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Persistence and Ecological Risk Associated with a Combination Ready Mix Sumiprempt Formulation in Soil under the Cover of Okra Crop

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ABTSTRACT: Sumiprempt, containing pyriproxyfen and fenpropathrin as active ingredients, has high potential for managing okra pests. Taking into account their potential hazards to non-target organisms and the associated environment, a field experiment was conducted to assess persistence and associated risk to various non-target organisms. Achieving efficient chromatographic separation of two pesticides can be challenging due to differences in their chemical properties, such as volatility, polarity, and structural characteristics. It is very time consuming, laborious and non-ecofriendly method due to the use of large amount of solvents in the extraction of the two pesticides simultaneously. Thus, by using GC-MS/MS, this study attempts to develop a rapid and less expensive QuEChERS method to extract and clean pyriproxyfen and fenpropathrin residues in soil simultaneously. Several factors were investigated in order to validate the effectiveness of the method, including the impact of spiking concentration, matrix effect (ME), repeatability between and within assays, reproducibility of results, and precision. For both analytes, the limit of determination (LOD) and limit of quantitation (LOQ) are 0.005 mg/kg and 0.01 mg/kg, respectively. The percentage recovery for both insecticides ranged between 87.8 and 97.7% with a relative standard deviation (RSD) below 7.09%. The foliar application of Sumiprempt formulation was applied at recommended (37.5 + 112.5 g a.i. /ha) and double recommended dose (70 + 225 g a.i. /ha) in the soil led to very low initial deposits of pyriproxyfen (0.029, 0.047 mg/kg) and fenpropathrin (0.054, 0.097 mg/kg) at respective doses. Both insecticides were not found to persist after 1st day of application. The risk quotient values for pyriproxyfen were in the range of 0.01-0.1 and for fenpropathrin greater than 0.1, implying that pyriproxyfen offers low risk whereas fenpropathrin offers moderate risk to soil invertebrates at both doses. This clearly indicates that longer persistence of these insecticides in soil can be toxic to organisms other than those that are targeted.

Keywords: Combination formulation, QuEChERS, risk assessment, pyriproxyfen and fenpropathrin.

INTRODUCTION

Agriculture uses a variety of pesticides formulations with different chemical structures to increase yields and reduce crop losses. However, no compound has yet been made to meet an ideal pesticide requirement which is flexible, safe, and non-hazardous chemicals. One of the potential possibilities that have come to the force is pesticide combination. When compared to utilizing a single active ingredient, combining pesticides with different mechanisms of action can provide a greater range of control (Mankar *et al.*, 2019). In order to reduce crop losses by pests, Sumitomo Chemical Co., Limited has launched a new ready-to-use insecticide formulation called Sumiprempt, which contains Pyriproxyfen 5% EC and Fenpropathrin 15% EC has good potential in the management of pests reported in okra. Pyriproxyfen 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy]pyridine is a juvenile hormone analogue that disrupts the growth of insects by mimicking a natural hormone. It's an insect growth regulator that is used to control houseflies, mosquitoes, and cockroaches for public health purposes by affecting young insects and eggs(Navickiene *et al.*, 1999). Adult insects are seldom poisoned by Pyriproxyfen. Instead, it prevents immature insects from maturing into adult forms by disrupting egg-laying and hatching. This stops the target insects from reproducing (Chang *et al.*, 2012). Sumitomo Chemical Co. Ltd. developed Fenpropathrin as a powerful pyrethroid pesticide for agricultural application. Fenpropathrin [(RS)-a-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate] is a racemic mixture of two optical isomers (R, S) because of the asymmetric nature of the benzyl a-carbon atom (Navickiene *et al.*, 1999). It has a strong insecticidal action against whiteflies and has low mammalian toxicity. As a result, it's utilized to control a wide range of mites and insects (Romeh *et al.*, 2014).

When pesticides formulation is applied to crop almost 99 % of the applied pesticides fall on the soil and it interacts with the soil matrix and the diverse array of organisms residing within it. The fate of any pesticide formulation in soil is influenced by various factors, including its chemical properties, environmental conditions (Zhang *et al.,* 2017), and interactions with soil microorganisms (Pérez-Lucas *et al.*, 2019). Both the insecticides present in sumiprempt formulation have low potential to volatilize into air when applied to dry soil but somewhat higher potential when applied to wet soils (Liu *et al*., 2017). It may be found in air surrounding the soil particles (Lewis *et al.*, 2016). Also, they have low water solubility but correspondingly strong tendency to bind to soil, leading to its presence in runoff sediments (Xiao *et al*., 2021). Suspended soil particles contaminated with pyriproxyfen and fenpropathrin can increase its toxic concentration in water bodies (Kanawi *et al*., 2013). Thus, soil act as the ultimate sink of the pesticide applied on the crop (Devillers *et al.,* 2005). Thus, it becomes necessary to evaluate the contamination levels of soils.

The pesticides present in the soil influences soil microbial communities, nutrient cycling, and overall soil health. Pesticide residues can also affect non-target organisms, including beneficial insects (Ndakidemi *et al.*, 2016), birds (Arya *et al.*, 2019), and soil-dwelling organisms like earthworms (Miglani and Bisht, 2020), which are crucial components of natural pest control and soil ecosystem functioning. The disruption of microbial communities can lead to imbalances in nutrient availability, hinder organic matter decomposition, and negatively impact soil fertility (Galand *et al.*, 2016). Thus, a comprehensive understanding of the ecological risks posed by pesticide formulation residues in the soil is essential for minimizing unintended consequences.

In this research paper, for the first time, we aim to delve into the ultimate fate of Sumipremt formulation in soil under the cover of okra crop and evaluate the associated ecological risks. By examining the processes governing pesticide degradation, and persistence, we will shed light on the potential long-term impacts on soil ecosystems and non-target organisms. Ultimately, this research aims to contribute to the preservation of environmental integrity, fostering a harmonious balance between agricultural productivity and ecological well-being.

MATERIALS AND METHODS

A. Chemicals, Reagents and Standards

Formulation under trade name Sumipremt (pyriproxyfen 5% EC + fenpropathrin 15% EC) was purchased from a local retailer while the certified reference materials of Pyriproxyfen (CAS No.- 95737-68-1) and Fenpropathrin (CAS No.- 39515-41-8) with a purity of 99.8% and 99.2% respectively, were acquired from Sigma Aldrich, Pvt, Limited. All the analytical organic solvents and reagents such as acetonitrile, acetone, sodium chloride, magnesium sulphate, and anhydrous sodium sulphate, were purchased from Merck (Darmstadt, Germany). Primary secondary amine (PSA) was supplied by Agilent Technologies Private Limited, Bangalore, India. Each of the chemicals used for the analysis was first subjected to glass distillation and then ran as reagent blank.

B. Field Experiment

The "Hisar 102" variety of Okra (*Abulmoschus esculents*) was raised following recommended agronomic practices at the Research Farm of ChaudharyCharan Singh Haryana Agricultural University, Hisar(29°10′ N latitude and 75°46′ E longitude) in a randomized block design. Sumipremt (pyriproxyfen 5% EC + fenpropathrin 15% EC) formulation was sprayed only once with the recommended (37.5 + 112.5 g a.i. ha^{-1}) and double the recommended (75 + 225 g a.i. ha^{-1}) on selected experimental plots with the knapsack sprayer. A buffer zone was left between the two fields, thus insulating one field's ecosystem from the other. Each treatment group was assigned three plots. Additionally, one of the experimental fields was left untreated to serve as a control. Approximate 200 g soil was collected using soil auger (up to 15 cm soil depth) randomly from five sites within each plot and mixed together. The samples were collected in triplicates randomly at 0 (2 h), 1, 3, 5, 7, 10, 15, 30 and 45 days after application (DAA). Samples were transported to the laboratory for the residue analysis. The soil was air dried under shade, powdered, sieved through a 2-mm sieve and stored in deep freezer (Bluestar India) at -18 °C until sample preparation and analysis. The soil collected from experimental field was characterized for various physico-chemicals properties as per the methods suggested by (Sherrod *et al.*, 2002). The soil was sandy loam in texture having 56% sand, 29.2% silt, 14.8% clay, EC 2.4 dS/m, pH 7.2 and organic carbon 0.98%.

C. Sample Preparation

Soil samples were taken in separate 50 mL centrifuge tubes and vortex with 30 mL AcN and distilled water respectively. The mixtures were shaken over rotary spin for 1h followed by centrifuge at 3500 rpm for 10 min. 5 mL of the supernatants were collected in separate centrifuge tubes. Primary secondary amine (PSA) is the frequently used sorbent, which can remove various polar pigments, some sugars and fatty acids. The supernatant was cleaned-up by using 4 g $MgSO_4$ + 0.5 g PSA. The content was vortex for 5 min before centrifuge at 3500 rpm for 10 min. The supernatants were concentrated to dryness and reconstituted with 3 mL in n-hexane for further analysis.

D. Instrumentation for Analysis

Pesticide analytes in samples were determined by GC-MS/MS (Shimadzu GC-MS TQ 8040) equipped with a capillary column (SH-Rxi-Sil MS column of 0.25 µm thick film having 30 m length and 0.25 mm internal diameter) using helium gas as the carrier gas at a constant flow rate of 1.5 mL min^{-1}. Samples were injected (1 µl) with an autosampler (20iAOC) in splitless injection mode. Temperature of the injection port was 250°C and programming of the oven temperature was done to optimize the working

conditions. The oven temperature programming began from 80^0C and remained at this temperature for 2 min, then start to increasing up to 180 °C at 20 °C/min ramp rate and attain the temperature of 300 °C, at rate of 5 °C/min and remains for 10 min. Pesticide residues could be confirmed and quantified by using GC-MS/MS in Multiple Reaction Monitoring (MRM) with a ESI(+) source of ionization throughout a scanning mass range of 40-1000 m/z,. Peaks in the total ion chromatogram of the sample recorded in MRM mode were detected based on their particular retention time (RT) and their characteristic ion peaks in the mass chromatogram. The analysis was carried out in a completely air-conditioned laboratory with a temperature of less than 22°C and a relative humidity of less than 60%.

E. Validation of Method

The method's linearity (R^2) was assessed by plotting the calibration curves (0.005-1 mg L^{-1}) of pyriproxyfen and fenpropathrin mixture standards. The LOD and LOQ values for each pesticide were set to their lowest concentrations, which produced peaks in the chromatogram three and ten times more intense than the noise, respectively. Accuracy and precision of the method have also been tested through recovery experiments in which the relevant control matrix was spiked at a concentration of 0.5, 0.25, 0.1, 0.05, 0.025, 0.01 mg kg^{-1} (n=3) and processed using the above-stated QuEChERSmethod. Precision was determined by calculating the relative standard deviation of intra-day repeatability (RSD_r) and inter-day reproducibility (RSD_R) assays. The selectivity of the method was evaluated by determining the presence or absence of any interfering peaks at the retention time of each insecticide. The robustness of a method was tested by making modest adjustments in mobile phase composition, detecting wavelength, and mobile phase flow rate. The matrix effect (ME) was also assessed by comparing the slope of the calibration curve based on matrix-matched okra standards to the slope of the pure solvent-based calibration curve. A steeper slope in the matrix calibration suggested matrix-induced signal enhancement, while a lower slope indicated signal suppression (Mondal *et al.*, 2017).

F. Soil Health Risk Assessment

The risk quotient for soil biota may be estimated using equation given below (Biswas *et al.*, 2019):

$$RQ_s = \frac{EC}{PNEC}$$

where EC is the average or maximum Pyriproxyfen and Fenpropathrin concentration in the soil (mg/kg) and PNEC is the predicted no-effect concentration used to evaluate acute toxicity. The PNEC is calculated by dividing the LC_{50} by a 1000-fold evaluation factor specific to earthworms (*Eisenia fetida*). If $RQ_s > 1$, then residues in the soil ecosystem are likely to have a harmful effect. Conversely, if $RQ_s < 0.1$, then the environmental risk would be low. Also, RQ_s between 0.1 and 1 indicate a medium risk, 0.01-0.1 low risk.

RESULTS AND DISCUSSION

A. Method Validation

The method was validated by employing the performance parameters of % mean recovery in relation to linearity, selectivity, accuracy, and precision of intra- and inter-assay analysis in spiked soil samples.

The method's quantification potential was evaluated using a linearity test, and the resultant coefficient of determination (R^2) demonstrated good linearity (0.999 and 0.999) between concentrations of Pyriproxyfen and Fenpropathrinand peak area over the calibration range of 0.005 to 1.00 mg/L (Fig. 1). The chromatographic behaviour of Pyriproxyfen and Fenpropathrin in the GC-MS/MS has been depicted in Fig. 2. The peaks for Pyriproxyfen and Fenpropathrin peaks were detected at R_t (retention time) values of 21.8, and 20.3 minutes, respectively, in the chromatogram obtained from the GC-MS/MS in MRM modes with a mass range of 40-1000 m/z. Using an ESI+ source for ionisation, scans were performed in a positive ion mode, yielding a fragmentation pattern for Pyriproxyfen with m/z 226, 136, 96, 78 and Fenpropathrin with m/z 265, 210, 172, 89. The LOQ and LOD were found to be 0.01 mg/kg and 0.005 mg/kg, respectively, which were in agreement with the values intended by Sushil *et al.*, (2017)and fulfilled the requirement of European Union, EU protocols. Similar operational conditions were found for Pyriproxyfen by Schenck *et al.* (2008), who employed GC-MS/MS in MRM modes with ESI+ ionisation source to generate a fragmentation pattern for the analyte with m/z 226.109 and 136 of the ions for confirmation of Pyriproxyfen. Cervera *et al.* (2010) also used GC-MS/MS to validate Pyriproxyfen by finding ions with m/z values of 136 and 226.Nasiri *et al.*, (2016)found very comparable conditions for confirmation and quantification of Fenpropathrin, with m/z 210 and 172. Considering the well-defined peaks (responses) of Pyriproxyfen and Fenpropathrin, GC-MS/MS was considered to be suitable for use in the present research.

Prior to any study, validation *via* recovery experiments for analytical methodologies at LOQ levels was performed on test samples of soil. The recovery chromatograms of Pyriproxyfen and Fenpropathrin are represented in Fig. 3. The test samples were processed using QuEChERS method to evaluate the residues and effectiveness of the method utilized. theQuEChERS method produce reliable results and chosen for present research due to its superior efficiency, low cost as well as reduced risk of exposure to solvents. The values of % Mean Recoveries for both insecticides at spiking levels of 0.5-0.01 mg/kg (n=3) for the QuEChERS method in soil samples ranging from 87.8 and 97.7% with RSD ≤ 7.09 % demonstrate the accuracy of the method by meeting the European Commission, 2002 guidelines for evaluating the accuracy of a procedure (% Mean recoveries must be in the range of 70-120% with the values of RSD ≤ 20 %).

The precision of the method was determined in two stages: intra-day assay (repeatability) and inter-day assay (within lab reproducibility) as provided in Table

1-2. The repeatability was represented by the $\%RSD_r$ of the data from three replicates tested on the same day using the same instrument. The intermediate precision (reproducibility) was represented by the $\%RSD_R$ of the findings of the analysis on three distinct days using the same instrument. The percent recovery values for Pyriproxyfen and Fenpropathrin intra-day assays of soil matrices processed by QuEChERS ranged from 87.8-94.3%, 88.2-92.9% with $\%RSD_r$ ranging from 3.49-6.36%, 3.48-6.02%, respectively (Table 1). The values of percent recovery for inter-day assays of soil matrices for Pyriproxyfen and Fenpropathrin ranged from 88.9-97.7%, 88.3-97.9% with $\%RSD_R$ were 4.61-6.18%, 4.80-7.09% respectively (Table 2). Our results were consistent with Ngolo *et al.* (2019) who validated an easy and efficient cleanup procedure for LC-MS/MS analysis of Fenpropathrin in soils with percent recovery ranging from 83.5-97.5% and %RSD varied between 0.69%-10.81% for the fortification levels of 0.01, 0.05 mg/kg. There was no significant difference in intra-day and inter-day assay recovery in spiked okra and soil samples processed using the QuEChERS method.

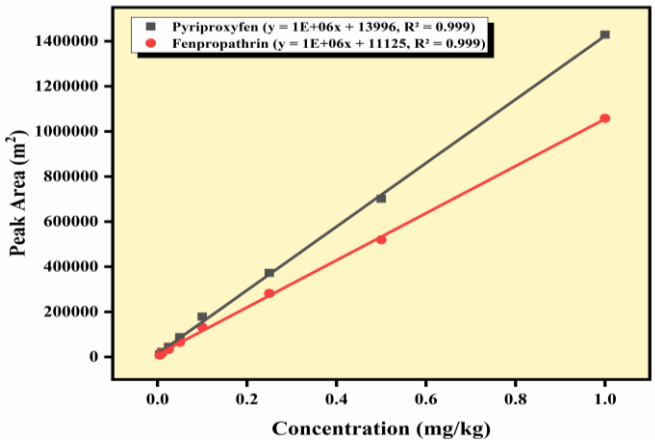

Fig. 1. Standard curve of Pyriproxyfen and Fenpropathrin on GC-MS/MS.

Table 1: Amount of Pyriproxyfen and Fenpropapthrin recovered from spiked soil samples processed on the same day by the QuEChERS (Method III).

Fortification level (mg/kg)	Pyriproxyfen		Fenpropathrin	
	Average Recoveries*±SD[a] (%)	RSD_r(%)[b]	Average Recoveries*±SD[a] (%)	RSD_r(%)[b]
0.50	89.0±5.66	6.36	92.9± 4.19	4.51
0.25	91.6±3.21	3.50	88.2±5.31	6.02
0.10	88.4±5.09	5.76	89.9±4.46	4.96
0.05	94.3±4.91	5.21	91.3±3.18	3.48
0.025	90.2±3.15	3.49	87.8±3.36	3.83
0.01	87.8±3.67	4.18	90.1±5.11	5.67

*Average of three replicates, [a] (± standard deviation), [b]RSD_r = Relative Standard Deviation for Repeatability

Table 2: Amount of Pyriproxyfen and Fenpropapthrin recovered from spiked soil samples processed on three different days by the QuEChERS (Method III).

Fortification level (mg/kg)	Day	Pyriproxyfen		Fenpropathrin	
		Average Recoveries*±SD (%)	RSD_R (%)	Average Recoveries*±SD (%)	RSD_R (%)
0.50	1	93.8±5.64	5.27	92.4± 6.49	5.85
	2	92.3±5.23		91.7±5.25	
	3	92.7±4.92		92.1±5.73	
0.25	1	91.9±6.56	6.10	90.1±7.56	7.09
	2	92.5±5.73		89.5±6.73	
	3	91.1±5.99		91.8±6.94	
0.10	1	88.9±4.16	4.61	90.3±5.38	4.80
	2	89.5±5.04		94.5±3.69	
	3	89.7±4.59		97.9±5.58	
0.05	1	89.0±5.89	5.05	96.4± 6.47	5.74
	2	91.5±3.56		94.7±5.38	
	3	97.0±5.39		93.1±5.29	
0.025	1	96.4±6.48	5.85	92.1±7.10	6.70
	2	91.2±5.45		88.3±6.09	
	3	93.1±5.55		94.0±6.87	
0.01	1	94.9±5.56	6.18	96.7±4.65	5.39
	2	95.5±5.12		94.9±7.43	
	3	97.7±7.59		92.6±3.21	

*Average of three replicates, [a] (± standard deviation), [b]RSD_R = Relative Standard Deviation for Reproducibility

Table 3: Residues of Pyriproxyfen (mg/kg) in soil from the okra field after the application of T_1 and T_2 doses.

Days after the treatment	Single dose (T_1=37.5 g a.i.ha^{-1})			Double dose (T_2=75 g a.i.ha^{-1})		
	Average residues*±SD[a] (mg/kg)	% Dissipation	RQ_s for earthworms	Average residues*±SD[a] (mg/kg)	% Dissipation	RQ_s for earthworms
0 (2h)	0.029±0.010	-	0.058	0.047±0.013	-	0.094
1	<LOQ	-	-	0.022±0.010	53.19	0.044
3	<LOQ	-	-	<LOQ	-	-
5	<LOQ	-	-	<LOQ	-	-

	Unacceptable risk (RQ_s> 1)		Moderate risk (RQ_s = 0.1-1)		Low risk (RQ_s = 0.01-0.1)		Negligible risk (RQ_s< 0.01)

LOQ = 0.01 mg/kg, LOD = 0.005 mg/kg; * Average of three replicates and[a] (± standard deviation)

Table 4: Residues of Fenpropathrin (mg/kg) in soil from the okra field days after the application of T_1 and T_2 doses.

Days after the treatment	Single dose (T_1= 112.5 g a.i.ha^{-1})			Double dose (T_2= 225g a.i.ha^{-1})		
	Average residues*±SD[a] (mg/kg)	% Dissipation	RQ_s for earthworms	Average residues*±SD[a] (mg/kg)	% Dissipation	RQ_s for earthworms
0 (2h)	0.054±0.012	-	0.293	0.097±0.010	-	0.527
1	<LOQ	-	-	0.061±0.016	37.11	0.331
3	<LOQ	-	-	<LOQ	-	-
5	<LOQ	-	-	<LOQ	-	-

	Unacceptable risk (RQ_s> 1)		Moderate risk (RQ_s = 0.1-1)		Low risk (RQ_s = 0.01-0.1)		Negligible risk (RQ_s< 0.01)

LOQ = 0.01 mg/kg, LOD = 0.005 mg/kg; * Average of three replicates and[a] (± standard deviation)

The selectivity was assessed by comparing the blank okra or soil sample with the working mix standard for peak interference. There were no interfering peaks at the retention time of each insecticide in the chromatogram of the fortified okra and soil matrices (Fig. 3). This indicated that the optimised method was selective. There was no matrix effect due to the soil matrix on the residues of pyriproxyfen and fenpropathrin. The robustness of the method was also studied by performing the same analysis with a small change in chromatographic conditions i.e. temperature of column and injector, the flow rate of mobile phase, relative humidity, etc. Due to these changes the variations in the GC – MS/MS analysis was ≤1.67 (less than 5%, according to the European Commission, 2002) indicating the robustness of the method. The results of linearity, accuracy, precision, selectivity, and robustness of our experiments complied with SANTE, and European Commission recommendations with the values of % mean recoveries falling in the 70–120% with less than 20% RSDs. Thus, the QuEChERS method was used to process the test samples and residue analysis of these processed samples was done using the optimized GC-MS/MS conditions.

B. Dissipation of pyriproxyfen and fenpropathrin in soil under field

Initial deposits of Pyriproxyfen and Fenpropathrin were found very low in the soil under the okra crop when ready-mix formulation (Pyriproxyfen 5% EC + Fenpropathrin 15% EC) applied @37.5 + 112.5 ga.i.ha^{-1} (T_1) and 75 + 225 ga.i.ha^{-1} (T_2) in the field of okra (Table 3). Both Pyriproxyfen and Fenpropathrin were completely dissipated from the soil after 1 and 3 days, respectively, following the application of T_1 and T_2 doses (Fig. 4-5). The low levels of Pyriproxyfen and Fenpropathrin(pyrethroid) residues detected in the soil are likely attributable to the fact that the ready-mix formulation was sprayed on the bushy okra crop, which covers the most land possible, rather than applied directly to the soil. High temperatures, volatilization, and uptake by the crop could have all contributed to the total loss of pesticides from the soil under the okra crop. In addition, there is no Pyriproxyfen or Fenpropathrin residue in the soil following harvest, indicating its safety for the next crop. Results were consistent with those reported by Ahlawat *et al.* (2021). They discovered that the soil under the tomato crop was uncontaminated by the premix formulation of β-cyfluthrin (pyrethroid), imidacloprid (systemic insecticide), and 6-CNA. In another study Chauhan (2019) reported that the residues of Fenpropathrin persisted for up to 15 and 30 days in soil under the chilli crop treated with 375 and 750 g a.i ha^{-1} doses, respectively. The half-life values of Fenpropathrin in the soil 6.95 and 7.94 days at the respective doses. It's possible that the discrepancies in outcomes are the consequence of using larger dosages of Fenpropathrin on the crop. Soil samples obtained from below an okra crop that had been treated with another pyrethroidbifenthrin @ 25 g a.i. ha^{-1} and 50 g a.i. ha^{-1} were found to have detectable levels of bifenthrin, according to a study by Kumari and Kumari, (2014)for a period of 7 and 15 days at the respective doses. Also, cypermethrin (pyrethroid) was shown to be persistent in soil samples collected from beneath an okra crop for 7 days by Uddin *et al.* (2016). These variations in outcomes may be attributable to the usage of different pesticides, meteorological circumstances, countries, and insecticide doses.

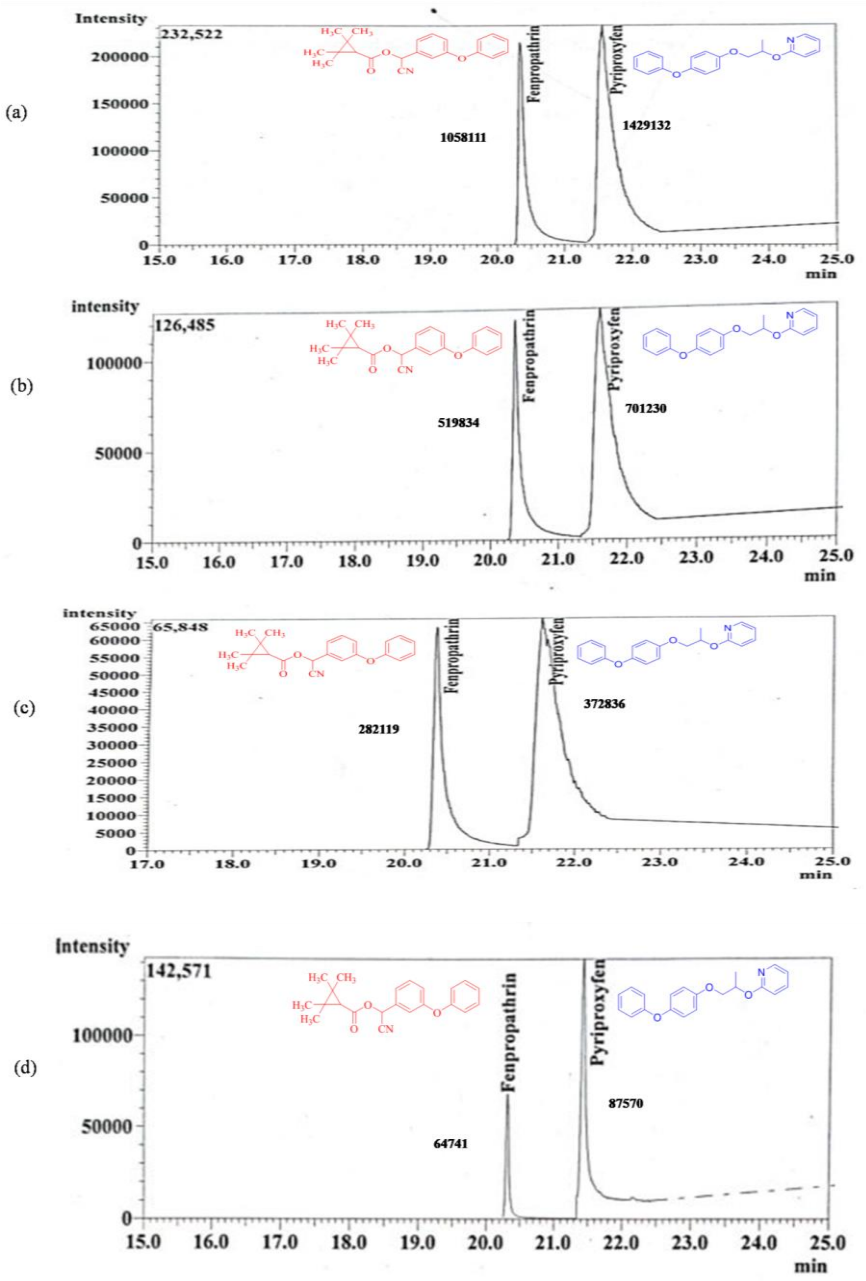

Fig. 2. Chromatogram of standard of Pyriproxyfen and Fenpropathrin on GC-MS/MS at (a) 1 mg/L (b) 0.5 mg/L (c) 0.25 mg/L (d) 0.05 mg/L

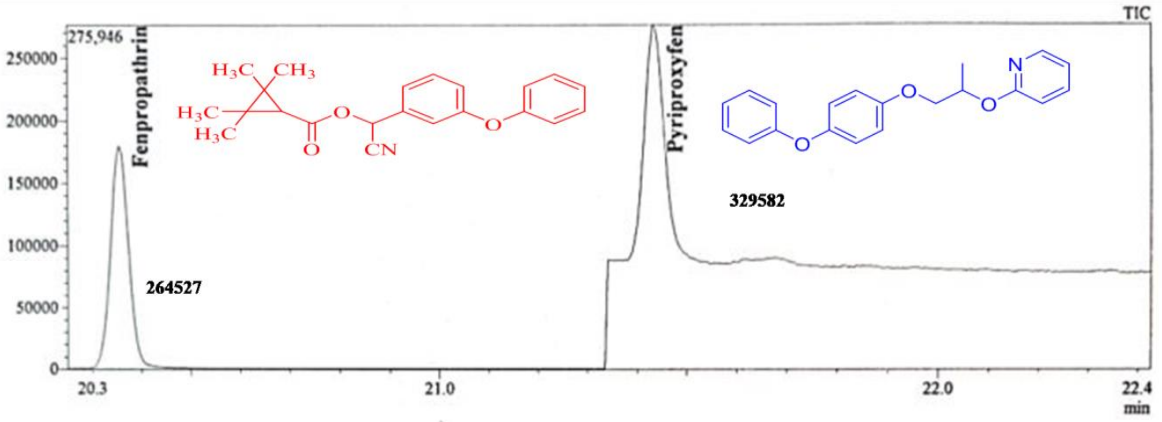

Fig. 3. Chromatograms of fortified soil samples at 0.25 mg/L processed by the QuEChERS method.

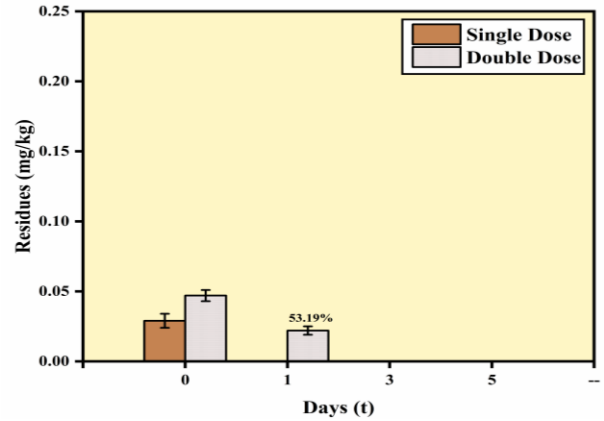

Fig. 4. Amount of residue (mg/kg) of Pyriproxyfen in soil under the okra crop at single (T_1) and double dose (T_2).

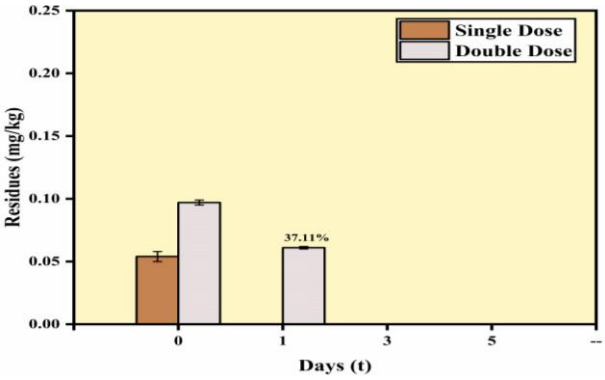

Fig. 5. Amount of residue (mg/kg) of Fenpropathrin in soil under the okra crop at single (T_1) and double dose (T_2).

C. Toxicity Risk Assesment

The risk quotient (RQ_s) was employed to estimate the possible harm that Pyriproxyfen and Fenpropathrin residues in soil may cause to earthworms (*Eisenia fetida*) (Table 3). The computed risk quotients (RQ_s) for earthworms were 0.058 and 0.094 for Pyriproxyfen residues of 0.029 and 0.047 mg/kg at T_1 and T_2, respectively, on day 0 of exposure (2 h after the application). The RQs value for residues with a concentration of 0.022 is 0.044 on double dosage (T_2) one day after application. The lower values of RQ_s (below 0.1) can be justified by the fact that the concentrations of the residues deposited by the drops of the ready-mix formulation (Sumiprempt) were less due to which there was no risk to the earthworms by these small concentrations of the Pyriproxyfen molecules. In contrast to our findings, a study by Liu *et al.*, (2019)reported the toxicity of Pyriproxyfen and its metabolites for the earthworms (*Eisenia fetida*). His findings differ from ours because the foliar spray deposits less amount of Pyriproxyfen molecules in the soil and poses a less negligible threat to the earthworms.

Risk quotient (RQ_s) values for Fenpropathrin residues of 0.054 and 0.097 mg/kg at T_1 and T_2 on day 0 (2 hours after the application) are 0.293 and 0.527, respectively (Table 4). The RQs value for 0.061 residues is 0.331 on double dosage (T_2) one day after application. The fact that all of the RQ values for Fenpropathrin residues at both dosages are between 0.1-1 indicates that there is a moderate threat posed to earthworms by the presence of Fenpropathrin. Approximately similar results were reported by Zhang *et al.* (2022) indicating that both the enantiomeric forms of Fenpropathrinposses a risk to the earthworms present in the soil. Similarly, it has also been reported that the first pyrethroidtefluthrin used for soil treatment also poses threat to the earthworms (*Eisenia fetida*) present in the soils (Wen *et al.*, 2020).

CONCLUSIONS

It can be concluded from the above results that the methodology used for the extraction and cleanup of soil samples was simple, sensitive, selective, and repeatable and could be extended for monitoring various formulations based on the above premix formulation. The application of sumipremt formulation results in a very low amount of pyriproxyfen and fenpropathrin residues in soil under the cover of the okra crop. Due to the high temperature and humidity conditions in the field, residues persisted only for 1 day after the application (DAA). These lower amounts of pyriproxyfen and fenpropathrin, however, posed low and moderate risks to earthworms. Consequently, if farmers do not comply with recommended doses, residues of pyriproxyfen and fenpropathrin will pose an unacceptable risk to earthworms in the soil under the okra crop.

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Conflict of Interest. None.

REFERNCES

Ahlawat, S., Chauhan, R., Malik, K., Yadav, S. S. and Kumari, N. (2021). Persistence and processing effects in reduction of residues of β-cyfluthrin + imidacloprid and its metabolite in hot pepper. *International Journal of Environmental Analytical Chemistry*, *101*(3), 411–421.

Arya, A. K., Singh, A. and Bhatt, D. (2019). Pesticide applications in agriculture and their effects on birds: An overview. *Contaminants in Agriculture and Environment: Health Risks and Remediation*, 129–137.

Biswas, S., Mondal, R., Mukherjee, A., Sarkar, M. and Kole, R. K. (2019). Simultaneous determination and risk assessment of fipronil and its metabolites in sugarcane, using GC-ECD and confirmation by GC-MS/MS. *Food Chemistry*, *272*, 559–567.

Cervera, M. I., Medina, C., Portolés, T., Pitarch, E., Beltrán, J., Serrahima, E., Pineda, L., Muñoz, G., Centrich, F. and Hernández, F. (2010). Multi-residue determination of 130 multiclass pesticides in fruits and vegetables by gas chromatography coupled to triple quadrupole tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *397*(7), 2873–2891.

Chang, C. S., Yen, J. H., Chen, W. C. and Wang, Y. S. (2012). Soil dissipation of juvenile hormone analog insecticide pyriproxyfen and its effect on the bacterial community. *Journal of Environmental Science and Health, Part B*, *47*(1), 13–21.

Devillers, J., Farret, R., Girardin, P. P. Rirvière, J.-L., and Soulas, G. (2005). *Indicateurs pour évaluer les risques liés à l'utilisation des pesticides*. Tec et Doc.

Galand, P. E., Lucas, S., Fagervold, S. K., Peru, E., Pruski, A. M., Vétion, G., Dupuy, C. and Guizien, K. (2016). Disturbance increases microbial community diversity and production in marine sediments. *Frontiers in Microbiology*, *7*(DEC), 1–11.

Kanawi, E., Budd, R., and Tjeerdema, R. S. (2013). Environmental fate and ecotoxicology of fenpropathrin. *Reviews of Environmental Contamination and Toxicology*, 77–93.

Kumari, S. and Kumari, B. (2014). Persistence of Bifenthrin in Soil under the Cover of Okra Crop. *Pesticide Research Journal*, *26*(1), 78–81.

Lewis, K. A., Tzilivakis, J., Warner, D. J. and Green, A. (2016). An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment*, *22*(4), 1050–1064.

Liu, H., Wang, P., Zhou, Z. and Liu, D. (2017). Enantioselective dissipation of pyriproxyfen in soils and sand. *Chirality*, *29*(7), 358–368.

Liu, H., Yi, X., Bi, J., Wang, P., Liu, D. and Zhou, Z. (2019). The enantioselective environmental behavior and toxicological effects of pyriproxyfen in soil. *Journal of Hazardous Materials*, *365*, 97–106.

Mankar, R. B., Kulkarni, U. S. and Madankar, S. S. (2019). Base line toxicity of combination insecticides against okra shoot and fruit borer, *Earias vittella* (Fabricius) (Lepidoptera : Noctuidae). *Journal of Entomology and Zoology Studies*, *7*(1), 652–655.

Miglani, R. and Bisht, S. S. (2020). World of earthworms with pesticides and insecticides. *Interdisciplinary Toxicology*, *12*(2), 71–82.

Mondal, R., Kole, R. K. and Bhattacharyya, A. (2017). Validation of Multiresidue Method for Analysis of 31 Pesticides in Rice Using Gas Chromatography-Tandem Mass Spectrometry. *Journal of AOAC International*, 1094–1101.

Nasiri, A., Amirahmadi, M., Mousavi, Z., Shoeibi, S., Khajeamiri, A. and Kobarfard, F. (2016). A multi residue GC-MS method for determination of 12 pesticides in cucumber. *Iranian Journal of Pharmaceutical Research*, *15*(4), 809–816.

Navickiene, S., Polese, L., Minelli, E. V. and Ribeiro, M. L. (1999). A simplified method for the determination of fenpropathrin residues in fruits. *Chromatographia*, *49*(3–4), 212–214.

Ndakidemi, B., Mtei, K. and Ndakidemi, P. A. (2016). Impacts of Synthetic and Botanical Pesticides on Beneficial Insects. *Agricultural Sciences*, *07*(06), 364–372.

Ngolo, P., Nawiri, M., Machocho, A. and Oyieke, H. (2019). Pesticide Residue Levels in Soil, Water, Kales and Tomatoes in Ewaso Narok Wetland, Laikipia, County, Kenya. *Journal of Scientific Research and Reports*, *24*(5), 1–11.

Pérez-Lucas, G., Vela, N., El Aatik, A. and Navarro, S. (2019). Environmental Risk of Groundwater Pollution by Pesticide Leaching through the Soil Profile. *Pesticides - Use and Misuse and Their Impact in the Environment*.

Romeh, A. A., Hendawi, M. Y. and others. (2014). Dissipation of fenpropathrin residues in squash fruits intercropped with garden rocket. *Afr. J. Agric. Res*, *9*(3), 307–309.

Schenck, J. J., Brown, A. N., Podhorniak, L. V., Parker, A., Reliford, M. and Wong, J. W. (2008). A rapid multiresidue method for determination of pesticides in fruits and vegetables by using acetonitrile extraction/partitioning and solid-phase extraction column cleanup. *Journal of AOAC International*, *91*(2), 422–438.

Sherrod, L. A., Dunn, G., Peterson, G. A. and Kolberg, R. L. (2002). Inorganic carbon analysis by modified pressure-calcimeter method. *Soil Science Society of America Journal*, *66*(1), 299–305.

Sushil, Reena Chauhan, S. B. and B. K. (2017). Gas Chromatographic Studies of Fenpropathrin on Chilli. *International Research Journal of Mathemathics, Engineering and IT*, *4*(1), 34–42.

Uddin, A., Hossain, M. S., Rahman, M. M., Rahman, S. and Choudhury, M. A. R. (2016). Cypermethrin Chemodynamics in Okra Crop Agroecosystem in Bangladesh. *Science Letters*, *4*(3), 185–189.

Wen, Y., Zhou, L., Li, D., Lai, Q., Shi, H. and Wang, M. (2020). Ecotoxicological effects of the pyrethroid insecticide tefluthrin to the earthworm *Eisenia fetida*: A chiral view. *Environmental Research*, *190*.

Xiao, J. J., Wang, F., Ma, J. J., Xu, X., Liao, M., Fang, Q. K. and Cao, H. Q. (2021). Acceptable risk of fenpropathrin and emamectin benzoate in the minor crop Mugua (Chaenomeles speciosa) after postharvest processing. *Environmental Pollution*, *276*, 116716.

Zhang, P., Yang, F., Shi, L., Yang, C., Chen, Q., Hu, X., Zhang, Z., Qian, K., Xu, Z. and He, L. (2022). Enantiomer-Specific Study of Fenpropathrin in Soil-Earthworm Microcosms: Enantioselective Bioactivity, Bioaccumulation, and Toxicity. *Journal of Agricultural and Food Chemistry*, *70*(41), 13152–13164.

Zhang, P., Yu, Q., He, Y., Zhu, W., Zhou, Z. and He, L. (2017). Chiral pyrethroid insecticide fenpropathrin and its metabolite: Enantiomeric separation and pharmacokinetic degradation in soils by reverse-phase high-performance liquid chromatography. *Analytical Methods*, *9*(30), 4439–4446.

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