

Dissipation kinetics, decontamination and risk assessment of cyantraniliprole in/on cabbage using UHPLC

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ABSTRACT: A simple and steadfast analytical method was developed and validated for the determination of cyantraniliprole residues in cabbage by using Ultra High Performance Liquid Chromatography (UHLC) – photo diode array detector. The method developed had a valid specificity (RSD < 5%), good linearity ($R^2 = 0.999$), recovery (90.96 – 95.92 %) and satisfactory repeatability. The Limit of Detection and Limit of Quantification values for cyantraniliprole in UHPLC were 0.015 and 0.05 μg g⁻¹, respectively. Under the optimized specifications, the developed method was utilized to examine the dissipation kinetics of from the collected field samples. The mean initial deposit of cyantraniliprole after the second spray was 1.67 and 2.15 μg g⁻¹ and reached BDL (0.05 μg g⁻¹) on 10 and 15 days with the calculated half-lives of 1.47 to 1.84 days at 60 g a.i. ha⁻¹ and 120 g a.i. ha⁻¹ doses, respectively. Boiling the cabbage (100 °C for 10 min) reduced 78.06 % of cyantraniliprole residues as a result of decontamination study. The dietary Risk quotient (RQ) was also negligible to humans.

Keywords: Cabbage, cyantraniliprole, decontamination, dissipation, half-life, risk assessment

INTRODUCTION

Cabbage (Brassica oleracea L. var. capitata) is one of the important crucifer vegetables grown and consumed worldwide. Consumption of cabbage either raw or processed in different ways is popular because of its antioxidant, anti- inflammatory and antibacterial properties (Rokayya et al., 2013). Fruitful cultivation of cabbage is impeded by a number of pests like diamond back moth (DBM), Plutella xylostella, leaf webber, Crocidolomia binotalis, cabbage webworm, Hellula undalis, tobacco cutworm, Spodoptera litura, aphids, Brevicorvne brassicae etc. (Srinivasan and Veeresh, 1986). Diamondback moth is a major constraint in the successful production which is reported to cause more than 90 per cent yield loss in areas of outbreak (Verkerk and Wright, 1997). For the speedy recovery and acceptable marketing, farmers resort to spraying a number of insecticides at frequent intervals. The major drawback of increased pesticide usage is the development of resistance in pests and residues in foodstuff. Thus monitoring of pesticide residues on food and use of pesticides with short half-life and waiting period at mandatory intervals is essential as it is less likely to build up after repeated applications.

Exploration of cyano-substituted anthranilic diamides lead to the second entry in the anthranilic class with the product, cyantraniliprole ((3-bromo-N-[4-cyano-2-methyl-6-[(methylamino) - hydroxy] phenyl]-1-(3-chloro-pyridine-2-yl)-1-H-pyridine-5-formamide) (Figure 1) (https://pubchem.ncbi.nlm.nih.gov/compound/Cyantraniliprole). Cyantraniliprole was developed with improved plant mobility and translaminar activity

(Mandal, 2012) that has excellent cross spectrum activity against a wide range of lepidopteran and sucking pests (Seiby *et al.*, 2013). This compound activates the insect ryanodine receptor that affects the calcium homeostasis by unregulated release of internal calcium ions leading to muscle paralysis and finally death (Cordova *et al.*, 2006). This paper reports a simple and reliable UHPLC method developed to detect the residues of cyantraniliprole in cabbage for which a field study was carried out.

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MATERIALS AND METHODS

Chemicals and reagents

The reference standards of cyantraniliprole (99.6 % purity) were purchased from Sigma Aldrich, Bangalore, India. Acetonitrile of LCMS and HPLC grade, sodium chloride and anhydrous magnesium sulphate of analytical grade were obtained from Merck, Mumbai, India. Primary secondary amine (PSA) (Bondesil 40 μm) and graphitized carbon black (GCB) were acquired from Agilent technologies, USA. HPLC grade water (18.2 M Ω) was collected with a Milli-Q water purification system. The commercial formulation of cyantraniliprole 10.6 OD was purchased from local pesticide shop at Coimbatore, India.

Preparation of standard solution

A primary stock solution (1000 μ g mL ⁻¹) of cyantraniliprole was prepared by dissolving 25.10 mg of analytical standards in 25 mL LCMS grade acetonitrile in a volumetric flask. An intermediate stock (100 and 10 μ g mL⁻¹) was prepped from primary stock solution

and further the working standards were prepared from intermediate stock. The standard solutions required for constructing calibration curve (0.01, 0.05, 0.1, 0.2, 0.4 and 0.8 μg mL⁻¹) were prepped from the intermediate stock by serial dilutions with LCMS grade acetonitrile. All working standard solutions were stored at -20°C before use.

Field experiment

Supervised field trial was executed in a farmer's field located at Naraseepuram village, Coimbatore, TamilNadu, India (11°N latitude and 76°E longitude). The field trial has been followed with good agricultural practices that had no previous application of cyantraniliprole and the treatment was made up of three replicated plots along with untreated control. Cabbage raised on the trail plots were sprayed with cyantraniliprole at 60 g a.i. ha⁻¹(recommended dose) and 120 g a.i. ha⁻¹(double the recommended dose), and the control plots were sprayed with water. Two spraying was given at 10 days interval during 50 percent head formation stage using hand operated knapsack sprayer using 500L/ha as spray fluid.

Sample collection and preparation

Cabbage head samples (2kg) were drawn at specified intervals starting from 0 (1 hr) to until 15 days after application. The head sample was collected randomly from each replicate of cyantraniliprole treated plots at 0 (1 h), 1, 3, 5, 7, 10, 15 and 21 days after the second application including the control sample. The collected samples were transported to the laboratory, chopped into small pieces, mixed thoroughly and homogenized with the help of high volume blade homogenizer.

Extraction and cleanup

The residues of cyantraniliprole were extracted from the cabbage by following the modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Anastassiades *et al.*, 2003). A representative homogenized sample of 10 g from each treatment were taken in a 50 mL poly-propylene centrifuge tube and 20 mL of acetonitrile was added to it and the mixture was hand shaken vigorously, followed by vortexing for 2 min. subsequently 1g of NaCl and 4 g of anhydrous MgSO₄

Table 1. Recovery of cyantraniliprole at different fortified levels in/on cabbage

Pesticide	Spiked concentration (µg/g)	Recovered concentration (µg/g)	Recovery (%) ± SD	RSD (%)	Horwitz ratio (HorRat)
Cyantraniliprole	0.05	0.045	90.96 ± 3.38	3.72	0.15
	0.25	0.229	91.52 ± 2.45	2.68	0.14
	0.50	0.480	95.92 ± 2.74	2.86	0.16

^{*}Mean of three replicates; SD - Standard Deviation, RSD- Relative Standard Deviation

Table 2. Persistence and dissipation of cyantraniliprole 10.6 OD residues in/on cabbage heads

		Resi	due (μg g ⁻¹)				
	Recommended	Double	the recommended				
Days after application	dose (120 g a.i.	· · · · · · · · · · · · · · · · · · ·	(60 11 1)				
	dose (60 g a.i. ha ⁻¹)						
	Mean*±SD	Dissipation (%)	Mean*±SD	Dissipation (%)			
Control	ND		ND	-			
0 (1 hr)	1.67 ± 0.118	-	2.15±0.125	-			
1	1.02 ± 0.096	38.59	1.32±0.107	38.73			
3	0.50 ± 0.063	70.17	0.70 ± 0.130	67.53			
5	0.17 ± 0.017	89.68	0.27 ± 0.034	87.42			
7	0.07 ± 0.012	95.78	0.14 ± 0.017	93.55			
10	BDL	100.00	0.06 ± 0.011	97.30			
15	BDL	100.00	BDL	100.00			

ND – Not detected; BDL – Below detectable level (0.05 µg g⁻¹)

were added to the sample mixture, vortexed for 2 min followed by centrifugation at 6000 rpm for 10 min. The supernatant (9 mL) aliquot was transferred into a test tube containing 4 g of NaSo₄. From this 6 mL of aliquot was transferred to a 15 mL prefilled centrifuge tube with 10 mg GCB, 100 mg PSA sorbent and 600 mg anhydrous MgSO₄. The mixture was vortexed for one minute and then centrifuged at 3000 rpm for 10 min and 4 mL of supernatant aliquot was transferred into a turbovap tube concentrated to dryness under a gentle stream of nitrogen by using the Turbovap LV set at 40°C. The residue was redissolved using acetonitrile (1 mL) and was filtered by 0.2 µm membrane syringe filter and transferred into a 2.0 mL UHPLC auto sampler glass vials for analysis.

Instrument parameters

The quantification of cyantraniliprole residues were performed by UHPLC (Shimadzu, i series 2020) equipped with diode array detector (SPD-M30A) and auto-sampler. Chromatographic separation was achieved with reverse phase - C18 (Agilent) column, 250 mm length x 4.6 mm id x 5 μ particle size in a column oven, at 40°C. The low pressure gradient condition employed with a mobile phase of acetonitrile and water (70:30, v/v) with a flow rate of 0.6 ml min⁻¹. The injection volume of 20 µl with the absorbance of 225nm, for cyantraniliprole was fixed with total run time of 10 minutes. Residues of insecticides were quantified by the comparison of peak area of standards with that of unknown or spiked samples run under identical conditions of operation. The cyantraniliprole was eluted at the retention time of 6.9 minutes.

Method validation

Linearity studies were performed by developing linearity curves of cyantraniliprole standard solutions with the concentrations of 0.01, 0.05, 0.1, 0.2, 0.4 and 0.8 µg mL⁻¹ with each three replications. The sensitivity of the method was evaluated by arriving the limit of detection (LOD) and limit of quantification (LOQ) by spiking the cyantraniliprole with selected matrices at the lowest concentration level meeting the analytical method requirements. The LOD and LOQ were determined by considering the signal to noise ratio of three and ten, respectively with regarding the background noise from the blank matrices. The method description for sample preparation was validated by recovery investigation. Recovery studies were carried out on blank matrix of cabbage (10 g) by spiking them with known quantities of standard cyantraniliprole solutions at three different concentrations (0.05, 0.25 and 0.5 µg g⁻¹) with six replications. The precision of the method was performed in terms of repeatability (Relative Standard Deviation) for each spiked levels of 0.05, 0.25 and 0.5 $\mu g \ g^{-1}$ of the matrix. The Horwitz ratio (HorRat) related to intralaboratory precision, which specifies the acceptability of a method with respect to reproducibility was calculated for cyantraniliprole as follows

HorRat = RSD/PRSD

Where, PRSD (Predicted RSD) = 2 $C^{-0.15}$, where C is the concentration expressed as mass fraction (10ng/g=10×10-9) (Paramasivam and Bhuvaneswari, 2020).

Statistical analysis

The cyantraniliprole residues were calculated using

$$\frac{As \times Cstd \times S1 \times Vs}{Astd \times Ws \times Asj}$$

Where, As - Peak area of the sample; Cstd – Concentration of the standard in (μg ml⁻¹); S1 – injected volume of standard (μl); Vs –volume of the sample (final extract in mL); Astd - Peak area of the standard; Ws - Weight of the sample in g; Asj- Aliquot of the sample injected in μl .

The insecticide degradation pattern was analyzed by applying seven transformation functions as suggested by Hoskin (1961) and Timme *et al.*, (21). The half-life was calculated using Pesticide Residue Half-life Calculator software developed by Department of Soil Science, Tamil Nadu Agricultural University, Coimbatore based on Regupathy and Dhamu (2001) and best fit degradation model was determined.

Decontamination of pesticides in/on cabbage

Cyantraniliprole residues in cabbage heads (collected 1 hour after application) were reduced by adopting six simple culinary practices as decontamination treatments. Treatments include T1 - control; T2 - washing in tap water for 2 min; T3 – washing with 2% tamarind water for 2 min; T4 - washing with 2 % baking soda for 2 min; T5 washing with 2 % lemon juice for 2 min and T 6 - boiling for 10 min. The decontamination solutions of tamarind, baking soda and lemon juice was prepared separately in 500 mL beaker and the cabbage samples were dipped in the solution for about 2 minutes and gently rubbed with hand. The same way tap water washing and boiling in 500 mL of water was also followed. After discarding the solutions the samples were air-dried on a filter paper to remove the moisture. The samples were subjected to analysis of residues following the above described method.

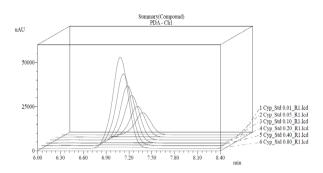


Fig. 1. Linearity chromatograms of cyantraniliprole in UHPLC-PDA

Dietary risk assessment

The estimated daily intake (EDI) of cyantraniliprole residues were calculated by multiplying the highest residue concentration (mg/kg) with the food consumption rate (kg/day) divided by the mean body weight of an adult. Risk quotient (RQ) is derived for the long term risk assessment of intakes, by dividing the EDI by the relevant acceptable daily intake (ADI) (mg/kg body weight (bw)/day). ADI of cyantraniliprole is 0.03 mg/kg bw/day according to FAO. The average body weight of an Indian adult according to National Instituite of Nutrition is 60 kg and recommended vegetable consumption of an Indian adult is 300 g/day (NIN, 2011). The risk for long term human dietary intake of cyantraniliprole is acceptable when RQ is less than 1 and unacceptable if RQ is more than one.

RESULTS AND DISCUSSION

Method validation

Efficiency of analytical method was evaluated based on linearity and recovery studies. Standard calibration curve of cyantraniliprole was constructed by plotting concentration against peak area in the range of 0.01 to 0.8µg g⁻¹ (Fig. 2). The concentrations injected observed linear signal with the r² value of 0.999 and the linear regression equations of cyantraniliprole was y = 140346x+ 84.633 (Fig. 3). Limit of detection (LOD) and limit of quantification (LOQ) of cyantraniliprole were determined as 0.015 and 0.05 µg g⁻¹, respectively. The results of the recoveries and relative standard deviations (RSDs) of cyantraniliprole carried out at the levels of 0.05, 0.25 and 0.5 µg g⁻¹ in cabbage is presented in Table 1. The mean recovery values of cyantraniliprole in cabbage heads ranged from 90.96 – 95.92%, with the standard deviation ranging from 2.68 - 3.72. Similar findings with the average percentage recoveries of cyantraniliprole from cabbage were 89.80 to 100.11% (Kumar et al., 2021) and 88.9 to 96.5% of recoveries from tomato fruits (Malhat et al., 2018) were also reported. Since the recovery

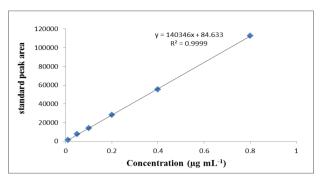


Fig. 2. Calibration curve of cyantraniliprole in UHPLC-PDA

percentage ranges within the acceptance limit of 80 and 120 % and relative standard deviation recorded less than 5.0 % which is in accordance with the SANTE guidelines (SANTE, 2020). The HorRat ratio for cyantraniliprole was below 0.5 for all the spiked concentrations (0.05, 0.25 and 0.5) which is acceptable according to AOAC guidelines (AOAC, 2016) that satisfy the intralaboratory precision and accuracy. Thus the method can be adopted for residue and dissipation study for cyantraniliprole in cabbage.

Dissipation of cyantraniliprole in cabbage

The results of persistence and dissipation of cyantraniliprole in/on cabbage sprayed at 60 and 120 g a.i ha-1 are presented in the Table 2. The mean initial deposits (1 hour after spraying) of cyantraniliprole on cabbage were found to be 1.67 and 2.15 µg g⁻¹ at recommended and double the recommended doses, respectively. At recommended dose, residues persisted upto 7 days and reached Below Detectable Limit (BDL) of less than 0.05 µg g-1 on 10 days after treatment. At double the recommended dose, the mean residues persisted for 10 days and reached BDL (0.05 ug g⁻¹) on 15 days after treatment. The initial deposits of cyantraniliprole in tomato were 0.751 and 0.841 mg/ kg in two different locations of Nile valley Delta region and it persisted upto 14 days after which the level has reduced below BDL (Malhat et al., 2018). Dissipation pattern of cyantranilprole in cabbage was computed following seven transformations and the best fit observed was first order kinetics for both the doses (Table 3). The statistical parameters like intercept (a), slope of regression lines (b) and half-life were presented in Table 3. The half – life values of cvantraniliprole on cabbage were found to be 1.47 and 1.84 days at recommended and double the recommended dose respectively. This was in close proximity with the findings in watermelon with the half-life of 1.1 days for cyantraniliprole (Hu et al., 2013). Close upon other reports with the half-life of cyantraniliprole were, 2.2 days in cucumber, 2.8 days

Table 3. Statistical parameters for dissipation pattern of cyantraniliprole 10.6 OD residues in/on cabbage heads

			•	•	•	•			D				
				Recommended	ed dose @ 60 g a.i. ha ⁻¹	a.i. ha ⁻¹			Doub	e the recomn	Double the recommended dose $(a 120~ m g~a.i.~ha^{-1})$	120 g a.i. ha	_
Function		æ	q	T Half	ı	\mathbf{r}^2	Modified r^2	æ	q	T Half	Ŀ	Γ^2	Modified r^2
First order		0.56	-0.47	1.47				0.70	-0.38	1.84			
	CC	0.82	-0.41	1.67	-0.99	0.99	0.99	0.87	-0.35	1.99	**66.0-	66.0	66.0
	TCL	0.30	-0.53	1.28				0.53	-0.41	1.69			
1.5th order		0.45	0.46	0.43				0.37	0.37	0.42			
	CC	1.46	0.70	1.35	96.0	0.92	-2.98	1.03	0.49	1.17	0.97	0.95	-6.54
	TCL	-0.50	0.22	-0.49				-0.29	0.25	-0.34			
2nd order		-1.56	2.12	-0.74				-2.23	1.80	-1.24			
	NCL	6.11	3.99	-4.42	0.90^{*}	0.81	-2.42	4.17	2.96	-4.87	0.91^{*}	0.82	-5.05
	Γ C Γ	-9.24	0.25	2.94				-8.62	0.65	2.40			
RF First order		1.01	-1.27	0.30				1.30	-1.23	0.32			
	NCL	3.50	-0.52	0.65	-0.95*	0.91	0.63	3.24	-0.79	0.54	-0.97	0.94	0.61
	Γ C Γ	-1.48	-2.02	-0.05				-0.64	-1.67	60.0			
RF 1.5th Order		0.14	1.17	0.02				-0.08	1.13	-0.01			
	NCL	2.29	2.37	0.65	$0.87^{\rm NS}$	0.76	96	1.63	1.95	-0.53	.680	0.78	-167714.4
	Γ C Γ	-2.02	-0.04	-0.61				-1.79	0.31	0.51			
RF 2nd order		-2.83	5.22	0.26				-3.91	5.24	0.56			
	NCL	10.67	12.76	3.22	$0.78^{\rm NS}$	0.62	-1.77	8.04	10.98	4.18	$0.78^{\rm NS}$	0.62	-0.95
	TCL	-16.32	-2.32	-2.63				-15.87	-0.50	-3.06			
Inverse PL		-0.37	0.40	5.75				-0.50	0.44	4.85			
	CC	2.60	0.97	20.26	0.79^{NS}	0.62	0.28	2.08	0.91	13.00	0.79 ^{NS}	0.63	0.33
	TCL	-3.33	-0.17	-8.75				-3.08	-0.03	-3.30			
UCL- Upper Confidence Limit; LCL - Lower Confidence Limit; *	ice Limit;	LCL-Lov	ver Confid	ence Limit; *Si	ignificant at 5	per cent leve	Significant at 5 per cent level; ** significant at 1 per cent level	1 per cent le	vel				

(Dong *et al.*, 2012), 2.6 days (Malhat *et al.*, 2018) in tomato and with a wide difference it was reported as 6.5 days in banana fruits (Qiang *et al.*, 2017).

Decontamination of insecticides in/on cabbage heads

The results of decontamination study revealed that, boiling for 10 min gives higher (78.06 %) reduction of residues of cyantraniliprole followed by washing with 2 % baking soda for 2 min (61.07 %), 2 min washing with 2 % lemon juice (51.16 %), 2 min washing with 2 % tamarind water (44.69 %) and 2 min tap water washing (34.01 %). In untreated sample the mean initial deposit of cyantraniliprole 10.6 OD @ 60g.ai / ha was 1.655µg g-1 (Table 4). In comparision with other diamides, chlorantraniliprole residues in cabbage and cauliflower heads were reduced upto 100% and 17 – 40 % by boiling and tap water washing, respectively (Kar et al., 2012). Cowpea pods on treatment with slaked lime minimized the chlorantraniliprole residues to the level of 90% (Vijavasree et al., 2013). Similarly, Vijavasree et al., (2015) reported about 90 % of chlorantraniliprole residues were brought down on brinjal fruits by treating the samples with 2 % slaked lime and common salt solution.

Dietary risk assessment

For calculating the risk assessment of cyantraniliprole in cabbage, grown under open field conditions the residue dissipation data were used. Dietary risk quotient (RQ) was calculated based on the highest residue concentration obtained from the treated recommended dose. The RQ was less than one from 0 (1 hour after application) days after application of cyantraniliprole, (Table 5) which indicates the cabbage heads from field were safe for consumption. Likewise, the dietary RQ was also less than one for chlorantraniliprole in okra fruits (Paramasivam and Bhuvaneswari, 2020).

CONCLUSION

A simple and efficient residue analytical method using UHPLC for detection and monitoring the residues of cyantraniliprole in cabbage was developed, validated and evaluated. The LOQ of the method for cyantraniliprole was below MRL (2mg/kg) as given by CODEX ALIMENTARIUS (2014). More than 80 % of cyantraniliprole residues were dissipated on 5 and 7 DAS and recorded BDL after 7 and 10 DAS. Dissipation of the insecticide followed first order reaction kinetics and the calculated half-life was 1.47 to 1.84. The dietary risk of cyantraniliprole at recommended doses were negligible to humans, since the RQ value is less than one so that the vegetable can be safely consumed.

Table 4. Effect of decontamination methods on cyantraniliprole residues at 60 g a.i. ha⁻¹in/on cabbage heads

	Residue (µg g-1)					
Treatment	Mean ± SD	Per cent Reduction				
T1 – Control (Samples without any treatment)	1.655 ± 0.045	-				
T2 - Washing with tap water for 2 minutes	1.092 ± 0.034	34.01				
T3 - Washing with 2 % tamarind solution for 2 min.	0.916 ± 0.028	44.69				
T4 - Washing with 2 % baking soda solution for 2 min.	0.644 ± 0.042	61.07				
T5 - Washing with 2 % lemon juice for 2 min.	0.808 ± 0.048	51.16				
T6 - Boiling with water for 10 minutes	0.363 ± 0.079	78.06				

Table 5. Dietary risk assessment of cyantraniliprole @ 60 g a.i. ha-1 and 120 g a.i.

		60 g a	.i. ha ⁻¹		120 g a.i. ha ⁻¹				
Days after treatment	Maximum residue (mg/kg)	EDI (mg/kg bw/day)	ADI (mg/kg bw/ day)	Risk quotient (RQ)	Maximum residue (mg/kg)	EDI (mg/kg bw/day)	ADI (mg/kg bw/day)	Risk quotient (RQ)	
0 (1 hr)	1.77	0.0089	0.03	0.2950	2.27	0.0114	0.03	0.3783	
1	1.11	0.0056	0.03	0.1850	1.44	0.0072	0.03	0.2400	
3	0.55	0.0028	0.03	0.0917	0.82	0.0041	0.03	0.1367	
5	0.18	0.0009	0.03	0.0300	0.29	0.0015	0.03	0.0483	
7	0.08	0.0004	0.03	0.0133	0.16	0.0008	0.03	0.0267	
10	BDL	-	-	-	0.07	0.0004	0.03	0.0117	
15	BDL	-	-	-	BDL	-	-	-	

ADI – Acceptable daily intake, EDI – Estimated daily intake

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