

Dissipation and Risk Assessment of Chlorantraniliprole Residues in Vegetable Cowpea

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ABSTRACT

A field experiment was conducted to study the rate of dissipation of insecticide chlorantraniliprole 18.5 % SC in vegetable cowpea. Chlorantraniliprole 18.5 % SC @ 0.30 mL L⁻¹ was sprayed at pod formation stage in vegetable cowpea and samples of cowpea pods were harvested at 0 (2hrs after spraying), 1,3,5,7,10,15 days after spraying and residues were estimated by using liquid chromatography tandem mass spectrometer (LC-MS/MS) at All India Network Project on Pesticides Residues, College of Agriculture, Vellayani, Kerala. The mean initial residue of chlorantraniliprole was found to be 0.42 µg g⁻¹. Residues of chlorantraniliprole were persisted up to five days.

Keywords: Dissipation, Chlorantraniliprole, Cowpea, Food safety, LC-MS/MS, Linearity and Recovery.

Cowpea or the yard-long bean (*Vigna unguiculata subsp. sesquipedalis* (L.) Verdc.) is a popular vegetable in India which is harvested at an immature stage of development and consumed. It is valued as a nutritional supplement to cereals and an extender of animal proteins. A major constraint in the cultivation of the crop is the threat posed by an array of insects among which the lepidopteran pod borers take a heavy toll of the crop in the country. Quite often, insecticides are required to protect the crop from these noxious pests and usage of insecticides during the fruiting stage may lead to accumulation of residues in the fruits. Certainly, the residue levels should not be allowed to exceed the maximum limits. Farmers' resort to spray various insecticides with short intervals resulted in resistance, secondary pest outbreak and pest resurgence along with destruction of natural enemies and environmental pollution. Increasing awareness of the potential impact of such toxic chemicals has led to the development of eco-friendly new molecules to ensure minimum risk to man and environment (Huan *et al.*, 2016). One such insecticide registered for the control of lepidopteran pests is chlorantraniliprole, a novel anthranilic diamide which interrupts normal muscle contraction in insects leading to their death. The insecticide is effective against insect populations that have developed resistance to other insecticide groups, thus representing an attractive new tool for integrated pest management programs. Categorized as a "reduced risk" pesticide, it is recommended as an alternative to pyrethroids for vegetables (USEPA 2008). The use of pesticides on any crop or commodity may lead to its residues remaining at the time of harvest. So, there are risks associated with the consumption of pesticide treated

crops. The continuous ingestion of these residues, though in small quantities, can result in their accumulation in the body causing adverse effects on human health. Thus, the knowledge of the nature and amount of chemicals, which remain in food commodities at the time of their consumption, is very important from public health point of view. The assessment of hazards from these small quantities of pesticides in food and environment, therefore, has become an important part of the overall risks/benefits evaluation before a pesticide is recommended for use (Bates, 1982). Since, literature pertaining to the persistence behaviour of chlorantraniliprole residues on cowpea is scanty, the present study was undertaken.

MATERIAL AND METHODS

The trial was undertaken in 2017 at farmers field, Kalliyoor Vellayani, Thiruvananthapuram to observe the dissipation pattern of chlorantraniliprole in vegetable cowpea. All the practices were followed as per KAU Package of Practices in raising the crop during experimental period. Chlorantraniliprole was purchased from local market and applied at pod formation stage as foliar spray @ 30 g a.i ha⁻¹ along with untreated control. Two sprays were given at 15 days interval.

Cowpea pods (2 kg each) sprayed with insecticide were collected from each plot at two hours, one, three, five, seven, ten and fifteen days after spraying and brought to the laboratory and processed immediately for residue analysis.

Cowpea (500 g) pods harvested from control plots were chopped and ground to a fine paste. Five replicates of 25 g representative samples of the fruits

were taken in 50 mL centrifuge tubes and spiked with 0.05, 0.25 and 0.50 mL of 10 $\mu\text{g mL}^{-1}$ working standard mixtures of the insecticides. The extraction and clean-up were done following the QuEChERS method (Anastassiades *et al.*, 2003) and quantified using UPLC-MS/MS under optimized conditions. The method which gave recovery of insecticides in the range of 70-120 per cent with a relative standard deviation less than 20 was considered to be the ideal method, the lowest spiking level of which was considered as LOQ (Table. 3).

The multiresidue estimation procedure recommended for vegetables as per QuEChERS method with suitable modification was adopted for extraction and cleanup of residues in cowpea. A representative sample of 25g of ground cowpea was taken in a 250 mL centrifuge tube. HPLC grade acetonitrile (50 mL) was added to the samples and homogenised with a high-speed tissue homogenizer (Heidolph Silent Crusher-M) at 14000 rpm for three minutes. This was followed by the addition of 10 g activated sodium chloride (NaCl) and vortexing for two minutes for separation of the acetonitrile layer. The samples were then centrifuged for five minutes at 2500 rpm and 12 mL of the clear upper layer was transferred into 50 mL centrifuge tubes containing 6 g pre-activated sodium sulphate and vortexed for two minutes. The acetonitrile extracts were subjected to clean up by dispersive solid phase extraction (DSPE). For this, 8 mL of the upper layer was transferred into centrifuge tubes (15 mL) containing 0.20 g PSA and 1.20 g magnesium sulphate. The mixtures were then shaken in vortex for two minutes and again centrifuged for five minutes at 2500 rpm. The supernatant liquids (5 mL each) were transferred to turbovap tube and evaporated to dryness under a gentle steam of nitrogen using a Turbopap set at 40 °C and 7.5 psi nitrogen flow. The residues were reconstituted in 2 mL of methanol for chlorantraniliprole filtered through a 0.2-micron filter (PVDF) prior to estimation in LC-MS/MS.

The chromatographic separation was achieved using Waters Acquity UPLC system equipped with a reversed phase Atlantis d C-18 (100 \times 2.1 mm, 5 μm particle size) column. The moisture phase consists of gradient system involving the following two eluent components: (A) 10 % methanol in water + 0.1 % formic acid + 5 mM ammonium acetate; (B) 10 % water in methanol + 0.1 % formic acid + 5 mM ammonium acetate was used as mobile phase for the separation of residues. The gradient elution was done as follows: 0 min isocratic 20 % B, increased to 90 % in 4 min, then raised to 95 % with 5 min and increased to 100 % B in 9 min, decreased to the initial composition of 20 % B in 10 min and hold to 12 min for re-

equilibration. The flow rate remains constant at 0.8 mL min^{-1} and injection volume was 10 μL . The column temperature was maintained at 40 °C. The effluent from the LC system was introduced into triple quadrupole API 3200 MS/MS system equipped with an electrospray ionization interface (ESI), operating in the positive ion mode. The source parameters were temperature 600 °C, ion gas (GSI) 50 psi, ion gas (GS2) 60 psi, ion spray voltage 5,500 V, curtain gas 13 psi.

The MS/MS conditions were optimized using direct infusion in to ESI source in positive mode to provide highest signal/noise ratio for the quantification ion of each analyte. Two MS/MS transitions were made in case of chemical interferences observed in the quantitation ion chromatogram and for qualitative purpose. The ion source temperature was 550 °C with ion spray voltage of 5500 V. Chromatographic elution zones were divided into appropriate number of time segments. In each segment corresponding MS/MS transitions were monitored using multiple reactions – monitoring (MRM) mode.

Based on the peak area of the chromatogram obtained for chlorantraniliprole, the quantity of residue was determined as detailed below.

Pesticide residue (mg kg^{-1}) = Concentration obtained from chromatogram by using calibration curve \times Dilution factor

Dilution factor =

Volume of the solvent added \times Final volume of extract

Weight of sample \times Volume of extract taken for concentration

The persistence of insecticides is generally expressed in terms of half-life (DT_{50}) *i.e.*, time for disappearance of pesticide to 50 per cent of its initial concentration. The residue data obtained at different intervals were subjected to statistical analysis to determine the half-life values of these insecticides on the treated substrate as per the procedure outlined by Hoskins (1961).

Calculation of theoretical maximum residue concentration involves the assumptions that all the pesticides legally allowed on a particular commodity will always be applied, that all residues are present at tolerance levels and that there is no post-harvest effect on residue levels. Therefore, to evaluate the risk assessment of insecticides, the TMRC was calculated by multiplying the maximum residue levels with average per capita daily consumption. Safety parameters were evaluated by comparing the Theoretical Maximum Residue Concentration (TMRC) with Maximum Permissible Intake (MPI) (Bhattacharya *et al.*, 2017).

Table 1. (Residue of chlorantraniliprole 18.5 % SC in cowpea pods)

Days after Spraying (DAS)	Chlorantraniliprole 18.5 % SC	
	Chlorantraniliprole	
	Mean residue \pm SD (mg kg ⁻¹)	Dissipation (%)
Before application	BQL	
0 (2 h after spraying)	0.42 \pm 0.02	
1	0.29 \pm 0.01	30.95
3	0.09 \pm 0.01	78.57
5	0.06 \pm 0.02	85.71
7	BQL	-
10	BQL	-
Half-life (Days)	1.66	

BQL – Below Quantification Level, Limit of Quantification (LOQ) - 0.05 mg kg⁻¹, SD – Standard Deviation

Table 2. Risk assessment of chlorantraniliprole 18.5 % SC in cowpea pods

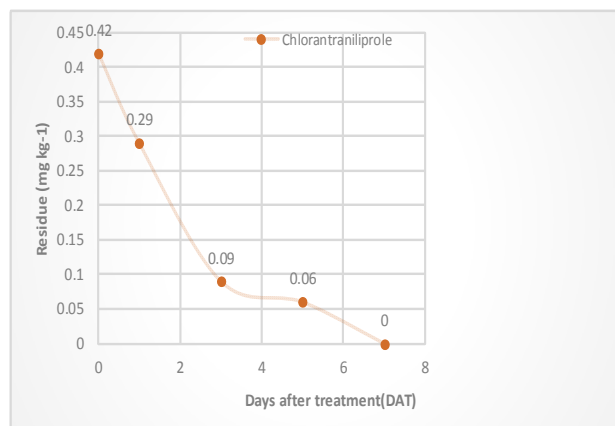
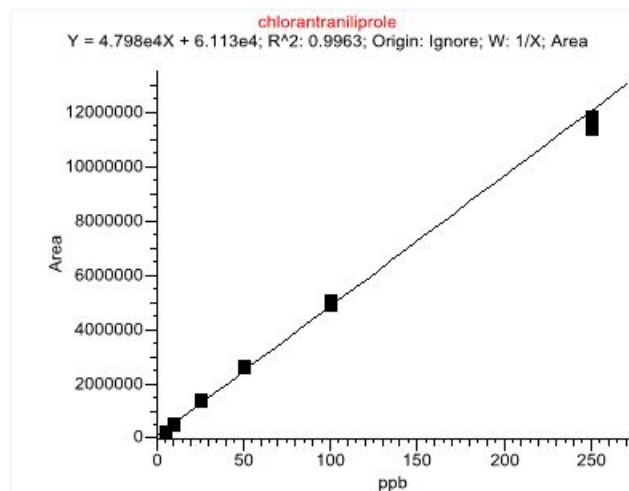
ADI (mg kg ⁻¹ bw d ⁻¹)	Average body weight (kg)	MPI * (μ g person ⁻¹ day ⁻¹)	Daily consumption rate (g day ⁻¹)	Interval (days)	MPI * (μ g person ⁻¹ day ⁻¹)	Average residue (μ g g ⁻¹)	TMRC (μ g person ⁻¹ day ⁻¹)
2	55	110000	90	0	110000	0.42	37.8
2	55	110000	90	1	110000	0.29	26.1
2	55	110000	90	3	110000	0.09	8.1
2	55	110000	90	5	110000	0.06	5.4
2	55	-	90	7	-	BDL	-

* MPI= ADI \times Average body weight \times 1000

Table 3. Recovery of chlorantraniliprole at different levels

Fortification levels (mg kg ⁻¹)						Ion Transitions	
0.05		0.25		0.5		Qualitative	Quantitative
Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)		
102.67	2.98	97.33	2.37	74.00	14.04	483.93>285.96	483.93>452.91

Limit of quantification (LOQ) – 0.05 mg kg⁻¹, RSD – Relative Standard Deviation

**Fig 1. Dissipation of chlorantraniliprole in cowpea****Fig 2. Calibration curve of chlorantraniliprole**

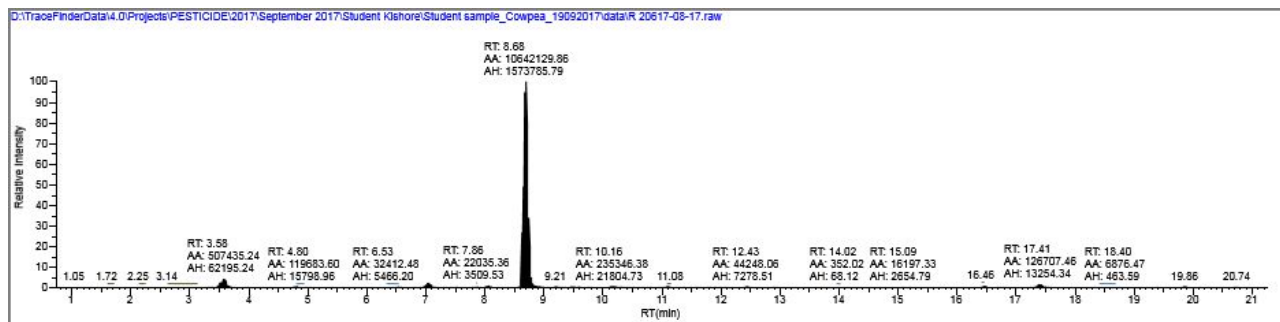


Fig 3. Chromatogram of chlorantraniliprole (Treatment)

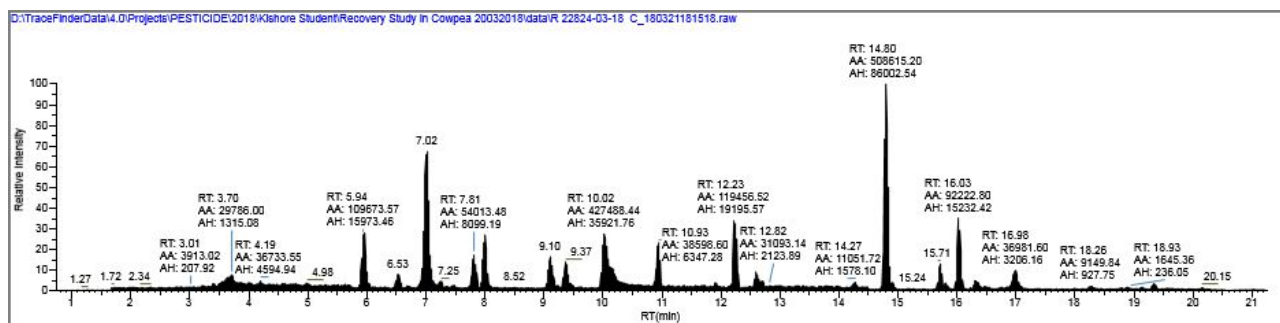


Fig 4. Chromatogram of cowpea spiked with chlorantraniliprole (0.05 mg/kg)

If TMRC value is less than MPI, the particular insecticide will not cause any health impact.

TMRC= Maximum residue level obtained at recommended dose on 0th day of application X total intake of food per day

MPI= Acceptable daily intake X average body weight (55) Kg of an adult of human being

Daily consumption value of cowpea was considered as 90 g d⁻¹ (Huan *et al.*, 2016).

The prescribed ADI values of insecticides were given by FAO/WHO.

RESULTS AND DISCUSSION

The initial deposit of chlorantraniliprole on cowpea fruits following application rate of 0.30 mL L⁻¹ was found to be 0.42 mg kg⁻¹, which dissipated to 0.29 mg kg⁻¹ on the first day of spraying, the extent of dissipation being 30.95 per cent. On the third day, 78.57 per cent of initial residue dissipated and the residue level became 0.09 mg kg⁻¹. The dissipation continued at a slower pace and on the fifth day, the residue reduced to 0.06 mg kg⁻¹ with a dissipation per cent age of 85.71 which reached below quantification level on seventh day and recorded a half-life of 1.66 days (Table 1). The studies on dissipation of chlorantraniliprole 18.5 % SC in cowpea are so scanty. However, more or less similar results were reported as 3.3 days in tomato (Malhat *et al.*, 2011) and 5 days in cauliflower curds (Kar *et al.*, 2013). Contradictory to our results, Studies on dissipation of chlorantraniliprole revealed 45 days in sugarcane (Ramanasubramanian *et al.*, 2012), 21

days in cowpea (Vijayasree *et al.*, 2013). These observations concluded that variation in the rate of dissipation is mainly due to the crop canopy changes, meteorological parameters existed in the experimental area, chemistry of insecticides, concentration of formulation etc. Dissipation of any compound depends on various factors including plant matrix, chemical formulation, agroclimatic conditions, physical phenomenon, application method and chemical degradation in which sunlight place an important role (Bhattacharya *et al.*, 2017).

ADI of chlorantraniliprole is 2 mg kg⁻¹. The mean residue of chlorantraniliprole in cowpea fruits from 0th to 5th day after spraying followed as 0.42, 0.29, 0.09 and 0.06 mg kg⁻¹ respectively. Maximum permissible intake (MPI) was 110000 mg kg⁻¹ bw d⁻¹, by taking 90g as daily consumption of cowpea fruits TMRC values were calculated. TMRC values from 0th to 7th day after spraying were 37.8, 26.1, 8.1 and 5.4 mg kg⁻¹ bw d⁻¹ respectively which were lower than the MPI of chlorantraniliprole (Table 2).

Risk assessment is the course to identify the potential menaces and the associated risks to life and health resulting from human exposure to chemicals present in food over a specific period (WHO, 2009). Consumer risk assessment is a crucial component in the regulatory approval of pesticides for use on food crops (Damalas and Eleftherohorinos, 2011; Huan *et al.*, 2015). The presence of harmful pesticide residues in food has caused a great concern among the consumers. Residue implication of foliar application

of chlorantraniliprole 18.5 % SC on cowpea crop has been evaluated by comparing theoretical maximum residue contribution (TMRC) of the pesticide with its maximum permissible intake (MPI).

CONCLUSION

The TMRC of chlorantraniliprole 18.5 % SC was found to be well below the MPI on cowpea pods even after first day of spraying. Hence the insecticide is safer to the consumers of vegetable cowpea under Kerala Agroclimatic conditions.

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