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Residue behavior and risk assessment of beta cyfluthrin 8.91% + imidacloprid 19.81% OD in chilli (*Capsicum annum* L.)

Anju Viswanathan K and Ambily Paul

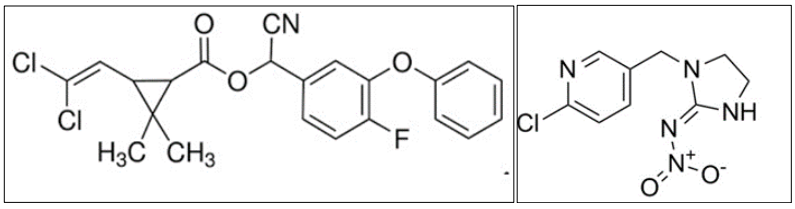
Abstract
A field experiment was conducted at Kalliyoor Panchayath of Thiruvanathapuram disrtrict to assess the dissipation and risk assessment of a combination insecticide, beta cyfluthrin 8.91% + imidacloprid 19.81% OD in chilli. The residue analysis conducted at Pesticide Residue Research and Analytical Laboratory at College of Agriculture, vellayani, Thiruvanathapuram revealed that, the insecticide beta cyfluthrin and imidacloprid had an initial residue of 0.49 and 0.18 mg Kg^{-1} respectively. Beta cyfluthrin reached LOQ on 15th day after spraying while, imidacloprid reached LOQ on 7th day after spraying. Risk assessment studies revealed that the TMRC value less than that of MPI, indicated the safety of particular chemicals.

Keywords: Risk assessment, beta cyfluthrin 8.91% + imidacloprid 19.81% OD, (*Capsicum annum* L.)

Introduction
Chilli, *Capsicum annum* L. is an important spice cum vegetable crop and a rich source of vitamin A, B, C and capsaicin. The largest producer of chillies in the world is India but productivity is only 1.93 tonnes per hectare (Geetha and Selvarani, 2017) [3] which has to be increased to a bench mark level of 5000 Kg ha^{-1} to compete the international market. Among the different constraints that lower productivity, the pest complex that attack chilli at different crop stage is the most important factor. The major sucking pest complex that attack chilli are mites, *Polyphagotarsonemus latus* Banks, thrips, *Scirtothrips dorsalis* Hood, aphids, *Myzus persicae* Sulzer, and *Aphis gossypii* Glove. The yield loss due to chilli thrips and mites are estimated to be the tune of 50 per cent (Halder *et al.*, 2015) [4]. In order to mitigate various pests of different feeding habits, farmers resort to individual application of different groups of insecticides and/ or acaricides with short spells, leads to deposition of huge amount of insecticides loads in crop as well as environment. This is evident from the frequent occurrence of insecticide residues in green chilli, higher mortality of beneficial arthropod fauna in chilli ecosystem and high cost of cultivation.

Recently, different pesticide firms have commercialized various insecticide mixtures which can take care of sucking pests *viz*, mite, thrips, aphids, whiteflies as well as leaf feeders/ chewing pests. Insecticide mixtures involve combinations of two or more insecticides having different mode of action into a single spray solution which entails exposing individual in an arthropod pest population to each insecticide simultaneously (Tabashnik, 1989; Hoy, 1998) [11, 5]. Beta-Cyfluthrin is an insecticide of synthetic pyrethroid group and having contact and stomach action. It acts on the insect's nervous system as sodium channel blocker (IRAC, 2018). In the pest, rapid excitation and impairment of coordination are the first visible symptoms of intoxication, followed by knockdown and death.

Imidacloprid is antagonist to the nicotinic acetyl choline receptor in the central nervous system. It disturbs the proper signal transmission system leading to excitation of nerve cell. Consequently a disorder of the nervous system occurs leading finally to the death of the treated insect. The chemical structure of beta cyfluthrin and imidacloprid are given below



a b

Chemical structure of a) Beta cyfuthrin b) Imidacloprid

The dissipation and risk assessment of the particular insecticide mixture on chilli under Kerala conditions are not much investigated. Hence, it is relevant to carry out such studies to generate information on residue behaviour and risk assessment of the insecticide mixture.

Materials and Methods

Persistence and degradation of insecticide residues in chilli

The studies on the persistence and degradation of the insecticide mixture in chilli fruits were done in the Pesticide Residue Research and Analytical Laboratory, College of Agriculture, Vellayani, Thiruvanathapuram, Kerala.

Method Validation

Before sampling of chilli fruits from insecticide treated plots, recovery experiments were carried out to assess the efficacy of extraction and clean up procedures adopted to standardize the procedure for insecticide residue estimation from chilli fruits. For conducting the experiment, control samples of chilli fruits were separately spiked at different levels with the certified reference material (CRM) of the insecticides described. The samples were extracted and cleaned up as per the QuEChERS method (Anastassiades *et al*., 2003) [1].

Preparation of standard insecticides

CRM of pesticides viz., betacyfluthrin and imidacloprid with 99.3, 99.9 per cent purity respectively were procured from M/s Sigma Aldrich. Stock solutions (1000 µg mL-1) of the insecticides were prepared by dissolving a weighed quantity of the analytical grade material in HPLC grade methanol. The stock solutions were serially diluted to prepare an intermediate stock of 100 µg mL-1. The intermediate stock solutions were further diluted with HPLC grade methanol to prepare working standard mixtures of each insecticides to be analyzed by positive electro spray ionization. The working standard mixtures were serially diluted to obtain 1.00, 0.50, 0.25, 0.10, 0.05, 0.01 and 0.005 µg mL-1 of analytical grade insecticides.

Fortification and recovery experiments

Chilli fruits (500 g) harvested from the control plots were cut and blended 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 (LOQ), 0.25 (2 x LOQ) and 0.50 (5x LOQ) mL-1 of working standard mixtures of the insecticides. The extraction and clean- up were done by following QuEChERS method and quantified by using GC and LC MS/MS under optimized conditions. The method which gave recovery of insecticide in the range of 70 to 120 per cent with a relative standard deviation less than 20 was considered to be the ideal method.

Field Experiment

The field experiment was conducted at Kalliyoor Panchayath of Thiruvanathapuram district during September 2018 to December 2018 with five replication in randomized block design. The crops were raised as per the recommendations given in the Package of Practices of Kerala Agricultural University (KAU, 2016) [7]. Beta cyfluthrin 8.91% + imidacloprid 19.81% OD @ 15.75+36.75 g a.i ha-1 was applied at fruit setting stage using a hand operated knapsack sprayer. A spray volume of 500 L ha-1 was used. Fresh chilli fruits (approximately 250 g) were collected randomly from each replicate at 0 (after 2 h), 1, 3, 5, 7, 10, 15 and 30 days after the application. The samples were collected in a polypropylene covers and brought to Pesticide Residue Research and Analytical Laboratory (PRRAL), College of Agriculture, Vellayani, Thiruvanathapuam for analysis.

Residue extraction and cleanup

The multiresidue estimation procedure recommended for vegetables as per QuEChERS method with suitable modification was adopted for extraction and clean- up of residues in chilli. The harvested fruits were blended in a high speed blender (BLIXER 6 vv Robot Coupe) and a representative sample of 25 g of chilli was taken in a 250 mL centrifuge bottle. HPLC grade acetonitrile (50 mL) was added to the samples and homogenized with a high speed tissue homogenizer (Heidolph Silent Crusher- M) at 1400 rpm for three minutes. This was followed by the addition of 10 g activated sodium chloride (NaCl) and vortexed for two minutes for the separation of acetonitrile layer. The samples were then centrifuged for 8 minutes at 2500 rpm and 16 mL of the clear upper layer was transferred into a 50 mL centrifuge tubes containing 6 g pre activated sodium sulphate and vortexed for 2 minutes. The acetonitrile extracts were subjected to clean up by dispersive solid phase extraction (DSPE). For this 12 mL of the upper layer was transferred into centrifuge tubes (15 mL) containing 0.20 g primary secondary amine (PSA) and 1.2 g of magnesium sulphate. The tubes were then centrifuged at 2500 rpm for 5 minutes. The supernatant liquid were transferred into turbovap tube (3 mL for LC compounds and 4 mL for GC compounds) and evaporated to dryness under a gentle steam of nitrogen using a Turbovap set at 40^{o} C and 7.5 psi nitrogen flow. The residues were reconstituted in 1.5 mL of methanol for imidacloprid and in 1 mL n- hexane for beta cyfluthrin, filtered through a 0.2 micron filter (PVDF) prior to estimation in LC- MS/MS and GC-ECD respectively.

Instrumentation

LC- MS/MS

The chromatographic separation was achieved by using Waters Acquity UPLC system equipped with a reversed phase Atlantis d c- 18 (100x 2.1 mm, 5µm particle size) column. The moisture phase consists of gradient system involving the following the eluent compounds *viz.*, (A) 10% methanol in water + 0.1% formic acid+ 5 mM ammonium acetate and (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 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^{o} C. The effluent from the LC system was introduced into triple quadrupole API 3200 MS/MS system equipped with an electro spray ionization interface (ESI), operating in the positive ion mode. The source parameters were temperature 600 ^{o} C, ion gas (GSI) 50 psi, ion gas (GS2) 60 psi, ion spray voltage 5,500 V, curtain gas 13 psi.

GC- ECD

Estimation of residues of beta cyfluthrin was performed using Gas Chromatograph (Shimadzu 2010 AT) equipped with Electron Capture Detector (ECD). Operating conditions of GC are, column, DB- 5 capillary (0.25µm film thickness x 0.25 mm x 30 mm), carrier gas- nitrogen, column flow- 0.79

mL min^{-1}, injector temperature - 250 ^{o} C and detector temperature used was 300 ^{o} C. The residues of beta cyfluthrin was confirmed in GC- MS (Shimadzu GC- MS QP 2010 Plus) with retention time of 61.10 min. Helium was used as carrier gas in GC- MS operated with Electron Impact Ionization (70 eV). In GC- MS injector temperature, column, column flow was similar to that of GC.

The MS/MS conditions were optimized by using direct infusion into ESI source in positive mode to provide the highest signal/ noise ratio for the quantification 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 ^{o} C with ion spray voltage of 5500 V in each segment corresponding MS/MS transitions were monitored using multiple reactions – monitoring (MRM) mode.

Residue quantification

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

$$\text{Pesticide residue (mg kg}^{-1}) = \frac{\text{Volume of the solvent added x Final volume of extract}}{\text{Weight of sample x Volume of extract taken for concentration}}$$

The persistence of insecticides are generally expressed in terms of half- life (DT 50) *ie.,* time for disappearance of pesticide to 50% of its initial concentration.

Risk assessment of insecticide mixture in chilli

Risk assessment was estimated by comparing the acceptable daily intake (ADI) value with maximum permissible intake (MPI) of residues of pesticide through the produce and the theoretical maximum residue contribution (TMRC). The MPI was obtained by multiplying the ADI with the average weight of Indian person, 60 Kg. (Katna *et al.,* 2017) [6]. An ADI value of 0.04 and 0.06 mg kg^{-1} body weight day^{-1} for beta cyfluthrin and imidacloprid was recommended by JMPR-WHO (2012) [12] was used for risk assessment

Result and Discussion

Validation of analytical method

Satisfactory recovery values obtained in the method validation study indicated the efficiency of the method adopted for extraction and cleanup of residues (Table. 1). The mean recovery of the two compounds *viz*. beta cyfluthrin and imidacloprid were within the acceptance range of 82 to 105.6 per cent at three levels of fortification. The repeatability of the recovery results as indicated by relative standard deviations, RSD < 20 per cent, confirmed that the method was sufficiently reliable for pesticide analysis.

Table 1: Per cent recovery of insecticides in chilli

Insecticides	Fortification levels (mg Kg^{-1})					
	LOQ		2 LOQ		5 LOQ	
	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
Beta cyfluthrin	87	7.39	82	14.23	105.6	7.03
Imidacloprid	96	2.08	84	4.17	84	4.76

Persistence and dissipation of beta cyfluthrin 8.91% + imidacloprid 19.81% OD in chilli

The persistence and dissipation of beta cyfluthrin 8.91% + imidacloprid 19.81% OD @ 15.75 +36.75 g a.i ha^{-1} on fresh chilli fruits are given in Table 2. The initial residual concentration of beta cyfluthrin is more than imidacloprid,that is 0.49 mg Kg^{-1} for beta cyfluthrin and 0.18 mg Kg^{-1} for imidacloprid. The dissipation of both the insecticide were slow on chilli fruits during initial days, but after 5th day onwards there was an increased dissipation. More than fifty percentage dissipation occurred for both the insecticide at fifth day after spraying. The dissipation pattern of both the insecticide followed first order kinetics. The residue of beta cyfluthrin reached limit of quantification at 15th day after spraying, whereas in imidacloprid, the residue reached LOQ on 7th day after spraying. More or less similar results was reported by Sahoo *et al.* (2012), where they found that a combination formulation of solomon 300 OD (beta cyfluthrin 9% + imidacloprid 21%) @ 120 g a.i ha^{-1} dissipated to below limit of quantification of 0.01 mg Kg^{-1} after 5 days for beta cyfluthrin and and 7 days for imidacloprid on okra plant. Similar findings was observed by Mandal *et al.* (2009) [8] on brinjal.

Table 2: Residue of beta cyfluthrin 8.91% + imidacloprid 19.81% OD in chilli fruits

DAS	Beta cyfluthrin 8.91% + imidacloprid 19.81% OD			
	Beta cyfluthrin		Imidacloprid	
	Mean residue ± SD (mg Kg^{-1})	Dissipation (%)	Mean residue ± SD (mg Kg^{-1})	Dissipation (%)
Before application	LOQ		LOQ	
0 (2 h after spraying)	0.49 + 0.008	-	0.18 + 0.001	-
1	0.34 + 0.028	30.61	0.17 + 0.006	5.55
3	0.33 + 0.029	32.65	0.13 + 0.001	27.77
5	0.16 + 0.021	67.34	0.07 + 0.000	61.11
7	0.08 + 0.016	83.67	< LOQ	
10	0.06 + 0.016	87.75	< LOQ	
15	< LOQ		< LOQ	
30	< LOQ		< LOQ	
Half-life (days)	4.20		3.80	

LOQ- Limit of Quantification, SD- Standard Deviation

Table 3: Risk assessment of beta cyfluthrin 8.91% + imidacloprid 19.81% OD on chilli ADI – Acceptable Daily Intake, MPI – Maximum Permissible Intake, TMRC- Theoretical Maximum Residue Concentration

ADI (mg kg^{-1} bw d^{-1})		Average body weight (kg)	Interval (Days)	Daily Consumption rate (g day $^{-1}$)	MPI (µg person^{-1} day^{1})		Average residue (µg g^{-1})		TMRC (µg person^{-1} day^{-1})	
Betacyfluthrin	Imidacloprid				Beta cyfluthrin	Imidacloprid	Beta cyfluthrin	Imidacloprid	Beta cyfluthrin	Imidacloprid
0.04	0.06	60	0	5	2400	3600	0.49	0.18	2.45	0.90
0.04	0.06	60	1	5	2400	3600	0.34	0.17	1.7	0.85
0.04	0.06	60	3	5	2400	3600	0.33	0.13	1.65	0.65
0.04	0.06	60	5	5	2400	3600	0.16	0.07	0.80	0.35
0.04	0.06	60	7	5	2400	3600	0.08	BDL	0.40	LOQ
0.04	0.06	60	10	5	2400	3600	0.06	BDL	0.30	LOQ
0.04	0.06	60	15	5	2400	3600	LOQ	LOQ	0.25	LOQ
0.04	0.06	60	30	5	2400	3600	LOQ	LOQ	LOQ	LOQ

Risk assessment is purely a theoretical calculation to ensure that the product is safe when offered for consumption based on the residue present. The primary aim of the risk assessment is to determine the safe levels of dietary exposure of agricultural chemicals to human beings and the environment. The repeated and prolonged use of pesticides on crops leads to high amount residues and the consumption of pesticide laden commodities may lead to adverse health related issues.

An average Indian consumes 5 g of fresh chilli in a balanced diet (NSSO, 2014) [9]. From the risk assessment table (Table 3) it was clear that the TMRC value from the 0 to 30 days after spraying is too behind MPI values, and thus it was safe for consumption even from the day itself after spraying. These observations are in accordance with the findings of Chahil *et al.* (2014) [2].

Conclusion

The dissipation pattern of both insecticide followed first order kinetics and reached LOQ at 15th day of spraying for beta cyfluthrin and 7th day of spraying for imidacloprid. Considering the TMRC values for beta cyfluthrin and imidacloprid, it was too lower than MPI from two hours after spraying itself. Thus it was considered safe for consumption from the day of spraying itself. Because of all these facts, the particular insecticide can be safely recommended for sucking pests of chilli without causing any health hazards to humans.

References

1. Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction / partitioning and "dispersive solid phase extraction" for the determination of pesticide residues in produce. J. AOAC Int. 2003; 86(2):412-431.
2. Chahil GS, Mandal K, Sahoo SK, Singh B. Risk assessment of mixture formulation of spirotetramat and imidacloprid in chilli fruits. Environ. Monit. Assess. 2014; 187:41-50.
3. Geetha R, Selvarani K. A study of chilli production and export from India. Int. J. Adv. Res. Innov. Ideas. Educ. 2017; 3(2):205-210.
4. Halder J, Kodandaram MH, Singh B, Rai AB. Bio-efficacy of some newer acaro-insecticides against yellow mite (*Polyphagotarsonemus latus* (Banks)) and thrips (*Scirtothrips dorsalis* Hood) in chilli. Pesticide Res. J. 2015; 27(2):171-174.
5. Hoy MA. Myths, models and mitigation of resistance to pesticides. Phil. Trans. Royal Soc. London Biol. Sci. 1998; 353:1787-1795.
6. Katna S, Patyal SK, Dubey JK, Chauhan BT, Chauhan A, Devi N, *et al*. Dissipation and risk assessment of combi product of mancozeb and carbendazim on apple at different locations in north India. Pesticide Res. J. 2017; 29:48-54.
7. KAU (Kerala Agricultural University). Package of Practices Recommendations: crops 15th editions. Kerala Agricultural University, Thrissur, 2016, 392p.
8. Mandal K, Chahil GS, Sahoo SK, Battu RS, Singh B. Dissipation kinetics of β-cyfluthrin and imidacloprid in brinjal and soil under subtropical conditions of Punjab, India. Bull. Environ. Toxicol. 2009; 84(2):225-229.
9. NSSO. Household Consumption of Various Goods and Services in India 2011-12, NSS 68th Round, National Sample Survey Office, Ministry of Statistics and Programme Implementation, Government of India, 2014.
10. Sshoo SK, Chahil GS, Mandal K, Battu RS. Estimation of β-cyfluthrin and imidacloprid in okra fruits and soil by chromatography techniques. J Environ. Sci. Health. 2012; 47(1):42-50.
11. Tabashnik BE. Managing resistance with multiple pesticide tactics: theory, evidence, and recommendations. J Econ. Entomol. 1989; 82:1263-1269.
12. WHO [World Health Organization]. Inventory of evaluations performed by the Joint Meeting on Pesticide Residues (JMPR). [Online]. Available, 2012. http://apps.who.int/pesticide-residues-jmpr-database/Home [12 Jan. 2017].