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Maximum residue limit and risk assessment of beta-cyfluthrin and imidacloprid in combination formulation on Tomato (*Lycopersicon esculentum*)

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Abstract : Field and laboratory experiments were carried out to study the persistence and dissipation of beta-cyfluthrin and imidacloprid in combination-mix formation on tomato. Initial deposits of beta-cyfluthrin ranged from 1.224 to 2.133 mg kg^{-1} and imidacloprid ranged from 1.447 to 2.383 mg kg^{-1} in tomato. Beta-cyfluthrin residues dissipated quickly and attained the below detectable level by 10 to 15 day. Imidacloprid residues are dissipated to below detectable limit (BDL) within 15 days after application. The Theoretical Maximum Residue Contribution (TMRC) values of initial deposits were quite low (beta-cyfluthrin, 0.245-0.427 mg $person^{-1}$ day^{-1}; imidacloprid, 0.289-0.477 mg $person^{-1}$ day^{-1}) than the maximum allowable concentration value (beta-cyfluthrin, 1.0 mg $person^{-1}$ day^{-1}; imidacloprid, 3.0 mg $person^{-1}$ day^{-1}). Based on the residue data, the Maximum Residue Limit (MRL) of 0.5 mg kg^{-1} is proposed for both beta-cyfluthrin and imidacloprid in tomato fruits under Indian conditions. Assuming if this MRL is fixed, the Theoretical Maximum Daily Intake (TMDI) was calculated as 0.1 mg $person^{-1}$ day^{-1} and % ADI (Acceptable Daily Intake) value of 10 and 3.33% also ensures the safety of beta-cyfluthrin and imidacloprid respectively. A waiting period of 3 days before harvest could ensures a sufficient margin of safety without any risk to human health.

Keywords : Combination-mix, beta-cyfluthrin, imidacloprid, MRL, risk assessment.

Introduction

A wide range of insect pests attack Tomato (*Lycopersicon esculentum*) and forms major limiting factor in its successful cultivation and improvement in yield. Among them tomato fruit borer, *Helicoverpa armigera* is an important pest which causes considerable loss in quantity as well as quality of tomato fruits[1,2]. Eventhough, the quick and effective control of pests by insecticides convinces the farmers but the lack of appropriate site specific management led the farmers to use insecticides excessively, which resulted in development of resistance in major agricultural pests. The development of resistance has limited the application of single insecticides and resort to tank mixtures. Mixing of two or more insecticides is very common among the Indian farmers though not recommended. Tank mixtures involve several problems like incompatibility of components, antagonism, separation of insecticides, coagulation and sometimes overdose of individual pesticide or both in combined form to crop that causes adverse effect on the crops as well as undue residues. These problems can be overcome by promoting ready mix or combination-mix formulations[3,4]. Combination-mix formulations are cost effective and have high efficiency on controlling the pests compared to single insecticides. The pre combination pesticide mixtures appear to be advantageous than single compound or tank mixtures since this gives broad spectrum activity, additive joint action, economy in pest control and application, multiple mode of action and overcoming or delayed resistance to pesticides. Recently introduced combination molecule (beta-cyfluthrin + imidacloprid) marketed under the trade name Confidor Ultra, 100 SC by Bayer Crop Science India that consists of two pesticide molecules in the ratio of 50 : 50. Beta-cyfluthrin, ((*SR*)-α-cyano-4-fluoro-3-phenoxybenzyl (1*RS*, 3*RS*; 1*RS*, 3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate) a halogen containing synthetic pyrethroid is a useful insecticide for wide range of activities like control of

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insect pests, horticultural pests, ectoparasites of livestock's and household pests. It is a broad-spectrum insecticide and acts primarily as a contact and stomach poison[5]. Imidacloprid (1-(6-chloro-3-pyridinylmethyl)-*N*-nitroimidazolidine-2-ylideneamine) has a unique structure composed of two nitrogen heterocyclic moieties. It is the first commercial representative of chloronicotinyl insecticide acting on nicotinic acetylcholine receptor poison[6,7]. In order to protect the consumer health and facilitating international trade, the primary objective is to develop Maximum Residue Limit (MRL). The possible exposure to residues of pesticides is an integral part of risk assessment process to ensure that the Acceptable Daily Intake of pesticides is not exceeded. The best assurance that the exposures to residues are within safe limits is obtained from dietary intake studies. The presence of pesticides residues in vegetables, fruits, and green leaves above the maximum limit is of concern to human health because of the toxic nature of the pesticides[8-10]. The objective of the study was to evaluate the persistence and dissipation of beta-cyfluthrin + imidacloprid in combination formulation on tomato. Besides, an attempt has been made to predict its total dietary intake through consumption of tomato fruits in order to assess whether it use might lead to consumer risk or not by generating food chemical concentration data (residues) from field trial.

Material and methods :

Chemical reagents : The organic solvents, acetone (AR), dichloromethane (AR), acetonitrile (HPLC grade), ethyl acetate (HPLC grade) were purchased from S.D Fine chem. Ltd. Technical grade pesticide standards were obtained from M/s Bayer Crop Science and stored in a freezer at -5 °C. Beta-cyfluthrin (50.66 mg; purity, 98.7%) and imidacloprid (50.50 mg; purity, 99%) dissolved in 50 ml of distilled acetone and acetonitrile respectively to obtain a 1000 $\mu g\ g^{-1}$ stock solution. Suitable aliquots were diluted serially to obtain the desired concentrations and the solutions were stored in refrigerator for further use.

Fortification : The efficiency of extraction, cleanup and estimation was determined by recovery studies. Untreated chopped fruits (50 g) cut into small pieces spiked with beta-cyfluthrin and imidacloprid solution at 0.1 and 0.2 mg kg^{-1} level and were shaken for 10 min to obtain uniform distribution of insecticide. Flasks were then kept overnight for equilibration. Three replicates were taken for each treatment including control and samples were analyzed for recovery.

Field studies : A field trial was conducted in randomized block design during 2007-08 in the experimental farms of Indian Agricultural Research Institute, New Delhi. Tomato seedlings (var. Pusa Sheetal) were transplanted on raised beds (4.5 × 0.5 m^2). Combination-mix (beta-cyfluthrin + imidacloprid, 1 : 1) [Confidor Ultra, 100SC; Bayer Crop Science India) @ 40 and 80 g a.i. ha^{-1} were sprayed separately on the crop at fruiting stage. The 4X dose of combination-mix (1 : 1, beta-cyfluthrin + imidacloprid) was also sprayed to assess the phytotoxicity of pesticides. The fluid rate was maintained at 500 L ha^{-1}.

Sampling : Tomato fruits (500 g) were collected randomly from each treatment at 0 (~ 1 h after spraying). 1, 3, 5, 7, 10, 15, 20, 25 and 30 days after spraying of insecticides. The fruits were cut into small pieces, mixed and a sub sample of 50 g was taken for residue analysis.

Extraction and clean up and estimation :

Beta-cyfluthrin :

Tomato samples (50 g) were macerated with 100 ml of acetone in a mixer blender for 2 min at high speed. The blended samples were transferred to 250 ml conical flasks by using acetone (2 × 20 ml) and shaken for 30 min in a mechanical shaker. The samples were then filtered through Buckner funnel under suction using Whatman No. 1 filter paper. The extracts were then concentrated under reduced pressure using rotary vacuum evaporator. The concentrated extracts were transferred to separating funnel and 100 ml saturated NaCl solution was added for salting out effect. Then the aliquot partitioned with 75 ml dichloromethane. The dichloromethane layer was collected after passing through activated anhydrous sodium sulphate. The process was repeated twice with dichloromethane (2 × 50 ml). The dichloromethane layers were pooled and concentrated to dryness using rotary vacuum evaporator. The residues were dissolved in 5 ml hexane-acetone (9 : 1) mixture. The residues in hexane-acetone (9 : 1) mixture was passed through pre-washed columns (45 × 1.5 cm) packed with 10 g of activated alumina over 2 cm layer of anhydrous sodium sulphate. After loading the residues the columns were eluted with 150 ml

of hexane : acetone (9 : 1) mixture. The eluates were evaporated to less than 2 ml at 40 °C with the help of rotary evaporator and transferred to 25 ml volumetric flasks and final volume made up to the mark by hexane : acetone (9 : 1). The residues were analysed by GLC equipped with Ni^{63} ECD detector.

Imidacloprid :

Samples were extracted using acetone as described in beta-cyfluthrin. The aqueous phase was partitioned with hexane (100 + 50 ml) and then with (50 ml) hexane-ethyl acetate (98 : 2, v/v). The organic phase was discarded to remove the co-extractives. Subsequently, the aqueous phase was extracted three times with dichloromethane (100 + 2 × 50 ml). The dichloromethane phase was washed with aqueous potassium carbonate solution (0.01 *M*, 50 ml) to remove the acidic co-extractives and then dichloromethane phase was dried by passing through 2 cm layer of anhydrous sodium sulphate. The sodium sulphate was washed with 30 ml dichloromethane. After evaporation of dichloromethane, the residues were dissolved in 2 ml of ethyl acetate. The residues in ethyl acetate were passed through column (45 × 1.5 cm) packed with 10 g of silica gel over 2 cm layer of anhydrous sodium sulphate. The columns were prewashed with 30 ml of ethyl acetate before loading the extract. After loading the residues, the columns were eluted with 10 ml of ethyl acetate and eluates discarded. The active ingredient was eluted with 50 ml of acetonitrile. The eluates were concentrated to dryness with rotary vacuum evaporator under reduced pressure. The residues were dissolved in 2 ml of acetonitrile and made up to 25 ml. The residues were analysed by HPLC equipped with UV-Vis detector (λ_{max} 270 nm) using C-18 column and mobile phase acetonitrile-water (60 : 40).

Dissipation of pesticides : The residue data of the pesticide were subjected to regression analysis and the regression equations were derived using the equation

$$\log C_t = \log C_0 + k_t$$

where, C_t is the residue concentration (μg g^{-1}) at time t (days) after pesticide application, K is the dissipation rate constant or the slope of the regression line and C_0 is the initial deposit concentration (μg g^{-1}) at zero time (days of application).

The residual half-life (RL50) in days was estimated using the equation

$$RL50 = \log 2/K$$

where K is the gradient of the regression line. It determines the speed of dissipation.

Bioefficacy of pesticides :

Bioefficacy of combination-mix (beta-cyfluthrin + imidacloprid) was also studied against tomato fruit borer (*Helicoverpa armigera*) under field condition. Five plants were randomly selected and tagged and the insects were counted before spraying in each treatments and replications, which serve as the pre-treatment. The post treatment counts were taken after one, seven and 15 days after spraying. The per cent reduction of pest population over control was calculated using modified Abbott's formula[11].

Percentage of population reduction =

$$\left[1 - \frac{\text{Post treatment population in treatment}}{\text{Pre treatment population in treatment}} \times \frac{\text{Pre treatment population in check}}{\text{Post treatment population in check}} \times 100\right]$$

Results and discussion

The retention time of beta-cyfluthrin under the present experimental conditions was 3.10 min. The limit of quantification (LOQ) of beta-cyfluthrin from tomato fruits was 0.01 mg kg^{-1}. The average recovery of beta-cyfluthrin from tomato fruits fortified at 0.1 and 0.2 μg g^{-1} was 87.2 and 90.1% respectively. Imidacloprid eluated a sharp peak at 2.31 min under the described field conditions. Cleanup by hexane partitioning successfully removed colouring materials from tomato fruits and no matrix interference observed in the HPLC analysis of imidacloprid from tomato fruits[21]. The limit of quantification (LOQ) was 0.05 mg kg^{-1}. The average recovery of imidacloprid from tomato fruits fortified at 0.1 and 0.2 μg g^{-1} was 91.5 and 93.84%, respectively.

The initial deposits of beta-cyfluthrin from combination-mix were 1.224 mg kg^{-1} and 2.133 mg kg^{-1} from 40 and 80 g a.i. ha^{-1} of combination-mix (1 : 1) respectively. The residues of beta-cyfluthrin from lower treatment of combination-mix dissipated to 0.724 mg kg^{-1} in one day after application and further dissipated to 0.463, 0.317, 0.117, 0.081 and 0.020 after 3, 5, 7 and 10 days after spraying (Table 1). The results of persistence studies showed that beta-cyfluthrin persisted up to 10 days from lower rate of application (40 g a.i. ha^{-1}) while up to 15 days from higher rate of application (80 g a.i. ha^{-1}). Similar trend of dissipation were reported in tomato[10,12,13,18], brinjal[9,20] and okra[14]. Data analysis by logarithmic plots of beta-cyfluthrin residues versus time, obtained by fitting the regression equation, showed that the dissipation of beta-cyfluthrin followed first order kinetics with half lives of 2.03 and 2.69 days respectively at the two application rates (Table 3). The initial deposits of imidacloprid on tomato from combination-mix (1 : 1) were 1.447 and 2.383 mg kg^{-1} from @ 40 g a.i ha^{-1} and 80 g a.i ha^{-1}, respectively. The initial residues declined from 1.447 to 0.677, 0.427, 0.280, 0.180, 0.080 and 0.24 mg kg^{-1} after 1, 3, 5, 7, 10 and 15 days after application. The dissipation of imidacloprid residues from combination-mix persisted up to 15 days (Table 2). The logarithmic plots fitted first order kinetics well at both rates with half lives 2.71 and 2.60 days respectively (Table 3).

Table 1. Persistence/residues of beta-cyfluthrin on tomato from combination-mix (1 : 1)

Days after application	T1		T2	
	Residues (mg kg^{-1})[a]	Percent loss	Residues (mg kg^{-1})[a]	Percent loss
0	1.224 (±0.095)	-	2.133 (±0.104)	-
1	0.724 (±0.094)	40.82	1.449 (±0.104)	30.65
3	0.463 (±0.083)	62.15	0.955 (±0.152)	54.32
5	0.317 (±0.033)	74.13	0.503 (±0.157)	75.92
7	0.167 (±0.033)	86.38	0.240 (±0.053)	88.52
10	0.081 (±0.019)	93.41	0.100 (±0.23)	95.23
15	0.023 (±0.025)	98.09	0.047 (±0.007)	97.77
20	ND	100.00	ND	100.00
25	ND	100.00	ND	100.00

T1, T2; 40 and 80 g a.i. ha^{-1} combination-mix (1 : 1); ND, Non detectable (<0.01).

[a]Mean of three replicates and standard deviation in parentheses.

Table 2. Persistence/residues of imidacloprid on tomato from combination-mix (1 : 1)

Days after application	T1		T2	
	Residues (mg kg^{-1})[a]	Percent loss	Residues (mg kg^{-1})[a]	Percent loss
0	1.447 (±0.149)	-	2.383 (±0.176)	-
1	0.677 (±0.088)	53.24	1.323 (±0.125)	44.47
3	0.427 (±0.045)	70.51	0.883 (±0.126)	62.93
5	0.280 (±0.048)	80.67	0.533 (±0.194)	77.62
7	0.180 (±0.038)	87.54	0.203 (±0.055)	91.47
10	0.080 (±0.027)	94.47	0.097 (±0.047)	95.94
15	0.017 (±0.028)	98.36	0.045 (±0.040)	98.04
20	ND	100.00	ND	100.00
25	ND	100.00	ND	100.00

T1, T2; 40 and 80 g a.i. ha^{-1} combination-mix (1 : 1); ND, Non detectable (<0.05 mg kg^{-1}).

[a]Mean of three replicates and standard deviation in parentheses.

Similar trend of imidacloprid dissipation was reported in different crops[10,15-17,19].

The prescribed acceptable daily intake (ADI) value for beta-cyfluthrin is 0.02 mg kg^{-1} of body weight[22]. Considering the average body weight of Indian person as 50 kg, the maximum permissible intake (MPI) or maximum allowable intake is calculated to be 1.0 mg $person^{-1}$ day^{-1}. The recommended vegetables in the balance diet of adult man are 200 g as proposed by Indian Council of Medical Research (ICMR). The Theoretical Maximum Residue Contribution (TMRC = Daily consumption of food commodity × residues in mg kg^{-1}) values calculated[10,15] from initial residues present in the samples ranged from 0.245-0.427 mg $person^{-1}$ day^{-1} for beta-cyfluthrin from combination-mix. It indicated that the calculated TMRC values were much lower than the MPI value (1.0 mg $person^{-1}$ day^{-1}). Based on the residue data obtained, MRL of 0.5 mg kg^{-1} was proposed for beta-cyfluthrin in tomato fruits under Indian conditions. On the basis of this MRL the Theoretical Maximum Daily Intake (TMDI) is found to be 0.1 mg $person^{-1}$ day^{-1} and % ADI was found as 10. Therefore beta-cyfluthrin from combination-mix appeared safe and therefore it appears that the consumption of tomato does not warrant any hazard or risk.

The prescribed acceptable daily intake (ADI) value of imidacloprid is 0.06 mg kg^{-1} body weight[22]. The maximum permissible intake (MPI) or maximum allowable

Table 3. Theoretical dissipation models, initial deposits (theoretical) and half life values of pesticides

Treatments	Level of treatment (g a.i. ha^{-1})	Correlation coefficient	Theoretical initial deposit (mg kg^{-1})	Half-life (days)	Regression equation
Beta-cyfluthrin	40	0.994	1.815	2.69	$Y = 0.259 - 0.112x$
	80	0.980	1.887	2.62	$Y = 0.276 - 0.115x$
Imidacloprid	40	0.986	1.749	2.71	$Y = 0.0243 - 0.111x$
	80	0.969	1.850	2.60	$Y = 0.269 - 0.116x$

concentration for a person weighing 50 kg was calculated and found to be 3.0 mg $person^{-1}$ day^{-1}. Theoretical maximum residue contributions based on these initial residues were found 0.289–0.477 mg $person^{-1}$ day^{-1}. Thus calculated TMRC from residue data on zero day was found to be lower than MPI (3.0 mg $person^{-1}$ day^{-1}) calculated from toxicological data. Considering the present data, MRL of 0.5 mg kg^{-1} was proposed for imidacloprid in tomato under Indian conditions. Based on this the TMDI of 0.1 mg $person^{-1}$ day^{-1} is obtained and % ADI was found to be 3.33. This again suggests suitability and safety of imidacloprid on tomato. Based on the residue data obtained Pre Harvest Interval (PHI) of 3 days is calculated and proposed for harvest of fruits.

Bioefficacy of beta-cyfluthrin and imidacloprid against tomato fruit borer was recorded on the basis of mean population of fruit borer (*Helicoverpa armigera*) per five plants. The results of the first day after spraying showed that combination mix (1 : 1) at 80 g a.i. ha^{-1} gave highest reduction (86.06%) of pest control followed by combination-mix at 40 g a.i. ha^{-1} (80.26%). The combination-mix formulation was found very effective up to 15 days (observation period) even after that it appears that it might control the insect pest population effectively for a longer period.

Combination-mix (1 : 1, beta-cyfluthrin + imidacloprid) at normal (40 g a.i ha^{-1}) and double (80 g a.i ha^{-1}) recommended dose efficiently controlled the tomato fruit borer (*Helicoverpa armigera*). Beta-cyfluthrin from combination-mix exhibited low persistence and dissipated to below detectable limit by 10–15 days after application. Imidacloprid from combination-mix persisted up to 15 days after application. Based on the residue data obtained from this field trial, MRL of 0.5 mg kg^{-1} was proposed for beta-cyfluthrin and imidacloprid in tomato. A waiting period of 3 days before harvest could ensures sufficient margin of safety. Therefore application of combination-mix (1 : 1, beta-cyfluthrin + imidacloprid) could be taken as safe from crop protection, consumption of tomatoes and environmental contamination point of view.

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