RESEARCH ARTICLE

Assessment of environmental impact: Dissipation of insecticides on chilli agroecosystem

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Abstract

The use of pesticides is inevitable in achieving food security. Pesticide residues in the food commodity create havoc on human beings' food safety. This study investigates the dissipation patterns, half-lives and safe harvesting time of five distinct insecticides, *viz*., imidacloprid, thiamethoxam, acetamiprid, flubendiamide and triazophos applied to chilli ecosystem by supervised field experiment with recommended and double the recommended dose along with untreated control. Analytical validation of the method was done by assessing the concentration specificity, linearity, recovery rates, detection limit and quantification limit to ensure the validity of the results. Results reveal variations in dissipation rates and half-lives, providing crucial insights for effective pest management in chilli cultivation. Imidacloprid exhibited rapid dissipation, reaching below detectable levels in five days with a half-life of 1.84 days. Thiamethoxam and acetamiprid followed suit, reaching below detectable levels in seven and five days, with half-lives of 1.62 and 1.28 days, respectively. Flubendiamide showed a longer persistence, taking seven days to dissipate, with a half-life of 1.77 days. While recording below detectable levels within 15 days, Triazophos exhibited a half-life of 2.71 days. These findings contribute to informed decision-making for sustainable pest management practices in chilli cultivation and a safe waiting period to ensure food safety.

Keywords

chillies; environment; half-life; pesticides, safe harvesting period

Introduction

Chilli cultivation is a highly profitable spice crop that thrives across various regions in India. Chilli (*Capsicum annum* L.) holds momentous economic value in India and serves as a spice that is utilized worldwide. It plays a decisive role in various culinary applications, being a key ingredient in daily curries, pickles and chutneys worldwide. India holds the top position as the leading producer of chilli globally, accounting for 42.2 % of the cultivable area and contributing to 21.4 % of the worlds' total production (1). Chilli cultivation is widespread in countries like Britain, Australia, New Zealand, South Africa, India, USA, Central American and South American nations, as well as various other Asian countries. India holds the top position globally as the prime producer and consumer, tailed by China and Pakistan. The distinctive commercial attributes of Indian chillies, notably their vibrant color and pungency, contribute to their global popularity. These chillies are mostly exported to east Asian nations (2). In India, capsicum cultivation takes place during the kharif, rabi (specifically in Jharkhand, Karnataka,

Maharashtra and Tamil Nadu) and summer seasons (especially in the hills of Tamilnadu, Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh) as a field crop. When grown under protected conditions, plant has demonstrated the ability to produce early and abundant yields, leading to increased profits for farmers (3). Thrips, specifically *Scirtothrips dorsalis* Hood, yellow mites like *Polyphagotarsonemus latus* (Banks) and the fruit borer *Helicoverpa armigera* (Hübner) pose significant threats to chilli production in terms of both quantity and quality. The impact of these pests results in a substantial reduction in chilli yield, with an overall decline of up to 34 % in fruit output attributed to the damage caused by thrips and mites (4). The key constraint in chilli production is chilli thrips (*S. dorsalis),* which causes yield loss varied from 60.5 to 74.3 % (5). Besides yield loss, this pest acts as a vector for acute viral diseases, often resulting in the complete failure of the crop.

To counteract these issues, numerous pesticides are commonly employed. However, the use of certain insecticides raises concerns as they may leave residues on the fruits, which could persist until harvest. This persistence of residues poses potential risks regarding food safety, environmental impact and human health. It is crucial to address these concerns through scientific research and careful consideration of pesticide usage, ensuring that effective pest control measures are balanced with minimizing adverse effects on the quality and safety of the final agricultural produce. The dissipation rate plays a pivotal role in predicting the environmental fate of pesticides. This research delves into the dissipation patterns of distinct insecticides *viz.*, imidacloprid, thiamethoxam, acetamiprid, flubendiamide and triazophos applied to chilli crops. The investigation comprehensively evaluates essential parameters, including concentration linearity, specificity, recovery rates, limits of detection and quantification and measurement uncertainty, ensuring a rigorous assessment of the analytical methodology employed. By scrutinizing the destiny of insecticides in this context, the study aims to enhance our comprehension of the intricate interactions between pesticides and chilli plants. This knowledge contributes to making well-informed decisions for pest management in chilli cultivation.

Materials and Methods

A supervised field experiment took place in Naraseepuram, Coimbatore, from November to December 2018, aimed at investigating the dissipation pattern of five distinct insecticides: imidacloprid, thiamethoxam, acetamiprid, flubendiamide and triazophos. All the pesticides were purchased from authorized local dealers. Before applying treatments, the field was carefully maintained free of any insecticidal residues without spraying any chemical insecticides for the management of pests. The cultivation of the Chilli variety (CO 2) followed good agricultural practices in a plot measuring 500 m^2, with a precise spacing of 60 × 45 cm. A Randomized Block Design (RBD) was employed to ensure a robust scientific methodology. Each insecticide had three treatments (X: Recommended dose, 2X: Double the recommended dose and control) with three replications,

enhancing result reliability. The application of all insecticides involved spraying with a hollow cone nozzle fitted with a knapsack sprayer. The trial was initiated by spraying insecticides at the fruit formation stage and repeated after 10-day intervals. The dosage and treatment details of the insecticides were imidacloprid (@ 50 and 100 g. ai. ha^{-1}), thiamethoxam (@ 15 & 30 g. ai. ha^{-1}), acetamiprid (@ 50 & 100 g. ai. ha^{-1}), flubendiamide (@ 20 & 40 g. ai. ha^{-1}) and triazophos (@ 96 & 192 g. ai. ha^{-1}) for X (recommended) and 2X (double recommended) doses, respectively. Chilli fruits were randomly harvested from each replication at designated time intervals: 0 (1 hour), 1st, 3rd, 5th, 7th and 10th days after the final application, encompassing the control sample. The gathered samples were transported and processed in the laboratory. The chilli fruits were chopped homogenized and a 500-gram was stored in glass bottles at -20°C for future utilization.

Certified pesticide standards (90 %) of imidacloprid, thiamethoxam, acetamiprid, flubendiamide and triazophos reference standards were procured from M/S Sigma Aldrich, Bangalore, India. HPLC and LCMS grade acetonitrile, sodium chloride (NaCl) and analytical-grade anhydrous magnesium sulfate (MgSO$_4$) were obtained from Merck India Pvt Ltd, Mumbai, India. Bondesil (40 μm) (PSA - Primary, secondary amine) and Charcoal (GCB - Graphitized Carbon Black) were acquired from M/S Agilent Technologies, USA. Type 1 water was sourced from the Millipore water purification system. LCMS grade acetonitrile was used for the preparation of primary (400 μg mL^{-1}), intermediate (40 μg mL^{-1}) stocks and working standards (0.005, 0.01, 0.025, 0.05, 0.1 and 0.2 μg mL^{-1}) of flubendiamide and its metabolites and stored at 4°C until utilization.

The modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (6) was employed to process samples. In this procedure, a 50 mL centrifuge tube containing 10 g of chilli sample with 20 mL acetonitrile, anhydrous MgSO$_4$ (4g) and NaCl (1g) were mixed thoroughly and centrifuged (6000 rpm; 10 minutes). After centrifugation, supernatant (9 mL) was taken and dehydrated using Na$_2$SO$_4$ (4 mg) and 6 mL of dehydrated supernatant was transferred to a 15 mL centrifuge tube with PSA (100 mg), MgSO$_4$ (600 mg), GCB (25 mg) and centrifuged (3000 rpm for 10 minutes). The upper extract (4 mL) was evaporated to near dryness using a nitrogen evaporator (turbovap LV). The final volume was made to 1 mL using acetonitrile and taken for LCMS analysis.

Method validation was done based on SANTE Guidelines (7). The linearity study involved injecting standard solutions at five different concentrations of insecticides with three replications. The signal-to-noise ratio of three is determined as the Limit of Detection (LOD) and ten as the Limit of Quantification (LOQ). To validate the method, recovery studies were conducted using untreated chilli fruit samples, spiked at LOQ, 5LOQ and 10LOQ concentrations with insecticides and replicated thrice, including control. After one hour of equilibration, residues were extracted and estimated following the previously mentioned method. Analysis of control chilli fruit samples confirmed that the blank, which did not possess, interfered with the target analyte. The recovery percentage was

calculated using the Equation 1 formula.

Recovery percentage =

$$\frac{\text{Residue quantified in the fortified sample}}{\text{Fortified level}} \times 100$$

(Eqn. 1)

Insecticide residue estimation was carried out using LCMS (Shimadzu, series 2020; SPD-M20A diode array detector, Agilent C18 column (250 mm length; 4.6 mm dia; 5μ particle size) within a column oven set at 40˚C). Nitrogen gas served as both the nebulizer and collision gas. The gas flow rate was 15 L min⁻¹ for drying gas and 1.5 L min⁻¹ for nebulizing gas. Temperature was 250˚C at the Desolvation Line (DL) and 200˚C at the heat block. Ion monitoring occurred in negative Single Ion Monitoring (SIM) mode through an Electrospray Ionization (ESI) interface. The instrument parameters are detailed in Table 1 and Standard chromatograms are given in Fig. 1-5. The quantification of residue was calculated using the Equation 2 formula,

Residue (ppm) =

$$\frac{\text{Peak area of sample}}{\text{Peak area of Standard}} \times \frac{\text{Standard concentration (ppm)}}{\text{Sample weight (g)}} \times \frac{\text{Volume of Standard injected (μL)}}{\text{Volume of Sample injected (μL)}} \times \text{Final volume (mL)}$$

(Eqn. 2)

The determination of half-life was conducted by utilizing the Pesticide Residue Half-life Calculator software (8), which also facilitated the identification of the most appropriate degradation model. The Equation 3 formula was applied to ascertain the safe waiting period, incorporating the Maximum Residual Limit (Codex MRL) (9).

Safe waiting period =

$$\frac{Log\ K2\ -\ \left[\frac{MLR}{Tolerance}\right]}{Log\ K1}$$

(Eqn. 3)

Where, K1 = slope of the regression line (b), K2 = Initial deposit.

Results and Discussion

The degradation behaviour of different insecticides in/on chillies was assessed through a supervised field trial conducted at farmers' holdings without exposure to insecticides except for target analytes. A recovery study was carried out and all the insecticides' per cent recovery was satisfactory in all spiked levels. Recovery of imidacloprid, thiamethoxam, acetamiprid, flubendiamide and triazophos

was 80 to 100 percent; hence the method is accepted for the study (Table 2).

The study examined the dissipation dynamics of imidacloprid 17.8 SL at two application doses: 50 and 100 g a.i ha⁻¹. The initial mean deposit for the X dose was 0.264 μg g⁻¹, reaching below detectable levels on the fifth day after application. In contrast, the 2X dose exhibited a higher initial deposit of 0.398 μg g⁻¹, with more than 80% of residues dissipating by the third day. Residues for the X dose dropped below detectable levels (0.01 mg kg⁻¹) within 5 days, while the 2X dose took 7 days. The decline behaviour of persistent residues was analyzed using seven transformations, with the first-order reaction identified as the best fit. The calculated half-lives were 1.84 days for the X dose and 2.17 days for the 2X dose (Table 3). Similar trends in imidacloprid residues, noting initial concentrations of 0.99 mg kg⁻¹ (10). Among seven transformations, imidacloprid residues in chillies followed the first-order dissipation kinetics model. The calculated half-life was 2.1 days, dissipated to below the LOQ after 14 days when applied according to authorized patterns. In open field conditions, our observed half-lives align closely with their results, with 2.90 days (X dose) and 3.14 days (2X dose) for imidacloprid (11). This concurrence supports the reliability and applicability of our findings, emphasizing the consistency of dissipation patterns across different studies. The results on imidacloprid dissipation in chillies are comparable with a half-life of 2.92 days in Chinese chives (12).

The initial mean deposit of thiamethoxam 25 WG @ 50 g a.i ha⁻¹ was 1.106 μg g⁻¹, dissipating to below detectable level on the fifth day after spraying (Table 4) . Whereas in 2X dose @100 g a.i ha⁻¹ it was 2.002 μg g⁻¹. Residues of thiamethoxam reached LOQ within 5 and 7 days at X and 2X dose, respectively and a significant portion of residues (> 80 %) disappeared within three days. Seven dissipation transformation patterns were calculated to assess the decline

Table 2. Percent recovery of insecticides in chillies

Insecticides	Spiked concentrations (μg/g)	Mean Recovery (%)	RSD (%)
Imidacloprid	0.01	92.67	2.92
	0.1	103.24	4.67
	0.5	97.34	3.43
Thiamethoxam	0.05	91.30	2.16
	0.25	98.14	4.20
	0.5	94.79	3.94
Acetamiprid	0.05	93.67	3.46
	0.25	94.63	4.49
	0.5	99.00	3.98
Flubendiamide	0.05	91.64	1.92
	0.25	86.89	2.61
	0.5	82.86	4.42
Triazophos	0.05	88.50	1.24
	0.25	89.97	4.42
	0.5	92.34	2.71

Table 1. Instrument parameters

| Insecticides	Imidacloprid	Thiamethoxam	Acetamiprid	Flubendiamide	Triazophos
Molecular mass	255.66	292	223.0	682	314
Mobile Phase	Acetonitrile: water (70:30)	Acetonitrile and 5 mM ammonium acetate: water (50:50)	Acetonitrile and 5 mM ammonium acetate: water (50:50)	Acetonitrile and water with 5 mM ammonium acetate (70:30)	Acetonitrile and water with 5 mM ammonium acetate (70:30)
Flow rate (mL min⁻¹)	0.5	0.5	0.5	0.8	0.8
Injected volume (μL)	10	10	10	20	20
Retention time (minutes)	1.5	1.23	1.5	8.26	7.12
Limit of quantification (mg kg⁻¹)	0.01	0.05	0.05	0.05	0.05
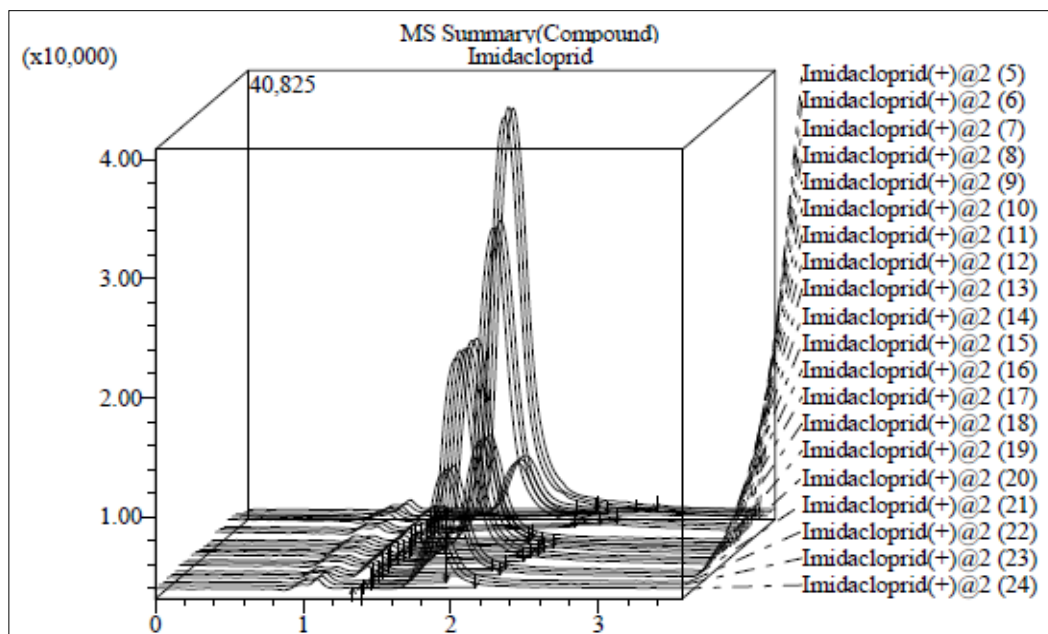
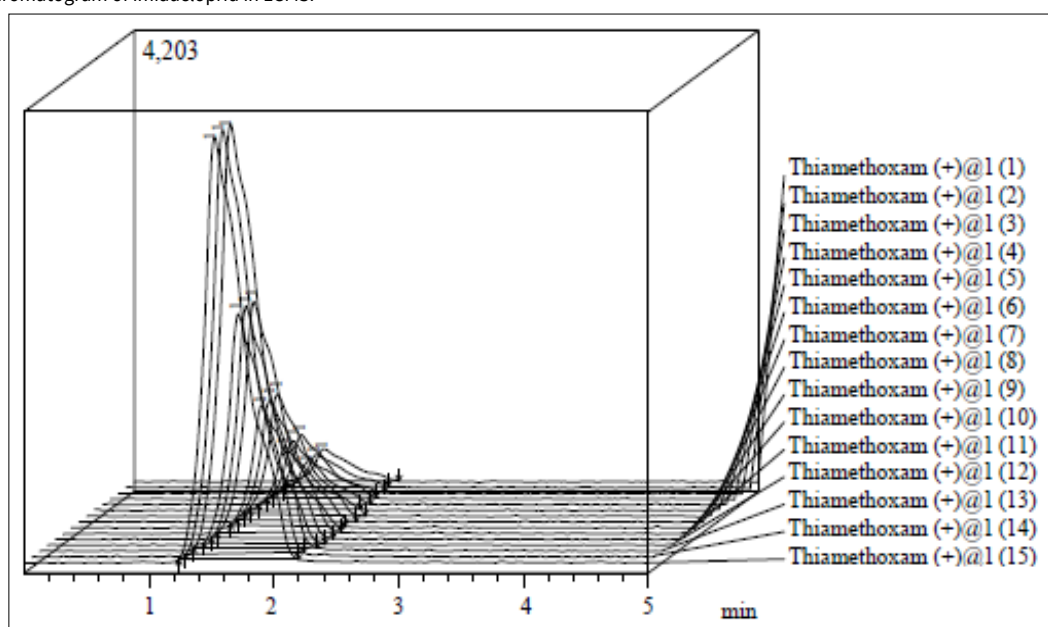**Fig. 1.** Standard chromatogram of imidacloprid in LCMS.

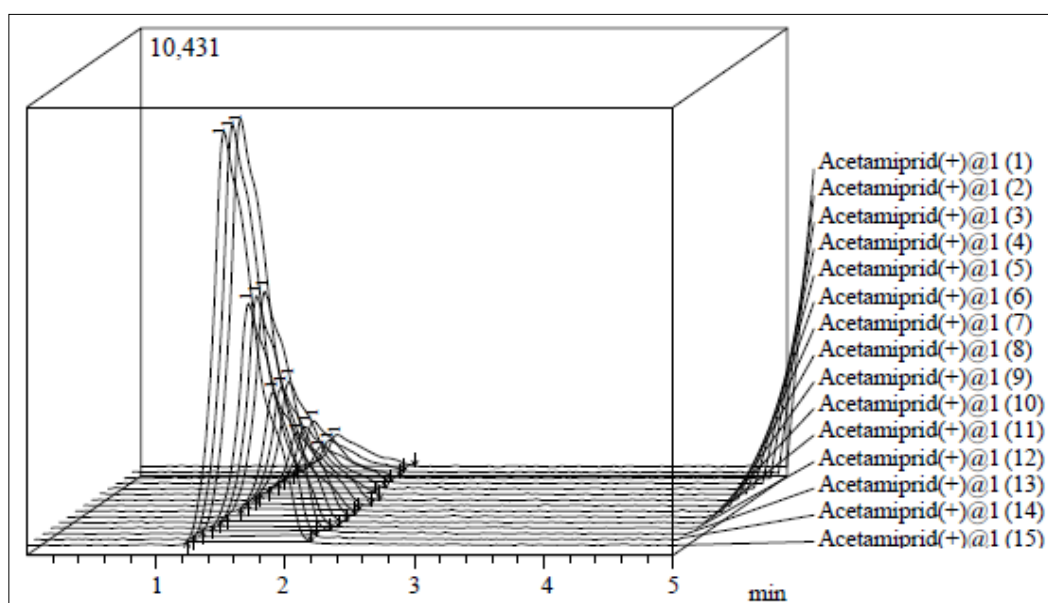**Fig. 2.** Standard chromatogram of thiamethoxam in LCMS.

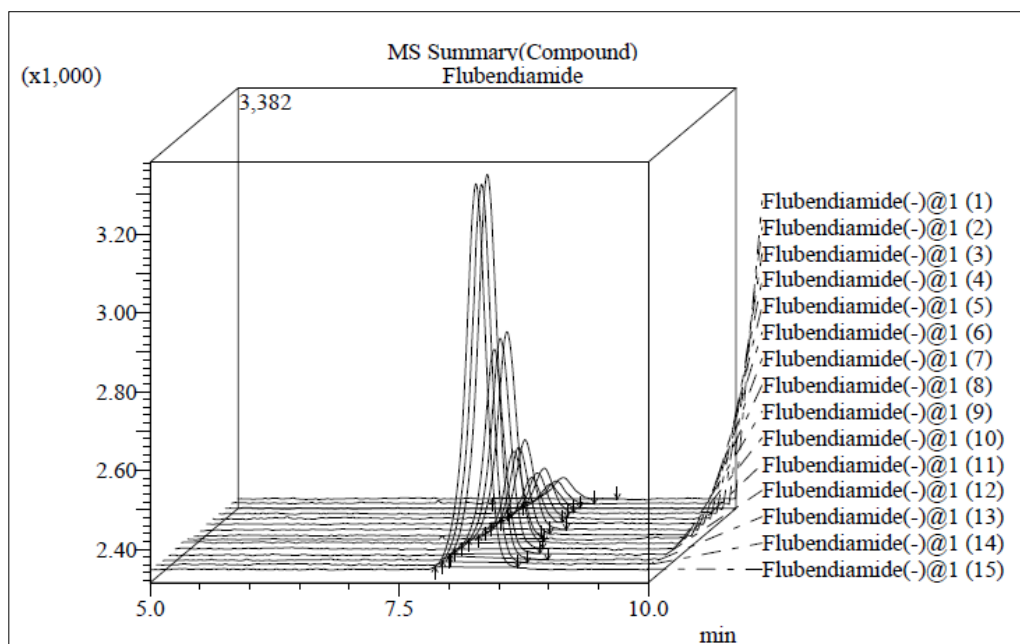**Fig. 3.** Standard chromatogram of acetamiprid in LCMS.

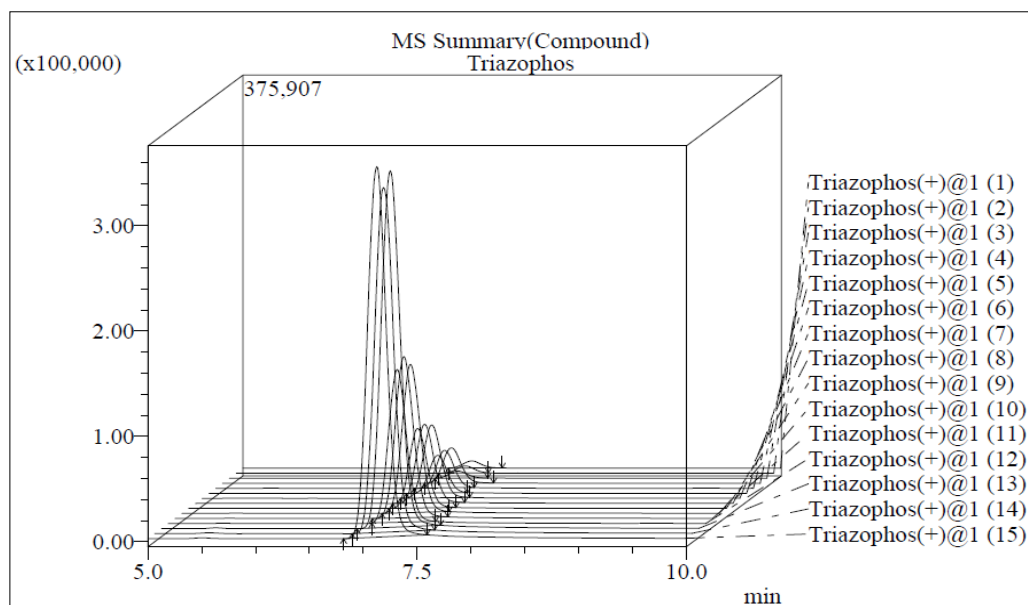**Fig. 4.** Standard chromatogram of flubendiamide in LCMS.

Fig. 5. Standard chromatogram of triazophos in LCMS.

Table 3. Residues of Imidacloprid in chillies after second application

DAT	Residue in mg kg^{-1}											
	Untreated Control				50 g a.i. ha^{-1}				100 g a.i. ha^{-1}			
	R1	R2	R3	Mean	R1	R2	R3	Mean	R1	R2	R3	Mean
0 (1 hr)	ND	ND	ND	ND	0.267	0.252	0.272	0.264	0.426	0.373	0.394	0.398
1	ND	ND	ND	ND	0.082	0.092	0.069	0.081	0.125	0.108	0.095	0.109
3	ND	ND	ND	ND	0.027	0.037	0.022	0.029	0.054	0.067	0.071	0.064
5	ND	ND	ND	ND	BDL	BDL	BDL	BDL	0.010	0.013	0.019	0.014
7	ND	ND	ND	ND	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

DAT: (Days after Treatment) BDL: Below Detectable level (0.01 mg kg^{1}), ND: Not Detected

Table 4. Residues of thiamethoxam in chillies after two applications

DAT	Residue in mg kg^{-1}											
	Untreated Control				50 g a.i. ha^{-1}				100 g a.i. ha^{-1}			
	R1	R2	R3	Mean	R1	R2	R3	Mean	R1	R2	R3	Mean
0 (1 hr)	ND	ND	ND	ND	1.102	0.990	1.225	1.106	1.938	2.247	1.820	2.002
1	ND	ND	ND	ND	0.487	0.419	0.523	0.476	0.925	0.827	0.926	0.893
3	ND	ND	ND	ND	0.115	0.185	0.201	0.167	0.214	0.373	0.372	0.319
5	ND	ND	ND	ND	BDL	BDL	BDL	BDL	0.093	0.063	0.083	0.080
7	ND	ND	ND	ND	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

DAT: Days after Treatment; BDL: Below Detectable level (0.05 mg kg^{1}); ND: Not Detected

behaviour of the thiamethoxam. The first-order reaction was observed as the best fit and the calculated half-life of thiamethoxam was 1.62 and 1.97 days for the X and 2X doses, respectively. The findings align with the earlier study on thiamethoxam in vegetable cowpeas (13). In their experiment, the initial residue in pods was 0.53 µg g^{-1}, persisted for three days and reached beneath quantification levels on the 5th day after application at a rate of 0.24 ml l^{-1}. This concurrence suggests consistent dissipation behaviour across different crops and application rates. Similar dissipation patterns in eggplant for four insecticides, including thiamethoxam were observed (14). The dissipation half-lives ranged from 3.4 to 14.5 days, with residues reaching <0.01–0.21 mg/kg after 7 to 10 days. A supervised field study on indoxacarb and thiamethoxam residues in chilli revealed that the initial deposits reached up to 5.93 mg kg^{-1} (15). Residues fell below the quantification limit (0.01 mg kg^{-1}) later 25 to 35 days, mirroring the dissipation trends observed in the current study.

The initial mean deposit of acetamiprid 20 SP @ 20 g a.i ha^{-1} (X dose) was 0.34 µg g^{-1}, dissipating to a detectable level on th^e fifth day after spraying. Whereas in 2X dose @ 40 g a.i ha^{-1} it was 0.549 µg g^{-1}. A significant portion of the acetamiprid residues (> 80 %) were dissipated on 3rd day after spraying and reached below the quantifiable level of 0.05 mg kg^{-1} within 5 days (Table 5). Among seven transformations, first-order kinetics was a fine fit for the persistent residues of acetamiprid and the half-life worked out was 1.28 and 1.87 days for X and 2X doses, respectively. Results are based on the earlier report of initial deposits when applying acetamiprid to kimchi cabbages (16). A half-life of 1.9 days was reported for acetamiprid on zucchini 0.87 to 1.46 days in okra fruits with no detectable residues after 7

days of application (17, 18).

The initial mean deposit was 0.646 & 1.292 µg g^{-1} for flubendiamide 39.35 SC @ 60 & 120 g a.i ha^{-1} and 1.013 & 1.390 µg g^{-1} for triazophos 40 EC @ 300 and 600 g a.i ha^{-1}. Residues reached below the limit of quantification on the 7th day for X dose of flubendiamide, the 10th day for 2X dose of flubemdiamide (Table 6) and the 15th day for both doses of triazophos (Table 7). Both insecticides followed first-order kinetics among seven transformations and the half-life value for X and 2X doses was 1.77 and 1.82 days for flubendiamide and 2.71 and 3.66 days for triazophos. The waiting period was calculated (FSSAI MRL value 0.1 ppm) as 4.7 days for X dose and 5.3 days for 2X dose of flubendiamide. Our findings are consistent with field and laboratory experiments on flubendiamide dissipation in field beans, which reported an initial deposit of 1.79 mg kg^{-1}, with residues declining to 0.06 mg kg^{-1} after the third spraying, reaching below detectable levels after ten days (19). Observed similar results in okra, with flubendiamide residues declining to below detectable levels on the 10th day after spraying at 48 g a.i. ha^{-1} (20). Flubendiamide dissipated below the limit of quantification by 25 and 30 days in x and 2x doses, respectively, with corresponding half-lives of 6.8 and 6.5 days in chillies (21). The present studys' findings align with the survey of the dissipation pattern of Triazophos on capsicum, in which residues reached to BDL in 10 and 15 days with 2.31 and 2.14 days half-life at the X and 2X dosages, respectively (22). Similar results were observed on capsicum, recommending a Pre-Harvest Interval (PHI) of twenty days for triazophos at 500 and 1000 g a.i ha^{-1} (23). Initial residue deposits of 0.90 and 1.85 mg kg-1 were observed on the brinjal crop sprayed at X and 2X

Table 5. Residues of acetamiprid in chillies after second application

DAT	Residue in mg kg^{-1}											
	Untreated Control				20 g a.i. ha^{-1}				40 g a.i. ha^{-1}			
	R1	R2	R3	Mean	R1	R2	R3	Mean	R1	R2	R3	Mean
0 (1 hr)	ND	ND	ND	ND	0.351	0.325	0.343	0.340	0.562	0.514	0.572	0.549
1	ND	ND	ND	ND	0.196	0.173	0.210	0.193	0.267	0.285	0.254	0.269
3	ND	ND	ND	ND	0.091	0.083	0.103	0.093	0.143	0.114	0.131	0.129
5	ND	ND	ND	ND	BDL	BDL	BDL	BDL	0.059	0.071	0.063	0.064
7	ND	ND	ND	ND	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

DAT: Days after Treatment; BDL: Below Detectable level (0.05 mg kg^{-1}); ND: Not Detected

Table 6. Residues of flubendiamide in chillies after two applications

DAT	Residue in mg kg^{-1}											
	Untreated Control				60 g a.i. ha^{-1}				120 g a.i. ha^{-1}			
	R1	R2	R3	Mean	R1	R2	R3	Mean	R1	R2	R3	Mean
0 (1 hr)	ND	ND	ND	ND	0.671	0.504	0.763	0.646	1.298	1.309	1.269	1.292
1	ND	ND	ND	ND	0.282	0.306	0.308	0.299	0.612	0.601	0.617	0.610
3	ND	ND	ND	ND	0.147	0.150	0.147	0.148	0.242	0.183	0.189	0.205
5	ND	ND	ND	ND	0.101	0.084	0.084	0.090	0.133	0.124	0.136	0.131
7	ND	ND	ND	ND	BDL	BDL	BDL	BDL	0.070	0.052	0.083	0.068
10	ND	ND	ND	ND	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

DAT: Days after Treatment; BDL: Below Detectable level (0.05 mg kg^{-1}); ND: Not Detected

Table 7. Residues of triazophos in chillies after two applications

DAT	Residue in mg kg^{-1}											
	Untreated Control				300 g a.i. ha^{-1}				600 g a.i. ha^{-1}			
	R1	R2	R3	Mean	R1	R2	R3	Mean	R1	R2	R3	Mean
0 (1 hr)	ND	ND	ND	ND	1.005	1.017	1.017	1.013	1.360	1.423	1.387	1.390
1	ND	ND	ND	ND	0.713	0.669	0.699	0.694	0.995	0.988	0.973	0.985
3	ND	ND	ND	ND	0.603	0.586	0.571	0.587	0.879	0.881	0.927	0.896
5	ND	ND	ND	ND	0.461	0.451	0.471	0.461	0.624	0.617	0.611	0.617
7	ND	ND	ND	ND	0.238	0.230	0.236	0.235	0.413	0.414	0.432	0.420
10	ND	ND	ND	ND	0.060	0.065	0.065	0.063	0.185	0.179	0.181	0.182
15	ND	ND	ND	ND	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

DAT: Days after Treatment; BDL: Below Detectable level (0.05 mg kg^{-1}); ND: Not Detected

doses of triazophos, respectively (24). Residues dissipated to BDL after 7 and 10 days, leading to a suggested PHI of seven days for residue-free brinjal.

Conclusion

In conclusion, this study delves into the dissipation patterns and half-lives of five key insecticides (Imidacloprid, thiamethoxam, acetamiprid, flubendiamide and triazophos) applied to chilli crops. The rapid dissipation of Imidacloprid, Thiamethoxam and Acetamiprid within a short timeframe highlights their efficiency in pest control. Flubendiamide exhibits a longer persistence, providing sustained protection against pests. While taking more time to dissipate, Triazophos underscores its effectiveness, especially at higher doses. These insights are crucial for optimizing pesticide application, ensuring effective pest management and minimal residues on harvested chillies. Even though double doses of insecticide spray are not supposed to be recommended, sometimes farmers may use a higher dose of insecticides to control economically essential pests quickly. This study generated valuable data for the projected issue. The study contributes valuable data for making informed decisions in sustainable chilli cultivation, balancing the need for pest control with environmental and food safety considerations.

Authors' contributions

BV conceptualized the idea, provided guidance, experimented, wrote an original draft and edited the manuscript. SSM performed the experiments, wrote an original draft and reviewed and edited the manuscript. PR helped review and edit the manuscript.

Compliance with ethical standards

Conflict of interest: There was no conflict of interest in the publication of this content.

Ethical issues: No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

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