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Development of Methodology for Estimation of Chlorantraniliprole Residues in Tomato

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ABSTRACT: Chlorantraniliprole is quite effective in management of fruit borer in tomato and approved for use in India. The continuous use of pesticides for protecting insect pests may further aggravate the problem of pesticide residues. The methodology for estimation of its residues is required. For this QuEChERS techniques with slight modification have been standardised in this study. The approach may be employed for quantitative analyte assessment in a variety of agricultural products whilst still minimising the usage of organic solvents, allowing the methodology to gain greater acceptance. The motto of this present study is to standardize and validate the QuEChERS method for the assessment of chlorantraniliprole residues in tomato. Aliquots of tomato samples were analyzed by using UHPLC- PDA with a C_{18} column. "Retention time" was observed to be 4.327 min. Mean recoveries of chlorantraniliprole in tomato samples spiked 0.5, 0.25 and 0.05 mg kg^{-1} varied about 85.33 to 87.60 per cent. LOQ and LOD were quantified to be 0.05 and 0.017 mg kg^{-1}. RSDr for chlorantraniliprole in tomato at 0.05 - 0.25 mg kg^{-1} was 2.949 to 7.306 per cent. RSD_R of chlorantraniliprole at 0.05 mg kg^{-1} was 4.671 per cent.

Keywords: QuEChERS; Chlorantraniliprole; Tomato; HPLC.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is a solanaceous vegetable with good source of nutrients and known to be widely grown throughout the world (Choudhary, 1996). Tomatoes are grown on 811 thousand ha in India, with the production of 21173 thousand tonnes and a productivity of 24.932 tonnes ha^{-1} (Anonymous, 2020). In India, fruit borer, *Helicoverpa armigera* (Hubner) is most serious pest and is responsible for huge economic losses by reducing the quantity, quality thereby market value (Singh *et al.,* 2011; Reddy and Zeharm, 2004). The pest is responsible for 50-80 per cent yield losses in tomato under favourable climatic conditions (Wade *et al.,* 2020).

Chemical control served as an important tool of pest management that was employed largely against the management of pests in tomato. This resulted in development of newer molecules with a unique mode of action for the safety of human beings as well as other beneficial organisms such as chlorantraniliprole on different plants, including tomatoes.

Chlorantraniliprole (3-Bromo- 4- chloro-2-pyridyl)-2-methyl-6 (methyl carbamoyl) pyrazole- 5-carboxanilide) structure shown in Fig. 1. It was introduced as novel insecticide with systemic action belong to group of anthranilic diamides and developed by DuPont in 2007 (Malhat *et al.,* 2011). The substantial difference among insect and mammalian ryanodine receptors makes chlorantraniliprole nontoxic to mammals. (Kar *et al.,* 2013; Lahm *et al.,* 2007; Cordova *et al.,* 2006). The mean larval population of fruit borer in plot treated with chlorantraniliprole reduced to 1.14 larvae/plant (Wasu *et al.,* 2020) and it is also effective in controlling whitefly, leaf miners, beetle and termite species.

It is well known fact that we are on risk when we go for the consumption of food commodities that are treated with pesticides, as the pesticide or their metabolites may be present on food commodities if not followed safe waiting period as well as good agricultural practices. The rational recommendation for an insecticide must need effective control of target pest as well as residues which are left on the produce should be toxicologically unobjectionable. To safe guard the problems arising out of pesticide use, it is always advisable for judicious use of pesticide.

MATERIALS AND METHODS

A. Reagents and chemicals

Pesticide reference standards chlorantraniliprole (purity 97.28 per cent) was obtained from Dr. Erhenstrofer, India and before use are stored at -4°C.

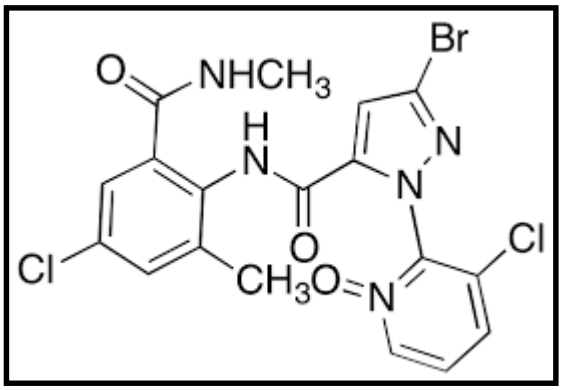

Fig. 1. Structure of Chlorantraniliprole.

Chlorantraniliprole (1 µg mL^{-1}) Standard stock solutions were prepared with regard to HPLC grade acetonitrile, then diluted to different concentrations and injected into the instrument to determine linearity by drawing a calibration curve. Other chemicals and reagents like Sodium chloride- E. Merck Ltd, Sodium sulfate. anhydrous- SD Fine Chemicals, Magnesium sulfate anhydrous- E. Merck Ltd, Primary. secondary amine (PSA)- Agilent Technologies. Solvents- HPLC grade Acetonitrile was procured and checked for its suitability by running reagent as a blank. And all the glassware was washed properly as per the standard operating. procedure to elude the contaminants during analysis.

B. Preparation of. standard solutions

A standard. a stock solution of 400 µg mL^{-1} was prepared by adding Certified Reference Material (CRM) of chlorantraniliprole in 25 mL of volumetric flask dissolved with acetonitrile. Additionally, sub stock solutions 100, 10, 2, 1, 0.5, 0.1 and 0.05 µg mL^{-1} were prepared by sequential dilutions from the stock solution with acetonitrile.

C. Residue analysis of tomato samples

"Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS)" techniques with. slight modifications were used for the preparation of tomato samples for residue analysis. (Anastassiades *et al.*, 2003). A homogenised tomato sample (15 g) was shifted to a 50 mL of polypropylene centrifugal tube and kept overnight. in refrigeration. The homogenized samples were taken and 30 mL of acetonitrile (HPLC grade) was added to each tube. Sodium chloride.(10 ± 0.1 g) was added to each tube and shaken well for 10 min at 50 rpm on rotospin.(Tarson®). Samples were kept in the centrifuge for 3 min at 2500 rpm. Sodium sulfate anhydrous (10g) added to remove moisture from an aliquot of acetonitrile. "Dispersive solid-phase extraction (DSPE)" has been followed for the clean-up process. For this process, a polypropylene tube containing "0.15 ± 0.01 g of PSA sorbent and 0.95 ± 0.01 g anhydrous $MgSO_4$" was prepared for 'an aliquot of 6 mL which was properly mixed by vortex spinix (Tarson®). Again kept in the centrifuge at 2500 rpm for 3 min and lastly, an aliquot of 3 mL was taken.

D. Estimation of chlorantraniliprole residues

The estimation of chlorantraniliprole was done through HPLC - PDA**.** The parameters for chlorantraniliprole were as follows:

Mobile Phase	:	Acetonitrile: HPLC water (70:30)
Column	:	C_{18}
Detector	:	PDA
Column temperature	:	40°C
Flow rate	:	0.3 ml min^{-1}
UV detector wavelength	:	260 nm
Injected volume	:	20 µl

The residues of chlorantraniliprole in tomato were matched with the "retention time" of respective standards, whereas, estimated by "peak area". "retention time" for chlorantraniliprole was observed to be 4.327 min., correspondingly when injected under above mentioned conditions. Quantification of residues (mg kg^{-1}) was calculated as:

$$\text{Residue level mgkg}^{-1} = \frac{\text{Pesticide Standard injected (ng)}}{\text{Peak height of standard injected}} \times \frac{\text{Peak height of the sample injected}}{\text{Sample extract injectd (µl)}} \times \frac{\text{Final volume of the sample extract (ml)}}{\text{weight of the sample (g)}}$$

RESULTS AND DISCUSSION

As chlorantraniliprole is quite effective in management of fruit borer in tomato and approved for use in India. The continuous use of pesticides for protecting insect pests may further aggravate the problem of pesticide residues. The methodology for estimation of its residues is required. For this QuEChERS techniques with slight modification have been standardised in this study.

Analysis of pesticide residues in various crop samples is usually based on methods involved in several steps that includes sampling, sample preparation including extraction, sample purification including clean up, and finally determination of residues by chromatography

techniques i.e. gas or liquid chromatography. In this study, the pesticide extraction is the 1st in sequence of method to assess the residue in different samples. Undesirable materials and other pigments are also extracted along with pesticides in the solvent. To avoid co extractives other than the target pesticide residues like plant pigment, removal of the toxin is accomplished prior to the assessment. To attain required sensitivity, removal of interfering substances if any must be carried out with one or more processes, commonly known as clean up. Clean up requirements differ per analysis scope and complexity of samples (Handa *et al*., 1999). Various type of extracting solvents needs to be selected prior to extraction of chlorantraniliprole in substrates and analysed in instruments *viz.* gas chromatographic (GC), high performance liquid chromatography (HPLC) etc. mentioned by various workers have been studied.

The method of determination for estimating of chlorantraniliprole residues in grain, vegetables and fruits using HPLC on a C_{18} column with clean up step, solid-phase extraction of $MgSO_4$ and PSA as adsorbent was supported by findings of Ambujakshi *et al*. (2018); Singh *et al*. (2012); Xu *et al.* (2010).

A. HPLC chromatograms of chlorantraniliprole

Chromatograms of chlorantraniliprole and tomato samples (spiked) at a wavelength of 260 nm are given in Fig. 2.

B. Tomato fortified with chlorantraniliprole @ 0.05 mg kg^{-1}; C. <LOQ

The bio-analytical approach indicated in the SANCO guidelines (Anon., 2021) was used to determine the quantitative assessment of chlorantraniliprole in tomato and the linear relationship generated for the calibration curve of chlorantraniliprole was shown in Fig. 3.

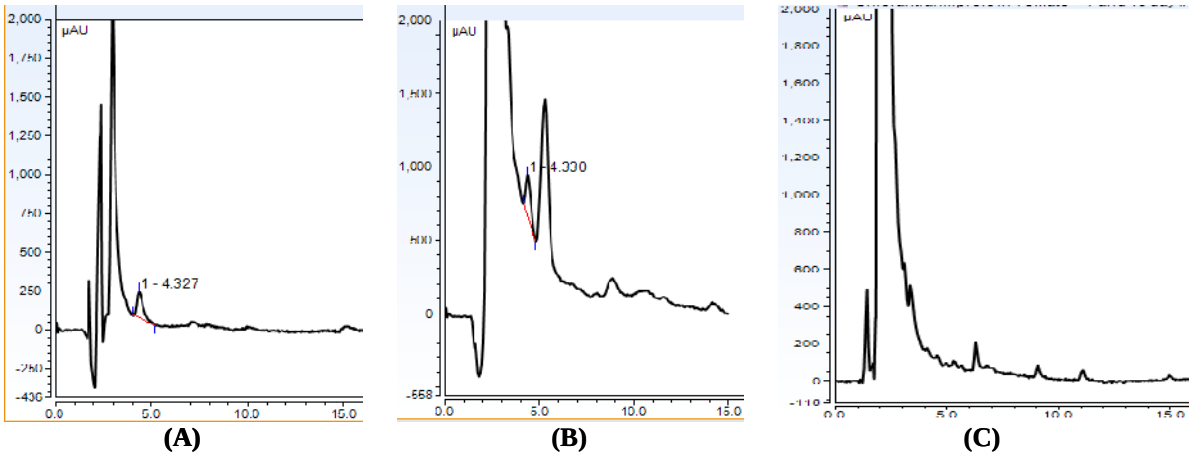

Fig. 2. HPLC chromatograms of **A.** chlorantraniliprole Standard @ 0.05 mg kg^{-1}.

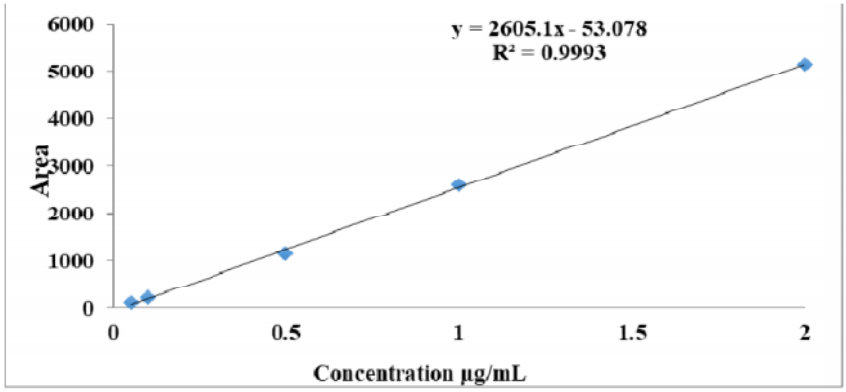

Fig. 3. Linearity curve of chlorantraniliprole standards.

C. Limit of detectability of chlorantraniliprole residues in tomato

The full-scale deflection was obtained with 5 ng of chlorantraniliprole. Terminal volume from processed tomato samples was 3 mL. The sample load of 20 µL for chlorantraniliprole was injected, respectively, to observed the maximum load of samples can be analysed without any interference peak in the area relating to the compound estimated. The LOQ (Limit of quantification) and LOD (Limit of detection) for chlorantraniliprole was found to be 0.05 mg kg^{-1} and 0.017 mg kg^{-1}, respectively.

For the purpose of assessing an analysis method of the relative standard deviation for repeatability (RSD_r)

spiking of chlorantraniliprole in tomato samples was done with 0.05, 0.25 and 0.5 mg kg^{-1} and the amount of residues recovered were. greater than 80 per cent. The within-batch. recovery and RSD_r of chlorantraniliprole at 0.05 - 0.5 mg kg^{-1} varied from 2.91 to 7.31 per cent (Table 1).

The between-batch recovery and the relative standard deviation. for reproducibility (RSD_R) of chlorantraniliprole were established at 0.05 mg kg^{-1} in tomato varied from 2.91 to 7.11 per cent (Table 2).

Table 1: Amount of chlorantraniliprole recovered from spiked tomato samples.

Spiked Level (mg/ kg^{-1})	Replicates		Percent Mean Recovery ± SD	RSDr
	Amount recovered	Percent recovery		
0.05	0.043	85.00	85.80 ± 2.498	2.911
	0.042	83.80		
	0.044	88.60		
0.25	0.219	87.60	87.60 ± 6.400	7.306
	0.240	94.00		
	0.212	81.20		
0.5	0.425	85.00	85.33 ± 2.517	2.949
	0.419	83.00		
	0.443	88.00		
RSDr = (Relative Standard Deviation (Repeatability), SD = (Standard Deviation)				

Table 2: Reproducibility for chlorantraniliprole at 0.05 mg kg^{-1}.

Substrate	Pesticide	Day	Amount recovered (%)	Standard deviation (%)	RSD_R (%)
Tomato	Chlorantraniliprole	1	85.80	2.498	4.671
		2	83.13	5.907	
		3	82.78	3.353	

*RSD_R = "Relative Standard Deviation" (reproducibility)

CONCLUSION

QuEChERS method was standardized and validated with slight modifications by following SANCO guidelines for assessment of chlorantraniliprole in tomato. Mean recoveries were found to be greater than 80 per cent. RSDr for chlorantraniliprole in tomato at 0.05-0.25 mg kg^{-1} was 2.949 to 7.306 per cent. RSD_R of chlorantraniliprole at 0.05 mg kg^{-1} was 4.671 per cent.

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Conflict of interest. None.

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