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Dissipation and risk assessment of ready-mix formulation volium flexi (Chlorantraniliprole and thiamethoxam) residues in cowpea

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Abstract
Dissipation of ready-mix combination insecticide Voliumflexi (Chlorantraniliprole 8.8% + thiamethoxam 17.5% SC) @ 150 g a.i ha^{-1} in cowpea was studied in 2017 at AINP on Pesticide Residues, Vellayani, Kerala Agricultural University. Samples were collected on 0, 1, 3, 5, 7, 10 and 15 days after spraying and residues were extracted by QuEChERS method and estimated by LCMS/MS. The results revealed that chlorantraniliprole was dissipated up to 7 days with an initial concentration of 0.27 mg kg^{-1} thereafter reached to below detectable level with a half-life of 5.34 days. Whereas, the residues of thiamethoxam were dissipated with initial deposit of 0.64 mg kg^{-1} and no residues were detected after 10^{th} day of application and half-life was 3.01 days.

Keywords: dissipation, chlorantraniliprole, thiamethoxam, maximum permissible intake (MPI), theoretical maximum residue concentration (TMRC)

Introduction
Cowpea (*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. 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. Increasing awareness of the potential impact of such toxic chemistries has led to the development of eco-friendly new molecules to ensure minimum risk to man and environment (Huan *et al.*, 2016) [1]. One such insecticide registered for the control of lepidopteron pests is chlorantraniliprole (3-bromo-N-[4-chloro-2-methyl-6-(methyl carbamoyl) phenyl]-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxamide) is a systemic insecticide, belonging to the diamide class of chemistry. Studies on the mode of action of chlorantraniliprole suggest that it generally causes lethargy and muscle paralysis, leading ultimately to the death of the insect. Categorized as a ‘‘reduced risk’’ pesticide, it is recommended as an alternative to pyrethroids for vegetables (IRAC, 2018) [2].

Materials and Methods
Method validation
Fortification and recovery experiment
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 µg mL^{-1} working standard mixtures of the insecticides. The extraction and clean-up were done following the QuEChERS method (Anastassiades *et al.*, 2003) [3] 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. 1).

Table 1: Risk assessment (TMRC) of chlorantraniliprole 8.8% + thiamethoxam 17.5% SC in cowpea pods.

Days after treatment	Chlorantraniliprole		Thiamethoxam	
	Residues (mg kg^{-1})	TMRC (µg person $^{-1}$ day^{-1})	Residues (mg kg^{-1})	TMRC (µg person $^{-1}$ day^{-1})
0	0.27	24.3	0.64	57.6
1	0.24	21.6	0.58	52.2
3	0.15	13.5	0.27	24.3
5	0.14	12.6	0.20	18.0
7	0.11	9.9	0.14	12.6

Table 2: Fortification levels (mg Kg^{-1})

Insecticides	Fortification levels (mg Kg^{-1})					
	0.05		0.25		0.50	
	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
Chlorantraniliprole	102.67	2.98	97.33	2.37	74.00	14.04
Thiamethoxam	84.00	8.25	101.33	4.56	77.83	12.22

Field experiment

Experiment was conducted in vegetable farm, Kalliyoor, Thiruvananthapuram during 2017 to observe the dissipation and persistence of combination product Voliumflexi (Chlorantraniliprole 8.8% + thiamethoxam 17.5% SC) @ 150 g a.i ha^{-1}. Cowpea pods (2 kg each) sprayed with insecticides 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 at AINP on Pesticide Residues, College of Agriculture, Vellayani, KAU.

Estimation of persistence and degradation of residues

Residue extraction

The multiresidue estimation procedure recommended for vegetables as per QuEChERS method with suitable modification was adopted for extraction and cleanup of residues in cowpea. The harvested fruits were macerated as such in a high-speed blender (BLIXER 6 *vivo* Robot Coupe) for three times and 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 homogenized 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 overtaxing 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 vortexes 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 Turbovap set at 40 ºC and 7.5 psi nitrogen flow. The residues were reconstituted in 2 mL of methanol for both chlorantraniliprole and thiamethoxam filtered through a 0.2-micron filter (PVDF) prior to estimation in LC-MS/MS and GC-MS respectively.

Instrumentation

LC-MS/MS

The chromatographic separation was achieved using Waters Acquity UPLC system equipped with a reversed phase Atlantis d C-18 (100 ×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 ºC. 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 ºC, ion gas (GSI) 50 psi, ion gas (GS2) 60 psi, ion spray voltage 5,500 V, curtain gas 13 psi.

Residue Quantification

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

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

$$\text{Dilution factor} = \frac{\text{Volume of the solvent added} \times \text{Final volume of extract}}{\text{Weight of sample} \times \text{Volume of extract taken for concentration}}$$

The persistence of insecticides is generally expressed in terms of half-life (DT50) *i.e.*, time for disappearance of pesticide to 50 per cent of its initial concentration.

Risk assessment of insecticide mixtures in cowpea

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 in the Indian context. Safety parameters were evaluated by comparing the Theoretical Maximum Residue Concentration (TMRC) with Maximum Permissible Intake (MPI) (Bhattacharya *et al.*, 2017) [4]. 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 0^{th} 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) [5].
The prescribed ADI values of insecticides were given by FAO/WHO.

Results

Table 3: Residue of chlorantraniliprole 8.8% + thiamethoxam 17.5% SC in cowpea pods.

Days after Spraying (DAS)	Chlorantraniliprole 8.8% + Thiamethoxam 17.5% SC			
	Chlorantraniliprole		Thiamethoxam	
	Mean residue ± SD (mg kg^{-1})	Dissipation (%)	Mean residue ± SD (mg kg^{-1})	Dissipation (%)
Before application	BQL		BQL	
0 (2 h after spraying)	0.27±0.01		0.64±0.023	
1	0.24±0.02	11.11	0.58±0.03	9.37
3	0.15±0.01	44.44	0.27±0.02	57.81
5	0.14±0.01	48.14	0.20±0.02	68.75
7	0.11±0.02	59.25	0.14±0.01	78.12
10	BQL	-	BQL	-
Half-life (Days)	5.34		3.01	

BQL – Below Quantification Level, Limit of Quantification (LOQ) - 0.05 mg Kg^{-1}, SD – Standard Deviation

Chlorantraniliprole

The initial deposit of chlorantraniliprole (two hours after spraying) was 0.27 mg kg^{-1}. One day after spraying, the residue reduced to 0.24 mg kg^{-1}, with a reduction of 11.11 per cent. On third day after spraying, the residue content was degraded to 0.15 mg kg^{-1} with a reduction of 44.44 per cent. The residues of chlorantraniliprole were 0.14, 0.11 mg kg^{-1} after 5 and 7 days after spraying with a dissipation% of 48.14 and 59.25 respectively. However, on tenth day after spraying, the residues reached below quantification level 0.05 mg kg^{-1}. The half-life recorded was 5.34 days.

Thiamethoxam

Thiamethoxam resulted in an initial deposit of 0.64 mg kg^{-1} on cowpea pods after two hours of spraying. One day after spraying, the residue degraded to 0.58 mg kg^{-1} with a reduction per cent of 9.37 per cent from the initial residue. The 57.81 per cent of the residue degraded on third day and the concentration of residue recorded being 0.27 mg kg^{-1}. However, on the fifth and seventh day after spraying the residue content degraded to 0.20 and 0.14 mg kg^{-1} respectively with a dissipation per cent of 68.75 and 78.12 respectively. The half-life of thiamethoxam calculated as 3.01 days.

Risk assessment of chlorantraniliprole 8.8% + thiamethoxam 17.5% SC in cowpea

Chlorantraniliprole

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 0.27, 0.24, 0.15, 0.14 and 0.11 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 24.3, 21.6, 13.5, 12.6 and 9.9 µg kg^{-1} bw d^{-1} respectively which were lower than the MPI of chlorantraniliprole (Table. 1).

Thiamethoxam

ADI of thiamethoxam is 0.08 mg kg^{-1}. The mean residue of thiamethoxam in cowpea fruits from 0th to 5th day after spraying followed as 0.64, 0.58, 0.27, 0.20 and 0.14 mg kg^{-1} respectively. Maximum permissible intake (MPI) was 4400 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 57.6, 52.2, 24.3, 18 and 12.6 µg kg^{-1} bw d^{-1} respectively which were lower than the MPI of thiamethoxam. Thus, the application of thiamethoxam in cowpea at the recommended dose does not pose any adverse health effect on the consumers (Table.1).

Discussion

Dissipation rate of insecticides is one of the most important parameters in assessing their potential hazards on the environment. However, specific studies on the dissipation and persistence of insecticide mixtures in cowpea are so meagre. The studies on dissipation of chlorantraniliprole 8.8% + thiamethoxam 17.5% SC are so scanty. The persistence of chlorantraniliprole and thiamethoxam in cowpea as single insecticide was studied in the present study. The result revealed that chlorantraniliprole and thiamethoxam as single insecticides dissipated within 7 and 5 days with half-lives of 1.66 and 1.37 days respectively. The results were in agreement with reported by Kar and his co-workers (2011) [6] as they found chlorantraniliprole @ 9.25 g a.i ha^{-1} was dissipated into BDL after 5 days with half-life of 1.36 days in cauliflower. Malhat and his co-workers (2012) [7] also reported the half-life of chlorantraniliprole as 3.30 and 3.66 days in tomato fruit and soil. Ahlawat and co-investigators (2019) [8] also reported the half-life of chlorantraniliprole as 1.18 days in capsicum at recommended dose of 30 g a.i ha^{-1}. Similar results were reported by Karmarkar and Kulshrestha (2009) [9] in tomato where thiamethoxam was dissipated up to 10 days with a half -life of 4 days. Contradictory to the present study, Vijayasree, (2013) [10] reported that chlorantraniliprole persisted up to 10 days in cowpea. Chauhan and his co-workers (2013) [11] reported thiamethoxam was persisted up to 15 days after application in okra with a half-life of 1.47 days. Chlorantraniliprole 8.8% + thiamethoxam 17.5% SC when applied as mixture, their residues persisted longer than when applied as single insecticides.

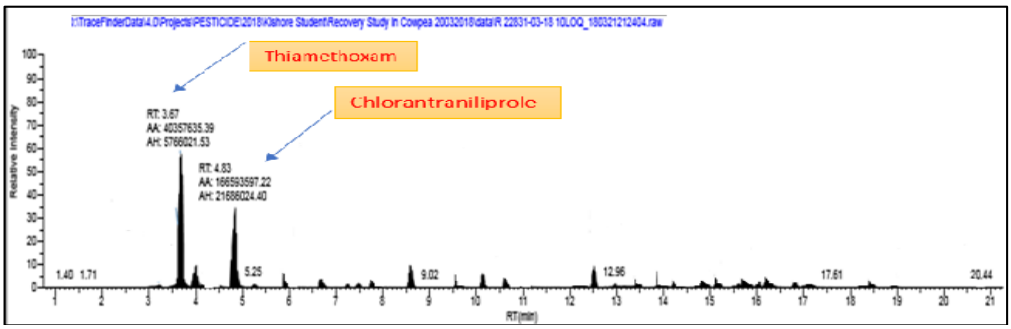

Fig 1: LC-MS/MS chromatogram of insecticides at LOQ

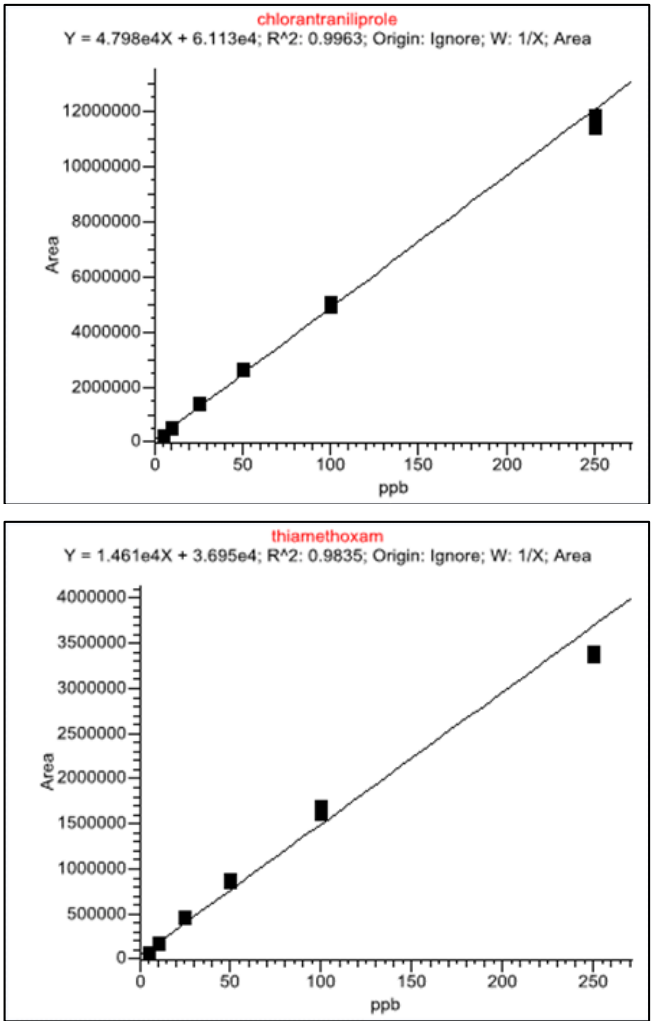

Fig 2: Calibration curves of chlorantraniliprole and thiamethoxam

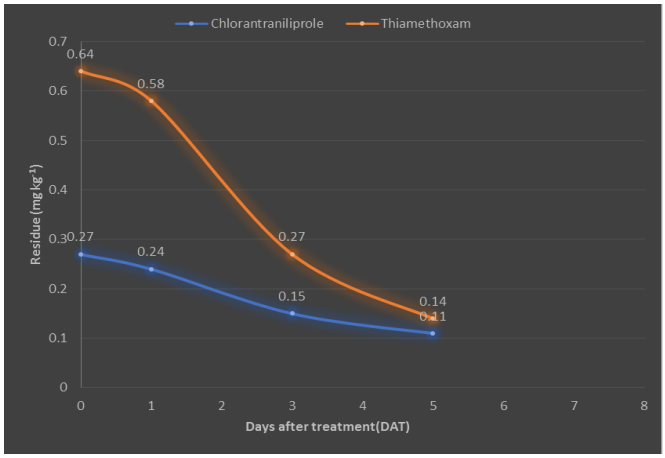

Fig 3: Dissipation of chlorantraniliprole and thiamethoxam in cowpea

Conclusion

Consumer risk assessment is a crucial component in the regulatory approval of pesticides for use on food crops. Theoretical maximum residual concentration (TMRC) of chlorantraniliprole 8.8% + thiamethoxam 17.5% SC was found to be well below the MPI on cowpea pods even at 2 hrs after spraying. Hence, the insecticide will not cause any adverse effect after consumption of cowpea pods. Therefore, the application of chlorantraniliprole 8.8% + thiamethoxam 17.5% SC at recommended dose seems to be safe from crop protection and environmental contamination and consumers point of view.

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