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DISSIPATION DYNAMICS OF CYANTRANILIPROLE, A DIAMIDE INSECTICIDE ON TOMATO UNDER PROTECTED CULTIVATION IN NORTH WEST INDIAN HIMALAYAS

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ABSTRACT

Present study was carried out with the aim to have an insight into persistence and dissipation kinetics of cyantraniliprole, an insecticide to diamide group, on tomato fruit grown under naturally ventilated greenhouse. Cyantraniliprole was evaluated at its recommended dose of 90g g a.i. ha^{-1}, and double the recommended dose i.e. 120 g a.i. ha^{-1} in the fruiting phase of the crop. Cyantraniliprole residues in tomato fruits were assessed using QuEChERS method and was analyzed in HPLC equipped with C_{18} column and UV-VIS detector. The initial deposits of cyantraniliprole on tomato fruits were 1.801 mg kg^{-1} and 3.628 mg kg^{-1} at 90 g a.i. ha^{-1} and 180 g a.i. ha^{-1} during cropping season 2021, whereas during 2022 cropping season the initial deposits were 1.787mg kg^{-1} and 3.603 mg kg^{-1} at 90 g a.i. ha^{-1} and 180 g a.i. ha^{-1}, respectively. The residues on fruits dissipated to half in 1.70 and 2.08 days, respectively in first season and 1.79 and 2.14 days in second cropping season and persisted for 10 days at 90 g a.i. ha^{-1} and 15 days at 180 g a.i. ha^{-1} during both the seasons. Based on the dissipation dynamics of cyantraniliprole on tomato, a pre harvest interval of 8-10 days was worked out from consumer's safety point of view.
***Keywords* :** Protected cultivation, cyantraniliprole, residue, tomato, pre-harvest interval, HPLC.

Introduction

For the quality and off-season production of high value crops, protected cultivation has recently surpassed open field cultivation in popularity. The favourable and stable environment prevalent in protected areas make pest management more challenging (Sood *et al.*, 2018; Kaushik *et al.*, 2021; Dash *et al.*, 2022). Modern agriculture relies heavily on pesticides to help growers to suppress pest infestation in various crops. However, widespread pesticide use has also prompted persistent public concerns over the adverse effects on non-target organisms and human health. In North West Indian Himalayas, greenhouse whitefly, *T. vaporariorum* is the key pest of tomato under protected cultivation and a number of chemical insecticides are being suggested for its management including cyantraniliprole. It is a diamide insecticide and is considered the most promising due to efficacy against whitefly and their lower toxicity to beneficial arthropods, mammals and pollinators (Schmidt-Jeffris and Nault, 2016; Zilnik *et al.*, 2021). Cyantraniliprole is effective against several insect pests (caterpillar, aphids, leafminer, psyllids, thrips, and whitefly) (Quinet *et al.*, 2019). Since diamide insecticides target ryanodine receptors in insects and cause an unregulated release of internal calcium reserves, insects exposed to these chemicals experience feeding cessation, lethargy, muscle paralysis, and ultimately death (Teixeira and Andaloro, 2013).

In the present study, persistence and dissipation kinetics of cyantraniliprole residue on tomato (*Solanum lycopersicum* L.) grown under naturally ventilated greenhouse was investigated. Tomato is one of the remunerative crops under protected environment and an economically important crop around the world. Central Insecticides Board and Registration Committee, (CIB & RC, 2019) has approved and recommended cyantraniliprole for managing whiteflies in tomato crop and proved to be very effective against adult and immature stages of whitefly, and for reducing transmission of plant viruses (Lahm *et al.,* 2007; Gravalos *et al.,* 2015; Wang *et al.,* 2017; Zheng *et al.,* 2017). The risk of pesticide residues in food products rises as a result of the excessive and repeated application of pesticides under protected farming to protect crops from various pests (Mansour *et al.,* 2009; Zhao *et al.,* 2020). Food contaminated with pesticide residues could pose a risk to both human health and the environment (Li *et al.,* 2018). Even in cases when applied in compliance with good agricultural practices, the pesticides may leave residues (Lazić *et al.,* 2012). Pesticide usage for the management of greenhouse pests is common, while dissipation takes slow and longer period in greenhouse conditions than in open field conditions (Guru and Patil, 2018). Although persistence and dissipation dynamics of anthranilic diamides on tomato has been well-studied in open field condition, however the information in greenhouse condition is lacking. The aim of the study was to determine the persistence and dissipation dynamics of cyantraniliprole and its pre-harvest intervals in tomato grown under protected cultivation for consumer's safety.

Material and Methods

Reagents and chemicals

Formulated (cyantraniliprole 10.26 OD) and standard analytical grade of cyantraniliprole (purity, 98.9%) were procured from M/s FMC India Pvt. Ltd.; anhydrous magnesium sulfate, sodium sulfate, sodium chloride and HPLC grade water were procured from Merck Pvt. Ltd. and HPLC grade acetonitrile was purchased from M/s Genetix Biotech Asia Pvt. Ltd., New Delhi.

Instrumentation

Cyantraniliprole residues were estimated on high-performance liquid chromatography (HPLC) LC-20AT equipped with C_{18} column and UV-VIS detector reverse (RP) C_{18} column (2.0 mm×25cm).

Experiment layout

The experimentation was carried out in naturally ventilated greenhouse of the Department of Entomology, CSK Himachal Pradesh Agricultural University, Palampur (HP) India, during the summer cropping seasons of 2021 and 2022. Over the course of the trial, the mean temperature and relative humidity under greenhouse were recorded as 30.8°C & 72.5% during 2021 and 29.7°C & 74.6% during 2022. Tomato F_1 hybrid (Palam Tomato Hybrid-1) raised under naturally ventilated greenhouse as per university standard package of practices (Anonymous 2018) except plant protection measures to avoid contamination. The plants were raised in raised beds (15 cm) of 30 cm width, spaced 70 cm apart at a spacing of 70×30 cm. Cyantraniliprole was sprayed on tomato crop at the recommended rate of 90 g a.i. ha^{-1} and double the recommended rate 180 g a.i. ha^{-1} using the battery-operated Knapsack sprayer fitted with hollow cone nozzle at 15 psi pressure. The tomato crop was sprayed twice at 15 days interval at the fruit development stage. Untreated plots were sprayed with water only. Each treatment was replicated thrice. The fruits and foliage were thoroughly covered with spray fluid to run-off stage. During the spray, care was taken that lower concentration was sprayed first, followed by higher concentration and all necessary precautions were taken to avoid the chances of drifting of spray fluid to another plots. After the second foliar application, tomato fruit samples (1 kg) from each replication were collected randomly at 0 (2 hrs after spray), 3, 5, 7, 10, 15 and 21 days interval. The samples were packed in polyethylene bags, labeled well and brought to laboratory for pesticide residue analysis.

Extraction and cleanup

Cyantraniliprole residue were extracted and purified from tomato fruits as per the method of Malhat *et al.,* (2018) with slight modifications. Tomato fruit sample (1 kg) was homogenized in a low-speed high-volume homogenizer. Homogenized tomato fruit (50g) were extracted in acetonitrile and 10 g of NaCl was added for phase separation. The supernatant was filtered using 10 g of activated sodium sulfate ($NaSO_4$) and hand shaken for 1 minute. This acetonitrile extract was evaporated to 5 ml using a rotary vacuum evaporator at 35°C and this was cleaned up with 90 mg of anhydrous $MgSO_4$ and 5 mg of activated charcoal. The final volume was made up to 5 ml with HPLC grade distilled acetonitrile and finally injected 20 µl into HPLC.

HPLC analysis

Cyantraniliprole residues were determined on liquid chromatography using acetonitrile and water (80:20) as mobile phase, run in an isocratic mode at a flow rate of 0.8 ml/min. Wavelength (λ_{max}) was set at 280 nm. Under these operating parameters, the retention time of cyantraniliprole was observed at 5.649 (Fig. 1). The different operational parameters of HPLC instrument used for residue estimation of cyantraniliprole are given in Table 1.

Table 1 : Operational parameters of HPLC instrument

Instrument Parameters	Cyantraniliprole
Mobile phase (v/v)	Acetonitrile:water (80:20)
Flow rate (ml/min)	Isocratic flow 0.8
Column	RP-18 end capped
Column length (mm)	250
Diameter (µm)	5
Detector	SPD-20A UV/VIS
Injection volume (µl)	20
Wavelength for detection (nm)	280
Start time (min)	0.00
End time (min)	10.00
Retention time (min)	5.649

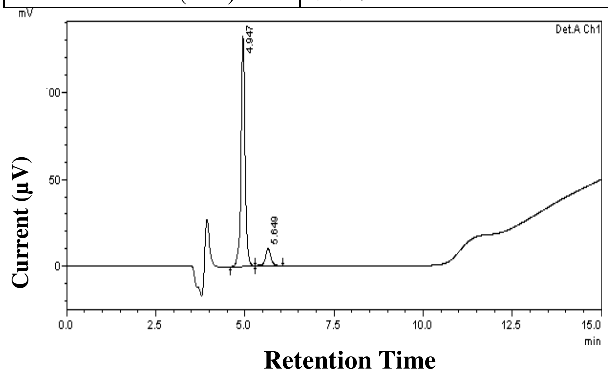

Fig. 1 : Chromatogram of cyantraniliprole standard (HPLC)

Data analysis

The residue data were subjected to statistical analysis according to Hoskins (1961) to compute the residue half-life and waiting period/pre-harvest interval. Log residues were regressed on time interval to determine the role of time after the spray on residue dissipation. Residue half-life (RL_{50}) was calculated as $T_{1/2}$=log2/b where, $T_{1/2}$=residue half-life (RL_{50}) in days and b=slope of regression equation. Waiting period/PHI was calculated on the basis of LOQ by using the formula: log of initial deposit–log of LOQ/b.

The per cent dissipation of the residue over the initial deposit was calculated for various sampling intervals as per the following mathematical formula.

$$\text{Per cent dissipation} = 100 - \frac{\text{Residue (mg kg}^{-1})}{\text{Initial deposit}} \times 100$$

Method validation

The efficiency of an analytical method is determined by the agreement between the true value of an analyte in the sample and the value obtained by its analysis. To establish the efficiency of the method validity, recovery studies were carried out by fortifying homogenized tomato fruit with cyantranilipole at five levels viz; 0.05, 0.1, 0.5, 1.00 and 2 mg kg^{-1} levels and each spiking level was replicated three times. The accuracy values were evaluated by using low to high concentrations of the pesticide. Linearity curve was established with concentrations of the standard and corresponding peak area. The linear range of the method was evaluated by calculating the regression coefficients (r^2). The precision of the method was expressed as the percent relative standard deviation (% RSD) for replicate analysis of the spiked samples. Percentage recovery and RSD were worked out as follows.

$$\text{Recovery (\%)} = \frac{\text{Concentration(mg/kg)in fortified samples}}{\text{Level of fortification}} \times 100$$

$$\text{RSD (\%)} = \frac{\text{Standard deviation (SD)in \% recovery}}{\text{Mean recovery}} \times 100$$

Limit of Detection and Limit of Quantification

Limit of detection (LOD) is the minimum concentration of the analyte that can be detected with acceptable certainty and is determined by considering a signal-to-noise ratio of three with reference to the background noise obtained for the blank sample. The limit of quantification determined as 3 times of LOD.

Results and Discussion

Method validation

The reliability of analytical method tested by spiking of untreated tomato fruit sample at different concentrations is being presented in Table 2. Recovery of cyantraniliprole was between 85.00 and 89.50%. The results are in close proximity with the investigation of Malhat *et al.* (2018) who recorded recovery of cyantraniliprole between 88.90 and 96.50% from tomato fruits. The findings of Sun *et al.* (2012) provide conformity to our results who achieved similar trends of recoveries of cyantraniliprole ranging from 88.30 to 91.80 % from puckchoi. The precision of the analytical method was determined by the relative standard deviation for its repeatability which ranged from 1.98–7.18 % for different spiking levels (Table 2). In our investigations RSD results met the requirement for the method validation as suggested by SANTE, 2017 which narrates the acceptable recoveries are ranging between 70 and 120%, with RSD% not to be more than 20%.

Good linearity with r^2 value of 0.94 and 0.98 were obtained for cyantraniliprole at recommended and double the recommend dose, respectively. The LOQ was calculated as 0.03 mg kg^{-1} and LOD was 0.009 mg kg^{-1}.

Table 2 : Recovery and relative standard deviation of cyantraniliprole from fortified tomato fruits.

Insecticide	Amount (ml) added in 50g sample	Average amount recovered (mg/kg)	Recovery (%) *Mean ± SD	RSD_r (%)
Cyantraniliprole	0.05 mg/kg	0.044	88.67 ± 0.002	3.45
	0.1 mg/kg	0.086	86..33 ± 0.007	7.78
	0.5 mg/kg	0.442	86.47 ± 0.011	2.43
	1 mg/kg	0.895	89.50 ± 0.018	1.98
	2 mg/kg	1.70	85.27± 0.015	0.88

*Mean of three replications
SD = "Standard Deviation"
RSDr = "Relative Standard Deviation" (Repeatability)

Persistence

The initial deposits of a pesticide depend upon a number of factors like concentration, formulation, weather conditions (Ebling 1963), whereas the dissipation dynamics of insecticides depends upon crop morphology, growth rate, and physicochemical properties of pesticides and prevailing weather factors. The dissipation rate and half-lives of pesticides provide an important index to assess the behavior of residues in plants. The dissipation pattern of cyantraniliprole in tomato fruit under protected cultivation is presented in Table 3. The average initial deposits of cyantaniliprole at the application rate of 90 and 180 g a.i. ha^{-1} were 1.801 and 3.628 mg kg^{-1} during the cropping season of 2021, whereas the corresponding values during 2022 cropping season were 1.787 and 3.703 mg kg^{-1} on tomato fruits, respectively. The half-life (RL_{50}) values of cyantraniliprole at the application rate of 90 and 180 g a.i. ha^{-1} were recorded as 1.7 & 2.08 days and 1.8 and 2.14 days during 2021 and 2022 cropping season, respectively. The initial residues reached to below quantification limit (BQL) on 15^{th} and 21 day at recommended and double the recommended dose, respectively during both seasons. Residues in samples collected on day 15 and 21 were below the LOQ of the method and therefore could not be further exploited.

A quick decline of cyantraniliprole residues was evident during the first 7 days after the treatment, on subsequent days the decline was very slow and resulted in slow dissipation rate of cyantraniliprole residues under protected cultivation in both the cropping seasons [Table 3 and Fig. 2 (a,b)]. The determined half-life is comparable and in agreement with findings of Malhat *et al.* (2018) who reported 2.6 days half-life value of cyantraniliprole on tomato at application rate of 75 g a.i. ha^{-1}. Sun *et al.,* (2012) also observed 2.9 days half-life value of cyantraniliprole deposits on pakchoi after the application at the rate of 60 g a.i. ha^{-1}. The half-life of cyantraniliprole on cabbage ranged from 3.5-4.8 days (Kumar *et al.,* 2021). Whereas least and longest half-life value of 2.4 and 2.9 days of chlorantraniliprole (diamide insecticide) were recorded correspondence to summer and winter season, respectively by Din *et al.,* (2015). The variation in half life and pre-harvest intervals (PHI) in both the seasons is attributed to prevailing temperature inside polyhouse. The minimum and maximum temperature varies between 37.3°C to 24.3°C during 2021 and 36.5°C to 22.9°C during 2022. Some studies indicated a negative correlation of air temperature with both half–life times and pre- harvest interval time, while a positive correlation with the relative humidity. Based on dissipation pattern, pre harvest interval/safe waiting period under greenhouse conditions, determined at LOQ of 0.03 mg kg^{-1} were worked out to be 8.34 and 9.9 days in 2021 and 8.8 and 10.2 days in year 2022 at recommended and double the recommended dose, respectively. Din *et al.,* (2015) also suggested a waiting period for chlorantraniliprole (diamide insecticide) as 6.0 and 7.4 days in summer and winter season, respectively on tomato fruits.

Table 3 : Persistence and dissipation pattern of cyantraniliprole in tomato under protected cultivation

Days after spray	Cyantraniliprole residue (mg kg^{-1})			
	2021		2022	
	Recommended dose (90 g a.i./ha)	Double the recommended dose (180g a.i./ha)	Recommended dose (90 g a.i./ha)	Double the recommended dose (180g a.i./ha)
0 (2hrs after spray)	1.801 ± 0.012	3.628 ± 0.004	1.787 ± 0.004	3.603 ± 0.007
3	1.315 ± 0.003 (27.00)	2.805 ± 0.014 (22.68)	1.307 ± 0.004 (26.86)	2.814 ± 0.007 (21.91)

5	0.754 ± 0.003 (42.67)	1.595 ± 0.004 (43.16)	0.724 ± 0.004 (44.61)	1.598 ± 0.004 (43.19)
7	0.255 ± 0.004 (66.17)	0.597 ± 0.003 (62.58)	0.228 ± 0.003 (61.10)	0.612 ± 0.012 (61.73)
10	0.031 ± 0.001 (87.97)	0.189 ± 0.010 (68.38	0.037 ± 0.001 (86.86)	0.212 ± 0.002 (65.34)
15	< BQL	0.034 ± 0.001 (81.98)	< BQL	0.038 ± 0.001 (82.08)
21	-	< BQL	-	< BQL
RL_{50} (days)	1.7	2.08	1.79	2.14
Regression equation	Y = -0.514x + 0.177	Y = -0.762x + 0.144	Y = -0.489x + 0.168	Y = -0.753x + 0.140
Regression coefficient (r^2)	-0.942	-0.985	-0.945	-0.985

Figures followed by ± signs indicate standard deviation of the mean value
Figures in parentheses denote per cent dissipation
BQL = Below quantification limit

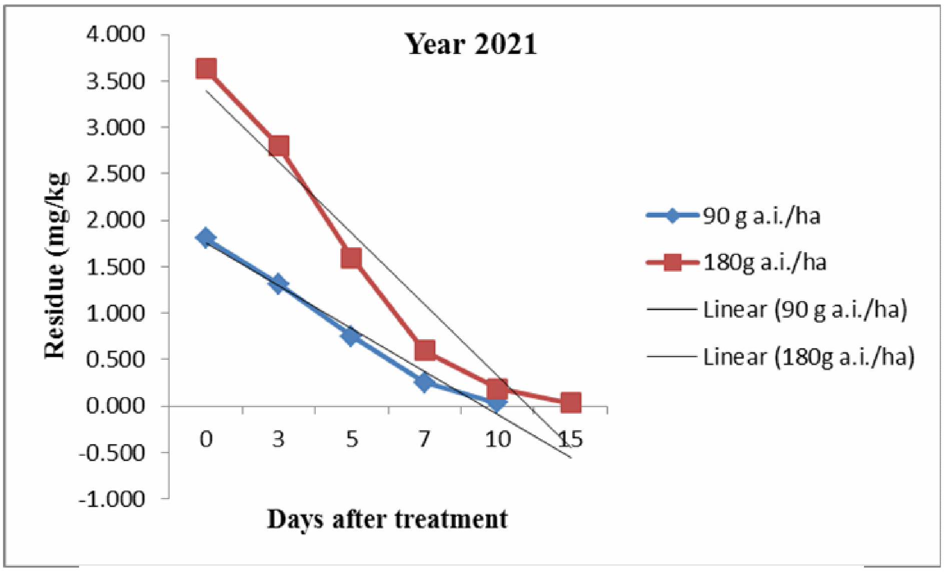

Fig. 2(a) : Dissipation of cyantraniliprole from tomato fruits in 2021

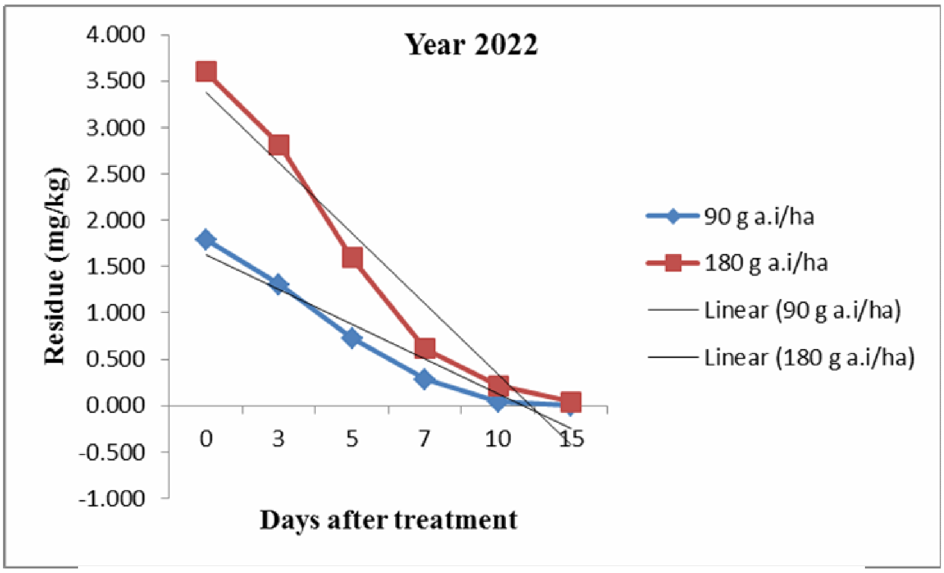

Fig. 2(b) : Dissipation of cyantraniliprole from tomato fruits in 2022

Conclusion

It is clear from the above study that the cyantraniliprole residue persisted for longer duration due to slow dissipation rate beyond 7th day. This could be due to the reason that persistence depends upon the weather parameters to hold insecticide deposit under greenhouse. The high r^2 value indicated that the cyantraniliprole showed first order kinetics. Faster dislodging and degrading capabilities of initial deposits of cyantraniliprole on tomato fruits due to the two treatments at the rate of 90 g a.i. ha^{-1} and 180 g a.i. ha^{-1} with the half-life of 1.7–1.79 days provide safety to human health. A PHI/safe waiting period of 8-10 days was suggested for cyantraniliprole on tomato from consumer's safety point of view.

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