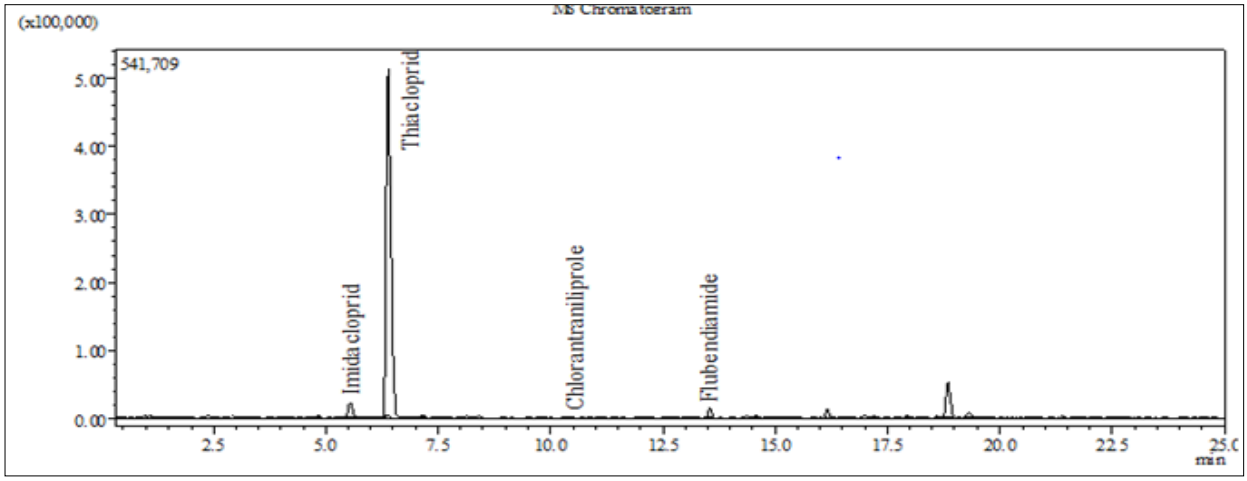

Plate 1: Chromatogram of brinjal plot sprayed with 100% RDP through drone sprayer

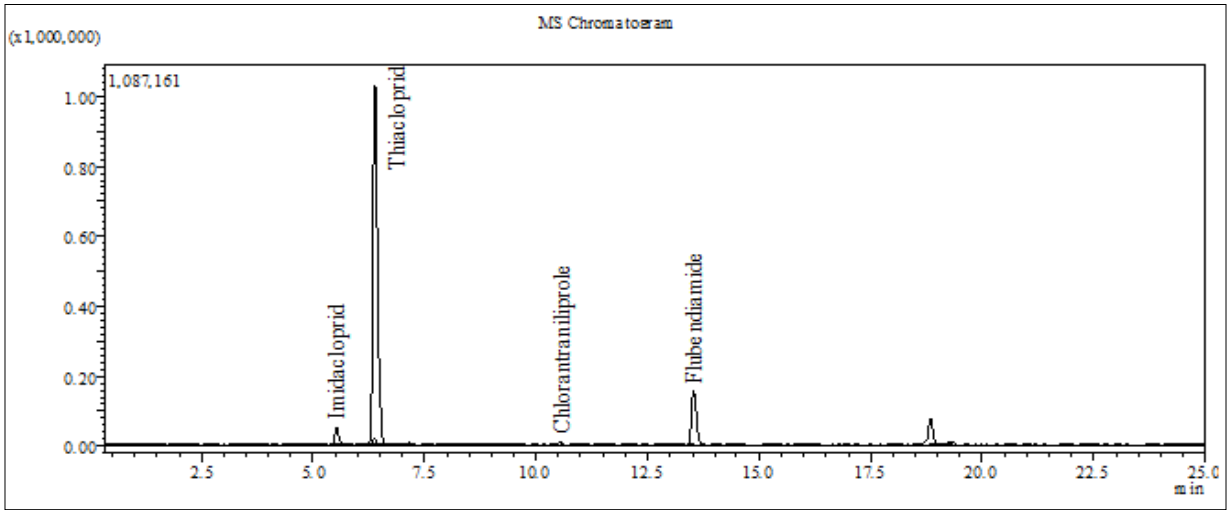

Plate 2: Chromatogram of brinjal plot sprayed with 100% RDP through drone sprayer

Conclusion

Drone-sprayed brinjal showed undetectable residues of Chlorantraniliprole, Flubendiamide, and Imidacloprid, with only Thiacloprid at 0.031 mg/kg—well below FSSAI limits. Knapsack spraying, however, yielded detectable but safe residue levels: Flubendiamide (0.036 mg/kg), Thiacloprid (0.062 mg/kg), and Imidacloprid (0.017 mg/kg). Chlorantraniliprole was undetectable with both methods, highlighting drones' potential in reducing pesticide residues.

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