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ANALYTICAL METHOD FOR DETERMINATION OF RESIDUES, DISSIPATION KINETICS AND HEALTH-RISK ASSESSMENT OF CHLORANTRANILIPROLE AND FIPRONIL IN MORINGA (*MORINGA OLEIFERA* LAM.) FLOWERS

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Abstract

A modified QuEChERS method was adapted to develop an analytical technique for the simultaneous detection, identification, and quantification of chlorantraniliprole and fipronil residues in moringa flowers. The employed LC-MS/MS instrument configuration exhibited excellent linearity ($r^2 > 0.99$) in case of both insecticides. Moreover, the method adhered to the SANTE guidelines in terms of recovery (70% - 120%), precision (RSD < 20%), and accuracy. The LOD and LOQ were set respectively at 0.007 and 0.025 mg kg^{-1}. Additionally, it demonstrated minimal matrix interference, with a recorded value of less than 20% for matrix interference. In addition to this, the method was employed to analyze the dissipation kinetics of chlorantraniliprole and fipronil on moringa flowers with the help of a supervised field trial. Subsequently, the half-lives and safe waiting periods were worked out for recommended dose (X) and double dose (2X) for both insecticides. The half-lives of the test insecticides applied to the moringa flowers were – 7.57 and 8.27 days for chlorantraniliprole at X and 2X dosages, and 6.36 and 6.97 days for fipronil, respectively. The data thus obtained, for the two chemicals were used to further compute the EDI (estimated daily intake) and hazard quotients (HQ) for both chlorantraniliprole and fipronil. Since MRL values were not available for the insecticides on edible flowers, the LOQ (0.025 mg kg^{-1}) was taken as the default MRL as per the recommendation of European Commission. It was found that, although both the insecticides were persistent in the moringa flowers, fipronil alone posed a threat to the consumers in causing acute safety hazard, at least up to one week of spraying.

Keywords : *Moringa oleifera*, chlorantraniliprole, fipronil, LC-MS/MS, residue analysis, dissipation kinetics, method validation.

1. Introduction

Moringa (*Moringa oleifera* Lam.) is a versatile plant that serves multiple purposes. As a food source, its leaves, pods, and flowers are consumed for their nutritional value, providing essential vitamins, minerals, and proteins (Anwar et al., 2007). Medicinally, *Moringa oleifera* is known for its potential health benefits, including anti-inflammatory, antimicrobial, and antioxidant properties (Farooq et al., 2012). Moreover, sustainable cultivation of *Moringa oleifera* and potential for combating malnutrition make it a valuable resource for food security and nutrition programs worldwide. Moringa flowers, derived from the *Moringa oleifera* tree, possess remarkable nutritional significance. From a consumption standpoint, flowers can be prepared in various ways. They can be cooked and incorporated into other dishes or fried using butter. Another option is to steep them in hot water to create a floral-infused tea that can be enjoyed as a beverage (Sandeep et al., 2018). Packed with essential vitamins and minerals, these delicate blossoms provide a valuable addition to a well-rounded diet. Moringa flowers are a rich source of vitamin C (Ahmed et al., 2016), which supports immune function, collagen production, and antioxidant activity. Moringa flowers possess hypocholesterolemic and anti-arthritic properties, and they have the potential to alleviate urinary problems and symptoms of the common cold. These flowers contain calcium, potassium, and amino acids, in addition to nectar, making them a valuable resource for beekeeping (Mishra et al. 2022). Moringa flowers are available throughout the drier parts of the year in the Indian sub-continent and hence consumed more often during these months. Moringa is attacked by about 78 different insect and non-insect pests (Kotikal and Math, 2016) and is hence continuously subjected to insecticides by the moringa growers. Moringa growing belts of Tamil Nadu, India – consisting of Theni, Madurai, Dindigul, Thoothukkudi and Tiruppur districts – were surveyed and results showed that chlorantraniliprole and fipronil were among the most commonly employed pesticides for pest management in the moringa belts (Ramesh et al., 2023a). Although no previous literatures are available on the residues on moringa flowers, some related studies on edible flowers reveal that these insecticides often exceeded MRL standards and posed acute and long-term effects on the human health (Wu et al., 2020; Gu et al., 2021; EFSA, 2018). The prime objective of the current research was to develop a reliable approach to measure the levels of insecticide residues and examine the dissipation patterns of chlorantraniliprole and fipronil on Moringa flowers. Additionally, the study aimed to assess the potential health risks associated with these insecticides.

2. Materials and Methods

2.1. Chemicals

The Certified Reference Material (CRM) of chlorantraniliprole with a purity of 98.3% was sourced from M/S Sigma-Aldrich in Bengaluru, India, while the CRM of fipronil with purity of 98.5% was obtained from Bayer AG, Crop Science Division, Frankfurt. The commercial formulations of chlorantraniliprole 18.5% SC (Coragen®, FMC India Pvt. Ltd.) and fipronil 5% SC (Regent®, Bayer Crop Science) were purchased from a local pesticide shop in Coimbatore, Tamil Nadu, India. Acetonitrile of HPLC grade was acquired from M/s. Sigma Aldrich in Bangalore, India, and acetonitrile (MS-grade) for mobile phase in LC-MS/MS was supplied by Sisco Research Laboratories in Mumbai, India. Sodium chloride (NaCl), anhydrous magnesium sulphate, and sorbents such as primary secondary amine (PSA), and graphitized carbon black (GCB) were obtained from various sources. Anhydrous magnesium sulphate was purified using acetone and baked in a muffle furnace at 600 °C for 4 hours to eliminate potential phthalate impurities. Formic acid of LC-MS grade with a purity

of 99% was acquired from Fisher Scientific Limited in Czech Republic. Ultra-pure water (Type-I water, 18.2 MΩ·cm @ 25 °C) was obtained from the Millipore Water Purification System.

2.2. Preparation of stock solutions

Individual stock solutions of chlorantraniliprole and fipronil, each with a concentration of 400 mg/l, were prepared in LC-MS grade acetonitrile (ACN) by accurately weighing 10.17 mg and 10.28 mg of the respective analytical standards and dissolving them in a calibrated, graduated 25 ml glass volumetric flask. Intermediate stock solutions of chlorantraniliprole and fipronil, with a concentration of 40 mg/l (40 ppm), were prepared by mixing 100 µl of the primary stock solution with 900 µl of LC-MS grade ACN. Similar procedures were followed to prepare intermediate solutions of 10 ppm and 1 ppm in LC-MS grade ACN. Working standard solutions in the range of 0.025 µl/l, 0.0625 µl/l, 0.125 µl/l, 0.1875 µl/l, and 0.250 µl/l were obtained by diluting precise volumes of the intermediate stock solutions in LC-MS grade ACN. These working standards were utilized for the linearity study and for spiking in the recovery study. All standard solutions were stored under refrigerated conditions (at -20°C) in a deep freezer until used. Matrix-matched standard solutions (0.025 mg/l, 0.0625 mg/l, 0.125 mg/l, 0.1875 mg/l, and 0.250 mg/l) were prepared using the moringa flower matrix.

2.3. Method validation

Adequate quantities of unsprayed moringa flowers were collected from an organic moringa field to study essential parameters for validating the residue analysis method. A modified QuEChERS method (Anastassiades et al., 2003) was developed specifically for moringa flowers. Five grams of homogenized sample were placed in a 50 ml polystyrene centrifuge tube. Homogenization of the moringa samples was achieved using a Robot Coupe mixer blender (Blixer 6VVA, France), and the excess samples were stored under appropriate refrigeration conditions (-20°C). Since the samples were in a semi-dry state, 10 ml of Millipore water was added to increase their moisture content. Additionally, 10 ml of LC-MS grade CH3CN was added. The tube was thoroughly shaken and vortexed for one minute. Subsequently, 4g of anhydrous magnesium sulfate ($MgSO_4$) and 1g of sodium chloride (NaCl) were added to the mixture, which was again shaken and vortexed. The mixture was then centrifuged at 6000 rpm for 10 minutes. Following centrifugation, 6 ml of the clear supernatant solution was carefully pipetted and transferred to an 18 ml centrifuge tube pre-filled with a sorbent mixture. The sorbents, namely anhydrous magnesium sulfate, primary-secondary amine (PSA), and Graphitized Carbon Black (GCB), were used in the proportions of 600 mg, 25 mg, and 10 mg, respectively. The tube was thoroughly shaken and vortexed for an additional minute. It was then subjected to centrifugation at 3500 rpm for 10 minutes. After centrifugation, a specific volume of the clear supernatant liquid or aliquot layer (4 ml) was pipetted into a clean glass test tube. The aliquot was subsequently dried under a stream of nitrogen gas at a temperature not exceeding 40°C and a pressure set at 30 psi using a turbovap system until complete dryness. Then, 1 ml of LC-MS grade acetonitrile was added to the dried matrix, allowing it to mix. The solution was filtered into 1.8 ml LC glass vials using glass syringes fitted with 0.22µ nylon membrane filters, and finally analyzed using LC-MS/MS.

2.4. Field experiments

The dissipation study was carried out during March 2022 - May 2022 in a farmer's field at Karamadai, Coimbatore, Tamil Nadu (11.24558° N, 76.9587° E; 353 m above MSL). In the field, the cultivation of the PKM-1 variety of moringa crop was carried out in adherence to good agricultural practices (GAPs), with no previous history of pesticide application.

The experiment was conducted within a designated area measuring 250 m^2, employing a square planting system for the cultivation of moringa crop, adopting a planting distance of 8 feet between rows as well as the plants. The application of chlorantraniliprole and fipronil was carried out using two different doses, namely the recommended dose (X dose) and twice the recommended dose (2X dose), in randomized plots with three replications. Chlorantraniliprole 18.5 SC was administered at single doses of 3 ml and 6 ml per 10 L of water, while fipronil 5% SC was applied at single doses of 3 g and 6 g per litre of water, respectively. Commercial formulations of chlorantraniliprole 18.5 SC (Coragen®, FMC India Pvt. Ltd.) and fipronil 5 SC (Regent®, Bayer Crop Science) were sprayed 30 days after flower initiation, when there are sufficient quantities of flowers available on the moringa crop. The spraying was carried out using battery-operated knapsack sprayers to ensure comprehensive coverage of foliage and flowers, with two applications performed at ten-day intervals. Samples were collected starting from the day of the second spray, including time points of 0 DAS (2 hours after the second spray), as well as subsequent days at intervals of 1, 3, 5, 7, 10, 15, 20, 25, and 30 days post-spraying. Throughout the study period, temperature observations ranged from 21.07 to 23.55°C for the minimum and 26.51 to 34.53°C for the maximum, while no rainfall occurred. The mean relative humidity during the experiment was recorded as 72.51%.

2.5. Instrumentation

The experimental setup involved a Waters Alliance LC-MS/MS system equipped with a C_{18}, 5 µm (4.8 x 250 mm) column. Two mobile phases, referred to as Mobile phase A and Mobile phase B, were used. Mobile phase A consisted of acetonitrile with 0.1% formic acid, while Mobile phase B consisted of type-I water with 0.1% formic acid. The mobile phases were mixed in a fixed ratio of 70:30, with acetonitrile representing mobile phase A and ultrapure water constituting mobile phase B. The flow rate was set to 0.5 mL min^{-1}, and the total run time for the analysis was 13 minutes. For detection of the compounds, an Acquity Tandem Quadrupole Mass Detector (TQD) with Electrospray ionization (ESI) interface from Waters, USA was utilized. The ionization of the two analytes differed, with chlorantraniliprole being positively ionized and fipronil showing negative ionization. The capillary voltage was maintained at 3.5 kV. The desolvation and cone gas flows were set at 1100 and 50 L h^{-1}, respectively, while the collision gas flow was 0.18 mL min^{-1}. The source block temperature was set to 150°C, and the desolvation temperature was set to 500°C. Nitrogen was used as the desolvation gas, and argon served as the collision gas. To introduce the compounds into the detector, a working standard concentration of 0.5 µg mL^{-1} was used for both chlorantraniliprole and fipronil. Mass Lynx Software was employed to calibrate the lens voltages, and the detection mode used was Multiple Reaction Monitoring (MRM) for the two compounds.

2.6. Authentication of method

The present investigation focused on examining the developed analytical methods for the detection of chlorantraniliprole and fipronil residues in the moringa leaf matrix. The assessment was conducted according to the guidelines outlined by SANTE (2019). Various parameters including linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, precision, accuracy, and matrix effect were examined. To evaluate linearity, a mixture of standards for the two insecticides was injected at five different concentrations ranging from 0.025 to 0.25 mg kg-1 in increasing order. The injections were replicated three times. The insecticide solutions were prepared using LC-MS grade acetonitrile. Calibration curves were

constructed for each analyte, determining the regression equation, intercept values, and slope. Matrix match samples were also analyzed across different concentration ranges to investigate the potential matrix effect. The LOD and LOQ values were calculated based on the standard deviations of the pesticides observed during the recovery tests, in addition to Student's t-test. Six leaf samples spiked with a combination of insecticides at a concentration of 0.025 mg kg^{-1} were separately analyzed using the standard methodology to obtain mean and standard deviation values. The standard deviation of the standard error of the intercept was calculated from the observations. This value was divided by the coefficient of the X-variable obtained from the data analysis. To obtain the LOD and LOQ, the resulting value was multiplied by factors of 3.3 and 10, respectively. Accuracy was defined by comparing the results of the recovery experiment, while precision was determined by observing the relative standard deviation during the analysis of the analytes. The recovery was validated by spiking the organic moringa leaf matrix at five different levels (0.025, 0.0625, 0.125, 0.1875, 0.250 mg kg-1) with seven replications and comparing it with control samples. To provide conclusive confirmation of a target compound initially identified by RT, the criteria specified in SANCO 12495/2011 (SANCO, 2011) were followed.

2.6.2 Matrix effect

In residue analysis of insecticides, the matrix effect refers to the influence or interference (suppression or enhancement of signal) caused by components present in the sample matrix on the analytical measurement of the insecticide residues (Rutkowska et al., 2019; Eslami et al., 2021). The sample matrix can contain various substances such as plant materials, fats, proteins, sugars, and other organic compounds that may impact the accuracy and reliability of the analysis. These matrix components can affect the ionization efficiency, chromatographic separation, and detection response of the target insecticides, leading to potential signal enhancement or suppression. Matrix effects are particularly important to evaluate in order to ensure accurate quantification and reliable results in residue analysis. Hence, the matrix effect for moringa flowers was evaluated at various concentration ranges and calculated using the following formula:

$$\text{Matrix effect (in \%)} = \left(1 - \frac{S}{MM_{std}}\right) \times 100$$

where,

MM_{Std} - Matrix match standard peak area

S – Solvent standard peak area

A negative value of the matrix effect (ME) indicates an average percentage suppression, indicating that the presence of matrix components in the sample matrix leads to a decrease in the measured peak area of the analyte. On the other hand, a positive value of the matrix effect represents enhancement in peak area, indicating that the matrix components contribute to an increase in the measured peak area of the analyte (Shabeer et al., 2019).

2.7. Calculations

The quantification of chlorantraniliprole and fipronil residues were performed utilizing the prescribed formula (Hoskins, 1961) and parameters obtained from the chromatogram, as follows:

$$\text{Residues } (\mu g/g) = \frac{A_{sp}}{A_{sd}} \times \frac{W_{sd}}{W_{sp}} \times \frac{V_{sd}}{A_{si}} \times V_s$$

where,

A_{sp} – Peak area of sample

A_{sd} – Peak area of standard

W_{sd} – Weight of the standard (in ng)

W_{sp} – Weight of the sample (in g)

V_{sd} – Volume of the standard injected (in µl)

A_{si} – Volume of aliquot injected (in µl)

V_s – Final volume of sample extract (in ml)

The values of the two areas, namely the sample peak area and the standard peak area, within the aforementioned parameters are determined based on the peaks observed in the chromatogram. Subsequently, the half-life, MRL (maximum residue limit), and safe waiting periods were calculated for chlorantraniliprole and fipronil on moringa flower matrix. The half-lives were determined using the formula 0.693/k, where 'k' represents the slope of the dissipation curve, estimated from the regression equation of the first-order reaction, thereby obtaining the slope value. Further, the safe waiting period, which denotes the maximum time interval during which the residue levels decrease below the MRL value, for the tested insecticides, was quantified using the following formula:

$$\text{Safe waiting period (In days)} = \frac{\text{Log } (C_0) - \text{Log (MRL)}}{k}$$

where,

C_0 = initial concentration of the residues

MRL = maximum residue limit for the commodity

K = dissipation constant (obtained from the slope of the dissipation curve)

Moringa oleifera flowers are a rather novel commodity which has come under the light in the recent times and hence, the MRL values for the same are not available for India. There has been no mention of the MRL of moringa flowers by the international regulatory authorities as well. Moringa flowers fall under the category of herbs and edible flowers as defined by the international bodies. And since the MRL values are not available, the lowest value of the test doses which could be quantified (LOQ) by the instrument (LC-MS/MS) in this experimental set-up (0.025 mg L^{-1}) could be taken as the default MRL. This is in accordance with the Article 12 of European Commission for procedures involving MRL data gap (European Commission, 2022). The same methodology has been employed for investigating the dissipation of neonicotinoid insecticides in pomegranate by Mohapatra et al. (2019) wherein, the standard MRL (maximum residue limit) values for these insecticides were not available. This approach has also been extensively discussed in a review by Charon et al. (2019), particularly focusing on substances that are not subjected to the MRLs established by regulatory authorities.

2.8 Hazard Quotient and assessment of risk

To evaluate the level of risk, the hazard quotient (HQ) is determined by evaluating the ratio of estimated daily intake (EDI) to the acceptable daily intake (ADI). The HQ value provides an indication of the relative safety of consuming a particular non-cancerous product (Eslami et al., 2021). If the HQ value exceeds 1, it signifies that the commodity is considered unsafe for consumption, while a value below 1 suggests it is safe. Due to the unavailability of ADI values for chlorantraniliprole and fipronil from the Food Safety and Standards Association of India, the ADI values established by the European Union (EU) were utilized instead. The ADI values for chlorantraniliprole and fipronil were respectively set at 1.56 (EFSA, 2020) and 0.00002 mg/kg body weight (FAO, 2016). It is worth noting that the average body

weight of a typical adult in the Indian context is approximately 55 kg (Mukherjee & Gopal, 2000). Moringa flowers unlike pods or leaves do not form an essential part of routine diet. The availability of moringa is also restricted to the dry months (Haldar and Kosankar, 2017). Considering the above, it could be concluded that moringa flowers are consumed about 30g (one serving) per person, per day. The same has been validated by the study carried out by Kavitha Shree et al. (2023). Based on the information provided, the estimated daily intake can be calculated as follows:

$$\text{EDI (mg/kg/day)} = \frac{\text{Maximum residue concentration (mg/kg) x Daily intake of food (kg/day)}}{\text{Average body weight of the adult (kg)}}$$

3. Results and Discussion

3.1. Optimization of instrument conditions

The triple-quadruple LC-MS/MS conditions were fine-tuned to detect and quantitatively determine the chlorantraniliprole and fipronil residues from moringa flowers. For chlorantraniliprole, the instrument operated in positive ionization mode (ESI+), while for fipronil, it operated in negative ionization mode (ESI-). Both analytes were analyzed using multiple reaction monitoring (MRM), and their parent ions, chlorantraniliprole (482.13) and fipronil (435.026), were detected in a single run at retention times (RT) of 7.21 and 10.68 minutes, respectively (Fig. 3). Simultaneously, the daughter ions for chlorantraniliprole (283.8 and 111.916) and fipronil (329.96 and 249.973) were also observed (Fig. 2). The identification of these compounds was based on the m/z ratio of the parent and daughter ions, and their specificity was confirmed by comparing the obtained area with the standard control (blank) run under the same instrument conditions.

3.2. Identification of compounds

The examination of pesticide residues in moringa flower samples required the utilization of various factors for identification. These elements consist of the pesticide retention time (RT) details in the chromatogram (Figure 4), the existence of two transitions in the tandem mass spectrum, and the ion ratio associated with these transitions. To determine the analyte's retention time, a calibration standard was employed.

3.3 Validation of the Method

To validate the method, five concentrations ranging from 0.025 to 0.25 mg kg^{-1} were assessed for both chlorantraniliprole and fipronil to construct a linearity curve.The instrument's response to the increasing concentrations (0.025, 0.0625, 0.125, 0.1875, 0.250 mg kg^{-1}) was recorded. The correlation coefficient (r^2) values exceeded 0.99 for both insecticides (Table 1), indicating a strong correlation and excellent linearity. Similar validation procedures were also performed to determine the recovery percentage and relative standard deviation (RSD) in the moringa flower matrix (Table 3 and Figure 3). The limit of detection (LOD) and limit of quantification (LOQ) values were established as 0.007 and 0.025 mg kg^{-1}, respectively (Table 2).

Moringa flowers, although deemed as a popular delicacy in most Indian states like Tamil Nadu and West Bengal, it is severely undermined by the scientific community, as indicated by the meagre amount of works carried out on the same. Moringa crop is subjected to regular insecticide sprayings, which lead to direct or indirect deposits on the moringa flowers, thus entering the diet of the consumer. The commodity belongs under herbs and edible flowers category, for which hardly a handful pesticides have been recommended for MRL, let alone for moringa flowers.

Since there is an MRL-gap, the limit of quantification (LOQ) value established in this study is considered as the default MRL, as recommended in Article 12 of European Commission Regulation (EC) No. 396/2005 procedures (EC, 2022). Recovery studies on moringa flowers yielded recoveries ranging from 95.74 to 106.74% for chlorantraniliprole and 89.99 to 113.48% for fipronil, which is well within the guidelines set by SANTE (2019), confirming the authenticity of the method. The precision of the arrived method, as indicated by the Relative Standard Deviation (RSD) observed in the recovery studies, remained within the prescribed values outlined in the SANTE guidelines across the entire range of concentrations where it did not exceed 8% for either analyte. Additionally, the impact of both insecticides on moringa flowers was assessed in terms of matrix effect, and it was determined to be negligible, measuring less than 20% (Table 4). Based on the above inferences, we can conclude that the developed technique is well-suited for the simultaneous identification of chlorantraniliprole and fipronil residues in moringa flowers. The method demonstrates excellent recovery and precision, ensuring accurate detection. Furthermore, the approach effectively minimizes interference from the matrix, thus enhancing the reliability of the results.

3.3 Residue dissipation kinetics of chlorantraniliprole and fipronil on moringa flowers

The investigations into the dissipation kinetics of chlorantraniliprole (Figure 1) and fipronil (Figure 2) on moringa flowers revealed that initial deposits of 2.088 and 4.254 mg kg^{-1} for X (recommended dose, 30 g a.i./ha) and 2X (double dose, 60 g a.i./ha) doses of chlorantraniliprole, respectively, observed on the day of spraying (two hours after spray) (Table 5). Similarly, fipronil exhibited initial residues of 1.190 mg kg^{-1} for X dose (75 g a.i./ha) and 2.460 mg kg^{-1} for double dose (150 g a.i./ha) (Table 6). Analysis of the dissipation reactions demonstrated that chlorantraniliprole reached below the limit of quantification (BLQ) only on the 25th day for the recommended dose and on the 30th day for the double dose. In the case of fipronil, BLQ values were achieved relatively swiftly, with the recommended dose reaching BLQ on the 15th day and the double dose on the 20th day. The half-lives of chlorantraniliprole were determined as 7.57 days and 8.23 days for the two different (X and 2X) doses, while fipronil exhibited half-lives of 6.36 and 6.97 days on moringa flowers. Both chlorantraniliprole (mol. weight = 483.1 g mol^{-1}) and fipronil (mol. weight = 437.15 g mol^{-1}) have high molecular masses, and positive log P values (octanol-water partition co-efficient or log K_{ow}) – 2.76 in case of chlorantraniliprole and 4.0 for fipronil (NCBI, 2023a; NCBI, 2023b), indicating their mobility within plant systems. Nature of crop/ plant surface and environmental factors such as temperature and windspeed may also contribute to the erratic degradation of fipronil (Singh et al., 2021). Moringa leaves and flowers contain more lipids in comparison with moringa pods (Sánchez-Machado et al., 2010). And hence, it could be theorized that both test insecticides – chlorantraniliprole and fipronil – having more affinity to hydrophobic molecules ($2.5 < k_{ow}$), are retained at a higher degree in case of flowers compared to pods, as confirmed by studies done on moringa pods by Ramesh et al. (2023b). This is also in alignment with the results obtained in the current study. The insecticides have been seen more persistent and recorded to have more initial residue in case of flowers in comparison with the moringa pods.

The current study provides valuable insights into residue analysis and risks associated in consumption of moringa flowers, which are consumed widely, throughout the country as a delicacy as well as a remedy for gastro-intestinal and

numerous other ailments. The dietary risk assessment, conducted by computing the hazard quotient (HQ) as the ratio between the variable Estimated Daily Intake (EDI) and a fixed quantity, Acceptable Daily Intake (ADI), revealed that the regular dose and double dose of chlorantraniliprole did not exceed the threshold. However, in the case of fipronil, the HQ values remained towards the upper side of the threshold value until 7th DAS in case of X dose and 10th DAS in case of 2X dose, subsequently receding to non-hazardous levels. This result suggests a potential acute health hazard associated with the consumption of fipronil-sprayed moringa flowers until at least a week of spraying. Safe waiting periods for both insecticides and doses were calculated using a default Maximum Residue Limit (MRL) of 0.025 mg kg^{-1}, indicating that a waiting period of 21.00 days and 26.62 days for chlorantraniliprole at X and 2X doses, respectively, and 15.39 and 20.05 days for fipronil, is necessary to ensure residue levels are below the MRL.

4 Conclusions

The core objective of this study was to investigate the degradation kinetics of chlorantraniliprole and fipronil on moringa flowers. To achieve this, we developed a modified QuEChERS method, which demonstrated favorable levels of accuracy, precision, and minimal matrix-effect. Due to the limited availability of research on moringa, there is a lack of data required to assess the persistence of these insecticides on moringa flowers and associated risks of acute and chronic consumption. In this study, we have made use of Maximum Residue Limit (MRL) of 0.025 mg kg^{-1} as a default standard for evaluating the potential risks associated with the consumption of insecticide-treated moringa flowers since there have been no works pertaining to residue analysis in moringa flowers or any other edible flowers for chlorantraniliprole and fipronil. Through supervised field trials, we extensively analyzed the dissipation kinetics to determine the half-lives of chlorantraniliprole and fipronil in moringa flowers and establish safe waiting periods. The calculated half-lives for the test insecticides on the sprayed moringa flowers were 7.57 and 8.27 days for X and 2X doses of chlorantraniliprole (Table 5), and 6.36 and 6.97 days for fipronil (Table 6), respectively. Although similar works have been conducted on moringa pods (Ramesh et al., 2023), there has been no record of researches carried out in moringa flowers, which is one among the popular summer snacks in the country. Moringa flowers, also a key ingredient in traditional medicines, demands more importance, particularly in case of residue monitoring. Reviews have recorded edible flowers like honeysuckle (*Lonicera japonica* Thunb.), which is a core ingredient in Chinese traditional medicines, had residues of fipronil (banned for use in herbal medicines in China) and chlorantraniliprole, leading to acute hazards in children as well as adults (Wu et al., 2020). Also, EFSA had reported that the MRLs were breached on multiple occasions in the import of edible flowers (EFSA, 2018). Moringa crop although a hardy crop, is susceptible to a plethora of pests and is constantly subjected to pesticide sprays. Thus, insecticide spraying in moringa, in particular fipronil, could contribute to potential acute hazards to the consumers due to longer retention of fipronil residues in moringa flowers, as demonstrated in this work. This work could launch further food-safety assessments and toxicological works in various economically important and consumable plant parts of moringa.

Declaration of competing interests

The authors report no competing interests to declare.

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Tables and Figures

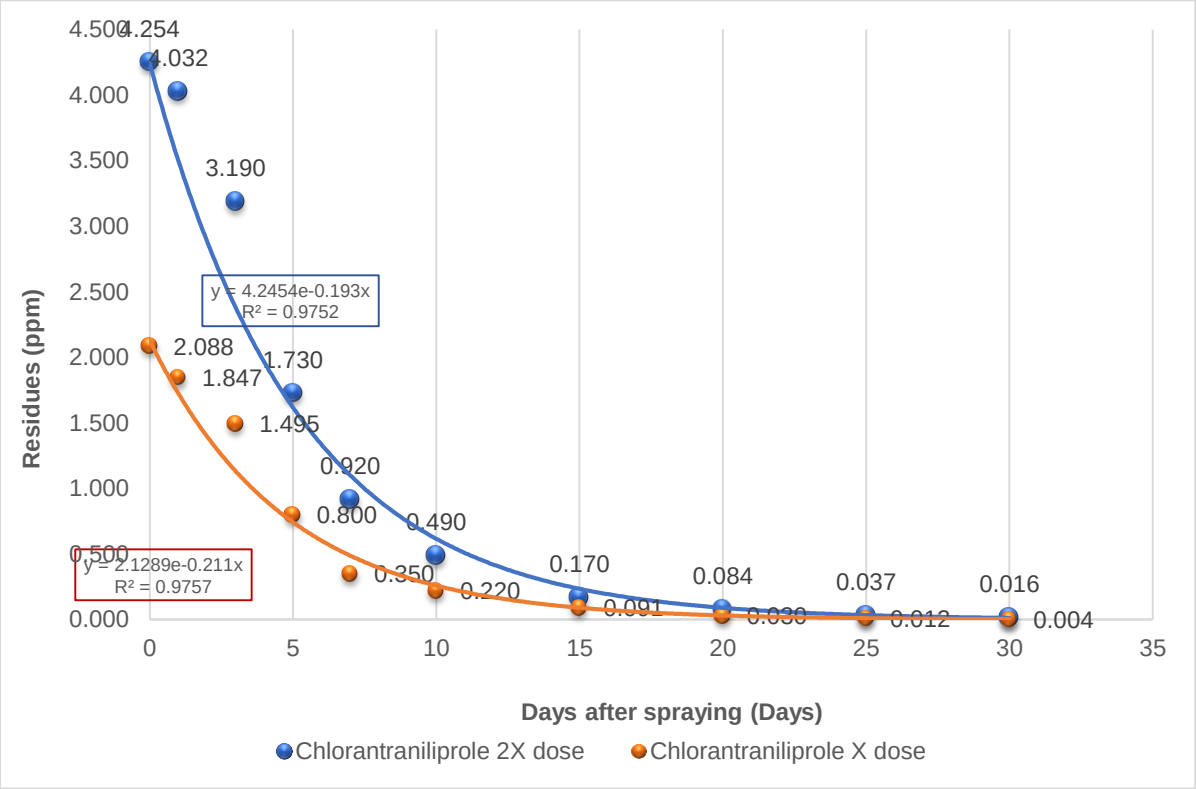


Figure 1. Dissipation pattern of chlorantraniliprole (X and 2X doses) on moringa flowers under field conditions

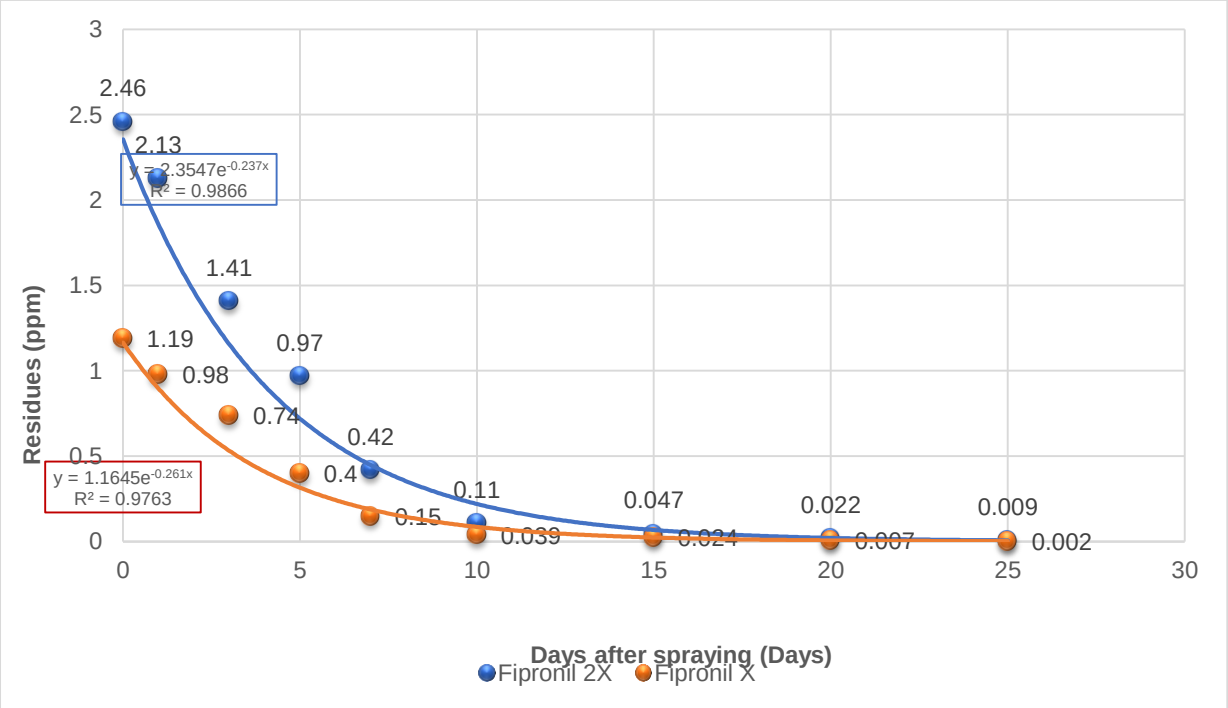
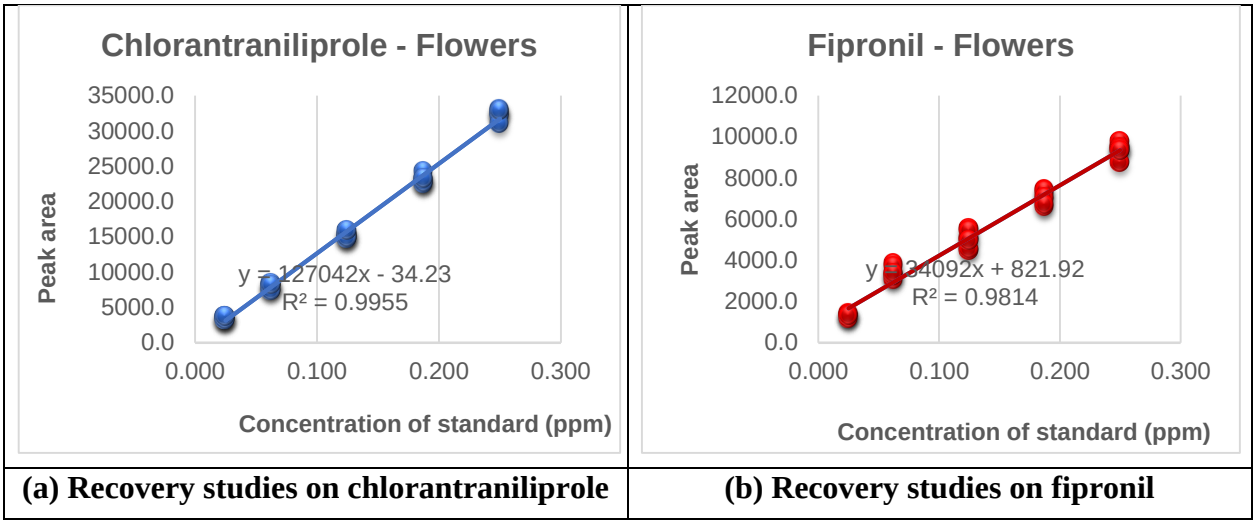
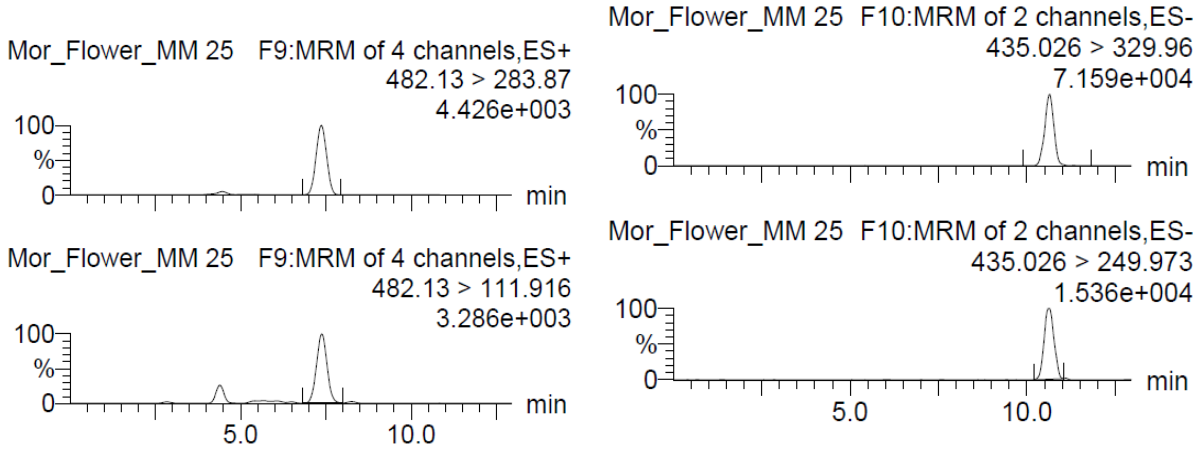


Figure 2. Dissipation pattern of fipronil (X and 2X doses) on moringa flowers under field conditions



(a) Recovery studies on chlorantraniliprole | **(b) Recovery studies on fipronil**

Figure 3. Recovery studies on chlorantraniliprole and fipronil in moringa flowers



(a) chlorantraniliprole **(b) fipronil**

Figure 4. Chromatograms of (a) chlorantraniliprole and (b) fipronil for moringa flower matrix-matched standard at LOQ (0.025 mg kg^{-1})

Table 1. MRM ions and linearity of the analyte standards in LC-MS/MS

Analyte	Parent ion	Quantifier ion	Qualifier ion	Concentration range (mg/kg)	Calibration curve	R^2 value
Chlorantraniliprole	482.13	283.87	111.916	0.025 – 0.25	124170x – 388.67	0.999
Fipronil	435.026	329.96	249.973	0.025 – 0.25	99826x +1357.80	0.991

Table 2. Linearity, LOD and LOQ of Moringa flower matrix match analyte standards

Analyte	Concentration range (mg/kg)	Moringa flower		LOD (mg/kg)	LOQ (mg/kg)
		Calibration curve	R^2 value		
Chlorantraniliprole	0.025 – 0.25	y = 127042x - 34.23	0.9955	0.007	0.025
Fipronil	0.025 – 0.25	y = 34092x + 821.92	0.9814	0.007	0.025

Table 3. Recoveries obtained at linear concentration levels for the two analytes

Matrix	Pesticides	Spiked levels (mg/kg)									
		0.025		0.0625		0.125		0.1875		0.25	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Moringa flower	Chlorantraniliprole	95.74	6.56	96.76	4.77	97.65	2.71	106.74	2.39	99.85	2.11
	Fipronil	113.48	6.51	89.99	8.42	96.18	8.05	94.81	4.49	95.55	3.37

Table 4. Matrix effect studies of chlorantraniliprole and fipronil on the moringa flowers

Matrix	Pesticides	Spiked levels (mg/kg)				
		0.025	0.0625	0.125	0.1875	0.25
		ME_1 (%)	ME_2 (%)	ME_3 (%)	ME_4 (%)	ME_5 (%)
Moringa flowers	Chlorantraniliprole	8.736	3.347	2.405	-6.316	0.146
	Fipronil	9.476	11.120	3.970	5.476	4.652

Table 5. Residues, dissipation percent and dietary risk assessment of chlorantraniliprole in moringa flowers – X and 2X doses

Chlorantraniliprole – X dose (30 g a.i./ha)						Chlorantraniliprole – 2X dose (60 g a.i./ha)					
Days after application	Residues* (mg/kg)	Dissipation (%)	Dietary risk			Days after application	Residues* (mg/kg)	Dissipation (%)	Dietary risk assessment		
			EDI (mg/kg bw)	ADI# (mg/kg bw)	HQ				EDI (mg/kg bw)	ADI# (mg/kg bw)	HQ
0	2.088	00.00	0.001139	1.56	0.00073	0	4.254	00.00	0.002320	1.56	0.00149
1	1.847	11.51	0.001008	1.56	0.00064	1	4.032	05.22	0.002199	1.56	0.00141
3	1.495	28.41	0.000815	1.56	0.00052	3	3.190	25.01	0.001740	1.56	0.00112
5	0.800	61.68	0.000436	1.56	0.00028	5	1.730	59.33	0.000944	1.56	0.00061
7	0.350	83.23	0.000191	1.56	0.00012	7	0.920	78.37	0.000502	1.56	0.00032

10	0.220	89.46	0.000120	1.56	0.00007	10	0.490	88.48	0.000267	1.56	0.00017
15	0.091	95.64	0.000050	1.56	0.00003	15	0.170	96.00	0.000093	1.56	0.00006
20	0.030	98.55	0.000017	1.56	0.00001	20	0.084	98.03	0.000046	1.56	0.00003
25	BLQ	-	-	-	-	25	0.037	99.13	0.000020	1.56	0.00001
30	BLQ	-	-	-	-	30	BLQ	-	-	-	-
Kinetic equation	y = -0.0915x + 3.3282					y = -0.0838x + 3.6279					
R^2 value	0.9947					0.9905					
Half-life (days)	7.57					8.27					
Safe waiting period (days)	21.00					26.62					

*Average of three replications; #EFSA (2020); BLQ - Below Quantifiable Limit; EDI - Estimated Daily Intake; ADI – Acceptable Daily Intake; HQ – Hazard Quotient

Table 6. Residues, dissipation percent and dietary risk assessment of fipronil in moringa flowers – X and 2X doses

Fipronil – X dose (75 g a.i./ha)						Fipronil – 2X dose (150 g a.i./ha)					
Days after application	Residues (mg/kg)	Dissipation (%)	Dietary risk			Days after application	Residues (mg/kg)	Dissipation (%)	Dietary risk assessment		
			EDI (mg/kg bw)	ADI# (mg/kg bw)	HQ				EDI (mg/kg bw)	ADI# (mg/kg bw)	HQ
0	1.190	0.000	0.0006491	0.0002	3.245	0	2.460	0.000	0.001342	0.0002	6.7091
1	0.980	17.647	0.0005345	0.0002	2.673	1	2.130	13.415	0.001162	0.0002	5.8091
3	0.740	37.815	0.0004036	0.0002	2.018	3	1.410	42.683	0.000769	0.0002	3.8455
5	0.400	66.387	0.0002182	0.0002	1.091	5	0.970	60.569	0.000529	0.0002	2.6455
7	0.150	87.395	0.0000818	0.0002	0.409	7	0.420	82.927	0.000229	0.0002	1.1455
10	0.039	96.723	0.0000213	0.0002	0.106	10	0.110	95.528	0.000060	0.0002	0.3000
15	BLQ	-	-	-	-	15	0.047	98.089	0.000026	0.0002	0.1282
20	BLQ	-	-	-	-	20	BLQ	-	-	-	-
25	BLQ	-	-	-	-	25	BLQ	-	-	-	-
30	BLQ	-	-	-	-	30	BLQ	-	-	-	-

Kinetic equation	y = -0.109x + 3.038	y = -0.0994x + 3.3493
R^2 value	0.9834	0.9809
Half-life (days)	6.36	6.97
Safe waiting period (days)	15.39	20.05

*Average of three replications; #FAO (2016); BLQ - Below Quantifiable Limit; EDI - Estimated Daily Intake; ADI – Acceptable Daily Intake; HQ – Hazard Quotient