



Ecological Risks of Chlorantraniliprole Residues in the Brinjal Cultivated Soil

**Abhinay ^a, Sharad Sharma ^a, Arvind Kumar ^b,
Rohit Kumar ^a, Anindita Paul ^{c*}, Jaydeep Halder ^{a*}
and Sujan Majumder ^{a*}**

^a ICAR-Indian Institute of Vegetable Research, Varanasi-221305, India.

^b Department of Chemistry, DSB Campus, Kumaun University, Nainital, India.

^c ICAR-National Institute for Research on Commercial Agriculture, Rajahmundry-533105, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Chlorantraniliprole is a relatively promising insecticide for controlling insect pests in vegetables. It is commonly used to manage the brinjal fruit and shoot borer (BFSB); however, its residues may pose serious health hazards and have adverse effects on flora and fauna. To address these concerns, insecticide monitoring in eggplant was conducted alongside soil monitoring, as some insecticides can leach into the soil and groundwater. A two-year study was undertaken in India to analyze the risks to the soil ecosystem. Samples were extracted using a modified ethyl acetate-based extraction method, which achieved significant recovery rates of 80.0–84.0% in soil. The risk quotient (RQ) values suggested negligible to low risk to earthworms and arthropods (e.g., *Aphidius rhopalosiphi*). Therefore, chlorantraniliprole can be recommended for use on brinjal as a low-risk insecticide, with minimal health hazards and environmental impact.

*Corresponding authors: E-mail: sujaniari@gmail.com; jaydeepento@gmail.com; aninditapaul20@gmail.com;

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1. INTRODUCTION

"India is the second largest producer of brinjal worldwide, after China. It is the fourth largest crop after potato, onion and tomato in terms of consumption in Indian scenario. According to National Horticulture Board, Ministry of Agriculture In India, it is cultivated on 0.73 million hectares with annual production of 12.8 million tonnes and a productivity of almost 17.5 tonnes ha⁻¹" (Indian Horticulture Database 2018). "There are more than 70 species of insects that attack brinjal among which the fruit and shoot borer (FSB) is the most destructive insect pest" (Subbarathnam and Butani, 1982). For the management of this pest, farmers primarily depend on the application of chemical pesticides. Considerable side effects are observed with indiscriminate use of chemical pesticides, including exposure to agricultural workers and end users. It also increases crop production costs, pesticide load in the environment, destruction of natural enemies, resurgence of insect pest, etc.

"Although several eco-friendly pest management options like the host plant resistance (HPR) and bio-control agents are available to control the insect pests" (Divekar et al., 2022; Dukare et al., 2021), "synthetic pesticides are the first choice of farmers. Farmers use insecticides inappropriately for managing BSFB", (*Leucinodes orbonalis* Guenee L.) which involves "applying pesticides more than the recommended dosage and applying them in calendar-based sprays. In India, farmers applied a "cocktail" of pesticides, including chlorpyrifos, cypermethrin, monocrotophos, and dimethoate, 20–30 times per crop season at a rate of approximately 26.7 L/ha. In general, the soil acts as a "purifying filter" that regulates the level of pesticide pollution in groundwater. The leaching tendency of a chemical into groundwater is significantly influenced by the soil profile, and soil organic content has an impact on pesticide persistence. Since the pesticides which are hydrophobic and lipophilic in nature, they can easily accumulate in biological tissues of species that are moving up the food chain and amplify their effects there" (Swackhamer et al., 1988; Vassilopoulou et al., 1993). Risks to human health and the environment are present when pesticide residues are found at detectable concentrations in soil, water (including groundwater and surface water), air, and even in commodities.

Chlorantraniliprole is a diamide group of insecticides and that has been successfully applied in suppressing many lepidopteran pests (Zhang et al., 2013; Kong et al., 2021). "Chlorantraniliprole demonstrated a distinct selectivity and safety for mammals because of structural variations between insect and mammalian ryanodine receptors" (Lahm et al., 2007). "Due to lower risk, it is approved as a substitute to pyrethroids for insect pest management in vegetables. However, pesticide residues (parent molecules or breakdown product or both) may remain in the plant produces which can cause threat to end users. In discriminant use of pesticides generally leave residues in the harvested products and, henceforth, during consumption of products potential risks can be associated with the end users. Even though the residues may in less quantities will definitely accumulate and magnify in the consumer's body, causing harmful effects on human health. Primary data on the extent of residues and safety intervals should be generated before any pesticide is recommended for field application. The environmental fate of chlorantraniliprole is influenced by various factors, including its chemical properties, application methods, soil characteristics, climatic conditions, and microbial activity" (Schmidt et al., 2016). "Chlorantraniliprole exhibits moderate persistence in soil, with reported half-lives ranging from several weeks to several months, depending on environmental conditions. Soil type, organic matter content, pH, temperature, and moisture levels can significantly affect its degradation rate and mobility" (USEPA 2008). "In aquatic environments, chlorantraniliprole can undergo hydrolysis and photolysis, although its persistence in water bodies may vary depending on factors such as temperature, pH, and sunlight exposure. Moreover, chlorantraniliprole and its metabolites may accumulate in sediment and biota, posing risks to aquatic organisms and ecosystem health" (Jactel et al., 2019). An effective decontamination technique can reduce the load of pesticides to be consumed by the end users. Limited information is generated on the pesticide residue dissipation kinetics in brinjal soil. In addition to having an adverse effect on flora and fauna, including people and their health, pesticide residues can persist as environmental contaminants in soil, water, and even the air. Insecticide monitoring in eggplants must be done simultaneously with soil monitoring since some insecticides can leach into the soil

and even groundwater. In the present work, studies were conducted to determine chlorantraniliprole residues in the soil, and the risk assessment against soil arthropods as well as earthworms.

2. MATERIALS AND METHODS

2.1 Preparation of Standard Solutions

Standard solutions were prepared by weighing 10 (± 0.1) mg reference standards of chlorantraniliprole dissolving in 10 ml of ethyl acetate resulting in a final concentration of 1000 $\mu\text{g mL}^{-1}$. A working solution of 10 $\mu\text{g mL}^{-1}$ was prepared in ethyl acetate by appropriate mixing of the individual standard stock solutions and further dilution, from which the calibration standard solutions at 0.01, 0.02, 0.05, 0.10 and 0.50 $\mu\text{g mL}^{-1}$ were prepared.

2.2 Field Experiment

Field experiments were conducted at the experimental farm of ICAR- Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India for two consecutive years in a randomized block design with four treatments and three replications. The chemical treatments for the management of borer pest of brinjal comprised of three dosages of chlorantraniliprole 18.5% SC at the rates of 20, 40 and 80 gm a.i. ha^{-1} , denoted as half of the recommended dose (RD half), recommended dosage (RD) and double of the recommended dosage (DD), respectively, and an untreated control was simultaneously maintained during the study. Insecticide applications were carried out using a high-volume knapsack sprayer fitted with a hollow cone nozzle.

2.3 Sampling

The soil samples were collected after final application of chlorantraniliprole following zig-zag pattern from each replication on 0 (2 h after spraying), 1, 3, 5, 7 and 10 days after the last spray. Samples were collected from 0–15 cm depth in the experimental field randomly. The samples were taken in sampling bags and kept in -20°C until analysis to avoid any degradation of the pesticide.

2.4 Extraction and Clean-up

The samples were prepared, extracted and cleaned-up by following earlier reported method with slight adjustments according to the nature of

the pesticide and type of the crop (Majumder et al., 2022a). A 10 gm sample was extracted with 10 mL of 1% acetic acid in ethyl acetate, along with 10 gm of anhydrous sodium sulfate. The mixture was vortexed for 2 minutes, followed by centrifugation at 5000 rpm for 5 minutes. The supernatant ethyl acetate layer (1.5 mL) was cleaned up by dispersive solid-phase extraction method (d-SPE) optimized. The extract was centrifuged at 10000 rpm for 5 min which was passed through a 0.22 μm Nylon 6,6 membrane filter and analyzed by GC- μECD . Soil samples were extracted following the same procedure, with the addition of 10 mL of water to 10 gm of soil. The mixture was allowed to stand for 20 minutes before adding the extracting solvent, ethyl acetate (Majumder et al., 2022b; Paul et al., 2021; Majumder et al., 2024).

2.5 Instrumentation

Gas chromatography with microelectron capture detector (μECD , ^{63}Ni) and fitted with a HP-5 capillary column was used for the detection of chlorantraniliprole.

2.6 Confirmation by GC/MS

The residues were further confirmed by injecting 1 μL of analytes to a QP-2010 Plus gas chromatography mass spectrometry system (GC-MS; single quadrupole, Shimadzu Corporation, Kyoto, Japan) equipped with autosampler. The column specification was ZB-5 (5% diphenyl, 95% dimethylpolysiloxane, 30 m (l) $\times$ 0.25 mm (id), 0.1 μm film thickness). The GC-MS separation of chlorantraniliprole was achieved by formulating an optimized oven temperature program that started initially at temperature of 100°C (hold for 0.5 min), ramped at the rate of ($@$) $30^\circ\text{C min}^{-1}$ up to 180°C (hold 1 min), increased to 240°C $@$ $10^\circ\text{C min}^{-1}$ (hold for 2 min), $@$ $10^\circ\text{C min}^{-1}$ up to 250°C min (hold for 1 min), and finally increased upto 280°C $@$ 2°C min^{-1} (hold 2 min). Under this condition resulted in a total run time of 35.04 min. The ion source temperature was 200°C and the interface was at 280°C . The detector voltage was set at 1 kV. The injector temperature was maintained at 250°C in a split injection mode (split ratio 10 and pressure 29.1 psi for 1 min) with injection volume of 1 μL and data acquisition was carried out in the selected ion monitoring (SIM) mode with specific m/z ions for selective identification of chlorantraniliprole the flow rate of helium gas was maintained at 3.14 mL min^{-1} with linear velocity of 64.4 cm s^{-1} . The mass spectrometer

was operated using electron impact ionization (EI, 70 eV).

2.7 Method Performance

The calibration curve of linearity for chlorantraniliprole in pure solvent and matrix with respect to concentration was obtained by establishing five calibration levels in the range between 0.01–0.50 mg kg⁻¹. The sensitivity of the method was determined in terms of limit of detection (LOD) and limit of quantification (LOQ) which decides as the smallest measured quantity in the soil matrix at which the signal to noise ratio (S/N) were 3:1 and 10:1, respectively. A recovery study was carried out on untreated (control) soil at five concentration levels: 0.01, 0.02, 0.05, 0.10, and 0.50 mg kg⁻¹, with six replicates for each concentration. The average recoveries within the range of 80-120% were accepted for the soil matrix, as per the SANTE guideline 2021.

2.8 Dissipation Kinetics

Chlorantraniliprole dissipation in samples was studied by subjecting the data to first-order kinetic equation i (Hoskins, 1961).

$$A_t = A_0 e^{-kt} \quad (i)$$

where, A_t denoted the concentration at time t , A_0 denoted the initial concentration, k denoted the rate constant for pesticide/insecticide dissipation, and t is the time.

For determination of the half-life ($t_{1/2}$) of the parent pesticide, the residue data were subjected to mathematical analysis as per the following equation ii.

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

2.9 Soil Ecological Risk

Ecological risk assessment of pesticides in terrestrial ecosystems has become one of the most important aspects in scientific pesticide evaluation in terms of biodiversity preservation. Soil ecological risk quotient (RQs) was calculated for earthworm (*Eisenia foetida*) and other arthropod (*Aphidius rhopalosiphii*), following the guideline given in the technical guidance document on risk assessment (European Communities 2003). The acute 14-day LC₅₀ and LR₅₀ value for earthworms and arthropods are >1000 mg/kg and 750 mg/ha respectively, considered for determining the RQ (PPDB 2017).

$RQ = EC/PNEC$, where EC = effective concentration (Ccanccapa et al., 2016).

Where, EC is the mean or maximum concentration of chlorantraniliprole detected in the soil samples and PNEC (Predicted No Effect Concentration) is calculated for acute toxicity, dividing the lowest short-term EC₅₀ or LC₅₀ by an assessment factor (AF), which is 1000 for this case. The risk ratios were classified into five risk levels: negligible risk (≤ 0.01), low risk (0.01), medium risk (0.1), high risk (1) and very high risk (>1) (Biswas et al., 2019).

3. RESULTS AND DISCUSSION

3.1 Method Validation

The analytical method was validated according to the SANTE guideline 2021 for estimation of chlorantraniliprole residues in soil. The percentage recovery was estimated at five levels for the soil matrix. The percentage recoveries at 0.01, 0.02, 0.05, 0.10, and 0.50 mg kg⁻¹ ranged between 80.00–85% (Table 1), with a relative standard deviation (RSD) of less than 20%, meeting the SANTE guidelines 2021. Chlorantraniliprole residue was detected in soil matrix at the time of 16.726 min i.e. retention (RT) (Fig. 1). The calibration curve with coefficient of determination (R^2) were 0.998 for solvent standard and 0.997 for matrix matched standard (soil) within the calibration range of 0.01-0.5 mg kg⁻¹ showed good linearity of the method. The LOD and LOQ were established at 0.005 mg kg⁻¹ and 0.01 mg kg⁻¹, respectively, for the soil matrix. The average matrix effect (ME) percentage were less than 13.57 % for soil. The method optimized data in the present study satisfied the internationally accepted pesticide residue estimation criteria for method validation and therefore the method is considered as well suitable for estimation of chlorantraniliprole residue in soil matrices. Recently Paul et al., 2021 had established a robust liquid chromatographic method for detection of multiple pesticides in tobacco matrix and achieved good screening detection limit at 5 ng g⁻¹ level which satisfactory fulfilled the internationally accepted guidelines. Similarly, a liquid chromatographic method developed by Majumder et al., 2021 showed upto 93.67% recovery for the residue estimation of acetamiprid and buprofezin pesticides in paddy matrix satisfied the international guidelines. Whereas Hingmire et al., 2015 had beautifully explained how matrix matched calibration method substantially reduce

the consideration of matrix induced false detection (Li et al., 2006).

3.2 Confirmative Analysis by GC-MS

The probabilities of false detection in plant matrix become crucial, as the interfering plant matrix compound may possess the same retention time of the intended pesticide compound. To avoid this chance of false detection a new GC-MS selective ion monitoring (SIM) method with confirmative identification based on quantifier-qualifier ions (m/z) ratio was employed. Based on the molecular breakdown recorded in the mass detector, six ions (m/z) namely, 112, 215, 243, 251, 278 and 280 were selected. However, this selection resulted in complex chromatogram and the base ion, 112, was found to present in several other molecules. Hence, the ion 112 was dropped and rest five ions (215, 243, 251, 278 and 280) were selected and total ion chromatogram was achieved and retention time of chlorantraniliprole was achieved at 15.62 min (Fig. 2). In this case also, it was observed that selection of 278 ion as a base peak and improved the identification of chlorantraniliprole as compared to 112 ion. Finally, 278 ion was selected as quantifier ion coupled with 243 and 280 ions as qualifier ions. (Fig. 2).

3.3 Residues Dissipation Kinetics in Soil

“The dissipation behaviour of chlorantraniliprole residues in soil were almost similar in both the consecutive year and follows good linearity of exponential simple first order dissipation kinetics with R^2 value of more than 0.928. In soil, after the last spray (2 h after application) the initial deposition of residues were 0.03, 0.04 and 0.08 mg kg⁻¹ for RD half, RD and DD respectively for the Year-I and for the Year-II, 0.04, 0.07 and 0.09 mg kg⁻¹ were found as initial deposition of residue for same doses stated above. The degradation of chlorantraniliprole was faster up to 3 DAA followed by slower degradation over the time period in all the doses. The half-lives of chlorantraniliprole residue in soil samples are in the range of 1.33-1.91 days across both the year

(Table 2). The dissipation pattern and percent reduction of residue in soil data were reparented in the Table 3.

Thus, the present investigation may help to ascertain the holistic approach in residue dynamics of chlorantraniliprole in brinjal where half-life values can be helpful to minimize the pesticide residue load in brinjal ecosystem.

In earlier reported literature, the half-lives of chlorantraniliprole in tomato fruits and soil came more than 3 days” (Malhat et al., 2012). Similarly, for other matrices like (Szpyrka et al., 2017) cabbage (Lee et al., 2019), maize straw (He et al., 2016), in pigeon pea (Kansara et al., 2021) the half-lives were estimated in between 2-2.5 days, 16-17 days, 10-15 days, 9-15 days, 4.95-5.78 days respectively Moreover Singla, Sharma, Mandal, & Kaur, 2020 studied that the chlorantraniliprole after its application in okra crop at 40 g a.i ha⁻¹, dissipation occurred below its limit of quantification after 7 days of spraying. In sugarcane ecosystem the half-life of chlorantraniliprole in soil varies between 6.50-6.81 days (Ramasubramanian et al., 2012; Sharma et al., 2014).

3.4 Soil Ecological Risk Assessment

“The environmental risk for earthworms and arthropods were predicted by calculating the risk quotient (RQ). The RQ values for earthworm (*E. foetida*) were in the range of 0.03–0.01 (RD half), 0.04 - 0.01(RD) and 0.08-0.02 (DD) for the year-2019 and for the year-2020, 0.04–0.01 (RD half), 0.07–0.01 (RD) and and 0.09-0.02 (DD) from 0 (2 h) to 7 days after application of chlorantraniliprole in the field soil. In case of arthropod (*Ahidius rhopalosiphi*), the RQ values were in the range of 0.04–0.01 (RD half), 0.05-0.01 (RD) and 0.11-0.03 (DD) for the year-I whereas, 0.05–0.01 (RD half), 0.09–0.01(RD) and 0.12-0.03 (DD) for the year-II from 0 (2 h) to 7 days after application of chlorantraniliprole in the field soil (Table 4). Thus, the RQ values indicate that there could be negligible risk ($RQ < 0.01$) to low risk ($0.01 \leq RQ < 0.1$) to earthworms

Table 1. Percentage recovery of chlorantraniliprole in soil

Level of fortification (mg kg ⁻¹)	Year-I		Year-II	
	% Recovery	% RSD	% Recovery	% RSD
Soil				
0.01	80.67	1.43	80.00	1.25
0.02	80.00	6.25	81.67	9.35
0.05	84.00	2.38	83.33	3.67
0.1	80.00	5.73	81.00	4.45
0.5	82.00	0.00	84.00	3.42

Table 2. Regression equation and half-life of chlorantraniliprole in soil

Doses	Year-I			Year-II		
	Regression equation	Coefficient of determination (R ²)	Half - lives (t _{1/2})	Regression equation	Coefficient of determination (R ²)	Half - lives (t _{1/2})
Soil						
RD half	$Y = 0.0295e^{-0.363x}$	0.9984	1.91	$Y = 0.037e^{-0.499x}$	0.9823	1.39
RD	$Y = 0.0775e^{-0.523x}$	0.9278	1.33	$Y = 0.1565e^{-0.635x}$	0.9291	1.09
DD	$Y = 0.1131e^{-0.445x}$	0.9664	1.56	$Y = 0.1749e^{-0.549x}$	0.9812	1.26

Table 3. Dissipation of chlorantraniliprole residue in soil

Days after spray	Year-I						Year- II					
	Half of the recommended dose (RD half)		Recommended dose (RD)		Double the recommended dose (DD)		Half of the recommended dose (RD half)		Recommended dose (RD)		Double the recommended dose (DD)	
	Residues (mg kg ⁻¹)	% decrease in residue	Residues (mg kg ⁻¹)	% decrease in residue	Residues (mg kg ⁻¹)	% decrease in residue	Residues (mg kg ⁻¹)	% decrease in residue	Residues (mg kg ⁻¹)	% decrease in residue	Residues (mg kg ⁻¹)	% decrease in residue
0	0.03	0.00	0.04	0.00	0.08	0.00	0.04	0.00	0.07	0.00	0.09	0.00
1	0.02	33.33	0.03	25.00	0.04	50.00	0.02	50.00	0.05	28.57	0.06	33.33
3	0.01	66.67	0.02	50.00	0.03	62.50	0.01	78.49	0.03	57.14	0.04	55.56
5	0.00	100.00	0.01	80.00	0.02	75.00	0.00	100.00	0.01	85.71	0.02	77.78
7	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.01	88.89
10	-	-	-	-	-	-	0.00	100.00	0.00	100.00	0.00	100.00

Table 4. Soil ecological risk assessment of chlorantraniliprole

Year-I																								
Earthworm (<i>E. foetida</i>)													Arthropod (<i>Ahidius rhopalosiphi</i>)											
DAYS	EC for RD half	LC ₅₀ (mg/kg)	PNEC (mg/kg)	RQs	EC for RD	LC ₅₀	PNEC (mg/kg)	RQs	EC for DD	LC ₅₀ (mg/kg)	PNEC (mg/kg)	RQs	EC for RD half	LC ₅₀ (mg/kg)	PNCE (mg/kg)	RQs	EC for RD	LC ₅₀	PNCE (mg/kg)	RQs	EC for DD	LC ₅₀	PNCE (mg/kg)	RQs
0	0.03	>1000	1.00	0.03	0.04	>1000	1.00	0.04	0.08	>1000	1.00	0.08	0.03	>750	0.75	0.04	0.04	>750	0.75	0.05	0.08	>750	0.75	0.11
1	0.02	>1000	1.00	0.02	0.03	>1000	1.00	0.03	0.04	>1000	1.00	0.04	0.02	>750	0.75	0.03	0.03	>750	0.75	0.04	0.04	>750	0.75	0.05
3	0.01	>1000	1.00	0.01	0.02	>1000	1.00	0.02	0.03	>1000	1.00	0.03	0.01	>750	0.75	0.01	0.02	>750	0.75	0.03	0.03	>750	0.75	0.04
5	0.00				0.01	>1000	1.00	0.01	0.02	>1000	1.00	0.02	0.00				0.01	>750	0.75	0.01	0.02	>750	0.75	0.03
7	0.00				0.00				0.00				0.00				0.00				0.00			
Year-II																								
Earthworm (<i>E. foetida</i>)													Arthropod (<i>Ahidius rhopalosiphi</i>)											
DAYS	EC for RD half	LC ₅₀ (mg/kg)	PNEC (mg/kg)	RQs	EC for RD	LC ₅₀	PNEC (mg/kg)	RQs	EC for DD	LC ₅₀	PNEC (mg/kg)	RQs	EC for RD half	LC ₅₀ (mg/kg)	PNCE (mg/kg)	RQs	EC for RD	LC ₅₀	PNCE (mg/kg)	RQs	EC for DD	LC ₅₀	PNCE (mg/kg)	RQs
0	0.04	>1000	1.00	0.04	0.07	>1000	1.00	0.07	0.09	>1000	1.00	0.09	0.04	>750	0.75	0.05	0.07	>750	0.75	0.09	0.09	>750	0.75	0.12
1	0.02	>1000	1.00	0.02	0.05	>1000	1.00	0.05	0.06	>1000	1.00	0.06	0.02	>750	0.75	0.03	0.05	>750	0.75	0.07	0.06	>750	0.75	0.08
3	0.01	>1000	1.00	0.01	0.03	>1000	1.00	0.03	0.04	>1000	1.00	0.04	0.01	>750	0.75	0.01	0.03	>750	0.75	0.04	0.04	>750	0.75	0.05
5	0.00				0.01	>1000	1.00	0.01	0.02	>1000	1.00	0.02	0.00				0.01	>750	0.75	0.01	0.02	>750	0.75	0.03
7	0.00				0.00				0.01	>1000			0.00				0.00				0.01			
10	0.00								0.00				0.00				0.00				0.00			

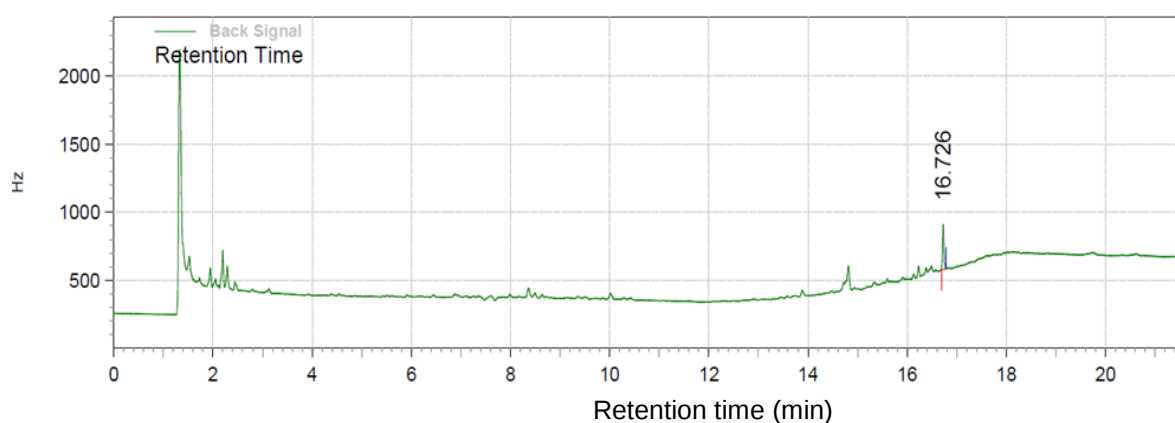


Fig. 1. Chromatogram of chlorantraniliprole in soil matrix

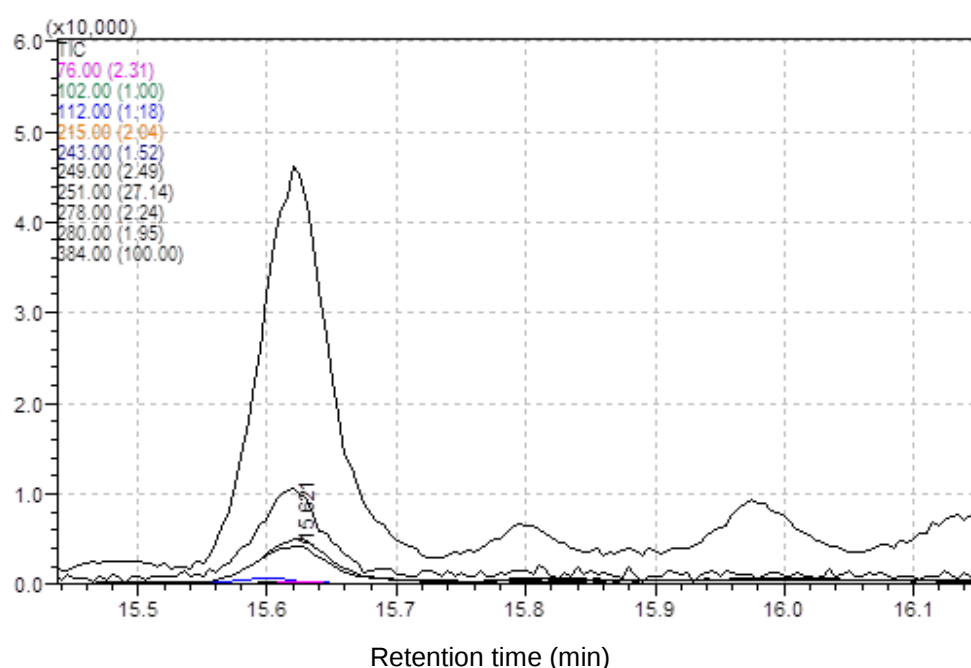


Fig. 2. Total Ion Chromatogram (TIC) of chlorantraniliprole in soil sample analyzed by GC-MS/SIM

due to the presence of chlorantraniliprole residues of all the three doses in soil and for arthropods also RQ could be negligible risk to low risk (except 0 days after final spray of DD). These finding also agreed with fipronil application in sugarcane" (Ccanccapa et al., 2016). Similar observations were recorded when pyridalyl was applied to tomato and cabbage (Jadav et al., 2020).

4. CONCLUSION

The modified method was effective in residues analysis of Chlorantraniliprole in

soil by GC- μ ECD which was further confirmed by GC-MS. The limit of quantification (LOQ) estimated 0.01 mg kg⁻¹ and % recoveries ranging from 80.0- 84.0 in the soil. The half-lives of chlorantraniliprole residue in soil less than 2 days for all the doses in both the year. The RQ values revealed that there could be a negligible risk (RQ < 0.01) to low risk (0.01 $\leq$ RQ < 0.1) to both earthworms and arthropods (except 0 days after final spray of DD), due to the presence of chlorantraniliprole residues at all three doses in soil.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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