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DETERMINATION OF TETRANILIPROLE AND ITS METABOLITE RESIDUES IN TOMATO

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ABSTRACT

Dissipation of tetraniliprole and its metabolite, chinazolinon in tomato was studied. Immature fruit samples were collected at specific intervals with three applications. Modified QuEChERS method was used and satisfactory recovery of 78.28 to 104.77 % was obtained. Good linearity (0.05 to 1.00 µg g^{-1}) with coefficient of determination (R^2) > 0.99 was recorded. The initial deposit at 60 (x) and 120 g a.i. ha^{-1} (2x) was 0.42 and 0.65 µg g^{-1} and reached the below limit of quantification on 5^{th} and 7^{th} days after spraying. The half-life period was 1.3 and 1.6 days and the safe waiting period was 4.5 and 5.8 days at x and 2x doses, respectively. The harvest time (20^{th} day) samples, mature fruit and soil were at BLQ in both the doses. The metabolite, chinazolinon was not detected in any of the sample analyzed. Theoretical maximum residue contribution (TMRC) was less than maximum permissible intake even on the day of spraying.

Key words: Tomato, soil, fruit, tetraniliprole, metabolite- chinazolinon, dissipation, half -life, safe waiting period, hazard index, modified QuEChERS method, linearity, coefficient of determination

Tomato is a major tropical and subtropical vegetable and in India, the area under cultivation and the annual production is about 781 thousand ha and 4976 thousand mt, respectively (NABARD, 2019). Tomato is susceptible to many insect pests and diseases and the major pest include fruit borer *Helicoverpa armigera* (Hubner), aphids *Aphis gossypii* (Glover), whitefly *Bemisia tabaci* (Gennadius), leaf eating caterpillar *Spodoptera litura* (F.), thrips, *Thrips tabaci* Lindeman, American serpentine leaf miner *Liriomyza trifolii* (Burgess) and two spotted red spider mite *Tetranychus urticae* Koch (Misra, 2010). Of these, the fruit borer is the most damaging, and numerous insecticides are employed for its control. One such insecticide with novel mode of action is tetraniliprole which showed an increased efficacy against lepidopteran caterpillars, dipterans and was found to be relatively safe to non-target organisms (Ramesh et al., 2016; Mahla et al., 2017). Tetraniliprole is an insecticide belonging to the phthalic acid diamide group developed by Bayer Crop Science (Bayer Annual Report, 2016). This compound binds and activates the ryanodine receptors and reduces intracellular calcium reserves, resulting in muscle paralysis and death (CIBRC, 2021). Repeated application of such insecticides in the later stage of fruit development may result in insecticide residues in the harvested fruits. This study aimed to determine the persistence of tetraniliprole and its metabolite chinazolinon residues in tomatoes under the Tamil Nadu Agroclimatic conditions and to estimate the dietary risk assessment of tetraniliprole.

MATERIALS AND METHODS

The formulation tetraniliprole 200SC, its certified reference standard (99.70% purity), and its metabolite chinazolinon (99.70% purity) (M/s. Bayer Crop Science, Mumbai), solvents viz., acetonitrile (Lichrosolv and Chromosolv grade), methanol (Chromosolv grade), salts viz., anhydrous NaCl and analytical grade sodium sulfate (M/s Merck Bangalore, India), anhydrous magnesium sulphate ($MgSO_4$) (M/s. Himedia Laboratory, Mumbai), primary secondary amine (PSA, 40 µm, Bondesil), graphitized carbon black (GCB) (M/s. Agilent, USA), LCMS grade formic acid (M/s. Sigma Aldrich., Bangalore) and membrane filter paper (0.45 and 0.20 µm, M/s. Pal life Science, Mumbai) were purchased. The ultra-pure type - I (18.2 MΩ) water was prepared in the laboratory using Merck (Direct - Q® 3) water purifier. A supervised field experiment was conducted at Muttathuvayal, Coimbatore (10.9624°N, 76.7445°E) to investigate the dissipation pattern of tetraniliprole in tomato from January to May 2017. Tetraniliprole 200 SC was applied at 60 (x) and 120 g a.i. ha^{-1} (2x) using power- operated knapsack sprayer thrice at ten days interval starting from fruit initiation stage. Each treatment was replicated thrice by following randomized block design (RBD) with a plot size of 25 m^2, with a separate plot for untreated control. After the third application, immature fruits were collected at 0 (within 2 hr of the last application), 1, 3, 5, 7, 10 and 15th day. During harvest time (20 DAT), mature fruit and soil samples were collected. One kg of immature tomato fruits was collected randomly from treated and untreated plots. Soil samples were collected from randomly chosen spots after clearing surface litters and samples were drawn at 0 to 15 cm depth using a conical trier. The foreign material (roots, stones, gravels and pebbles) in collected soil were removed and a subsample of 250 g was shade dried. The collected fruit and soil samples were labeled, packed and brought to the laboratory.

Insecticide stock solution was prepared in a 25 ml class A volumetric flask. Tetraniliprole and its metabolite, chinazolinon were weighted accurately to 10.03 mg within a volumetric flask and using acetonitrile, volume was made up to the mark to get 400 µg g^{-1} and stored in -20^0C for further use. Six repeated injections of tetraniliprole and chinazolinon at concentrations ranging from 0.05 to 1.0 µg g^{-1} were used to determine the linearity. The limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-background noise ratio. To determine the accuracy of the method, tetraniliprole and its metabolite, chinazolinon residues was fortified at 0.05, 0.25 and 0.5 µg g^{-1} with three replications in a 10 g tomato fruit and soil and processed for further analysis as mentioned below. Recovery (%) was computed and the precision was defined as % relative standard deviation (RSD). The fruit samples collected for residue analysis were chopped and blended in a high-volume blade homogenizer, Robot CoupeÒ. The residues of tetraniliprole and its metabolite were processed using the modified QuEChERS (dispersive solid phase extraction) method (Meenambigai et al., 2017).

Tetraniliprole and its metabolite chinazolinon were examined for residues using the liquid chromatography mass spectrometry (Shimadzu- LCMS-2020) system equipped with electrospray ionization (ESI) fitted with a reverse phase Shimadzu shim-pack GIST-HP C18 column, 100 x 3.0 mm with 3 µ particle size. Tetraniliprole and its metabolite chinazolinon were quantified using mass spectra of 543 m/z (-ESI) and 528 m/z (+ESI), respectively. A low-pressure gradient programme was set with the mobile phase ratio of A: degassed water with 0.1% formic acid (60%) + B: degassed methanol 0.1% formic acid (40%) and A: degassed water with 0.1% formic acid (70%) + B: degassed methanol 0.1% formic acid (30%) with the flow rate of 0.6 and 0.8 ml min^{-1} for tetraniliprole and its metabolite chinazolinon, respectively. The column temperature was 40^0C and the injection volume 20 µl. The MS parameters were; interface temperature 350°C; DL temperature 250°C; heat block temperature 200°C; nebulizing gas flow was 1.5 l/ min. and dry gas flow 15 l/ min. The retention time was 8.7 and 11.4 min for tetraniliprole and its metabolite, respectively showing clear separation of parent and its metabolite by the method. The risk assessment associated with the consumption of tetraniliprole treated tomato was determined by comparing theoretical maximum residue contribution (TMRC) with maximum permissible intake (MPI) (FAO, 2009). The daily consumption of tomatoes for Indian men and women (200 g) as well as the average human body weight of an Indian men (60 kg) and women (55 kg) were referred from NIN (2011). The ADI of tetraniliprole is 0.06 mg kg^{-1} body weight day^{-1} (Anonymous, 2019). The residue data was statistically analyzed to calculate the half-life (Hoskins, 1961). The safe waiting period was determined using the formula mentioned by Handa et al. (1999); safe waiting period = [log (A)-log (LOQ)]/K

RESULTS AND DISCUSSION

A simple and easy method was validated for extracting tetraniliprole and chinazolinon residues from tomato fruit and soil. Good recovery (70 to 120%) and RSD (20%) were obtained, satisfying the SANTE, 2017 guidelines. Calibration curve was obtained by injecting five different levels of both tetraniliprole and the metabolite chinazolinon (0.05, 0.1, 0.25, 0.5 and 1.0 µg g^{-1}) with six replications (Fig. 1). Both tetraniliprole and its metabolite chinazolinon exhibited good linearity (R^2 > 0.99). LOD (signal to noise ratio of 3) and LOQ (signal to noise ratio of 10) of tetraniliprole and its metabolite chinazolinon in tomato fruit and soil were determined to be 0.01 and 0.05 µg g^{-1}, respectively. The recovery percentage of tetraniliprole and chinazolinon in tomato fruit and soil ranged from 78.28 to 104.77 with the RSD of 2.91 to 15.32% (Table 1). Kaushik et al. (2019) reported the recovery as 94.00 to 120.80 for tetraniliprole and 92.80 to 119.60% for chinazolinon in tomato and soil, respectively using non QuEChERS method for cleanup. The calculated residues of tetraniliprole and chinazolinon are depicted in Table 2. The initial tetraniliprole deposits on tomato fruits were 0.42 and 0.65 µg g^{-1} at X and 2X doses, respectively. On the third day after spray, more than 50% of the initial deposit dissipated and reached Below Limit of Quantification (BLQ) (0.05 µg g^{-1}) at 5th and 7th days after treatment in recommended and double doses, respectively. A graph was computed based on the sampling intervals and log

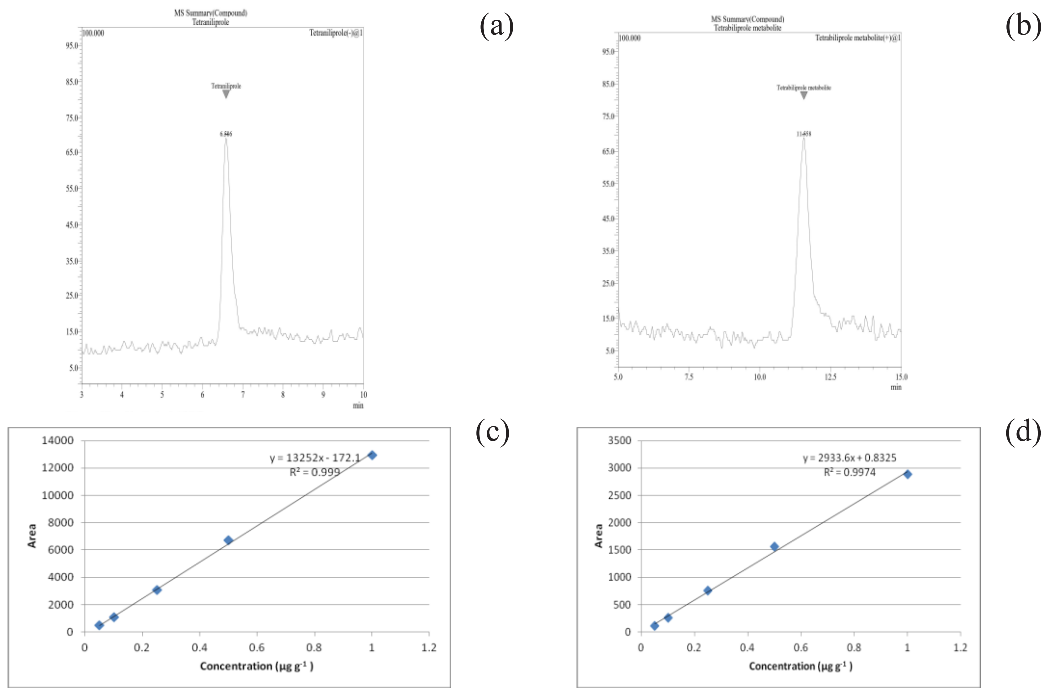

Fig. 1. The Standard chromatogram of tetraniliprole (a) & chinazolinon (b) at LOQ level and linearity chromatogram of tetraniliprole (c) & chinazolinon (d)

Table 1. Recovery of tetraniliprole and its metabolite in tomato fruit and soil

Fortification ($\mu g\ g^{-1}$)	Tetraniliprole		Chinazolinon	
	Fruit	Soil	Fruit	Soil
	*Mean % recovery (RSD %)	*Mean % recovery (RSD %)	*Mean % recovery (RSD %)	*Mean % recovery (RSD %)
0.05	101.19 (2.91)	102.16 (6.93)	87.06 (15.32)	94.81 (6.43)
0.25	86.39 (5.17)	104.77 (5.02)	94.63 (8.48)	90.58 (2.91)
0.50	78.28 (5.96)	96.37 (7.86)	93.29 (6.90)	85.06 (6.40)

*Mean of three replications

Table 2. Dissipation kinetics and dietary risk assessment of tetraniliprole in tomato

Days after spraying	Tetraniliprole 200SC 60 g a.i. ha^{-1} (X dose)			Tetraniliprole 200SC 120 g a.i. ha^{-1} (2X dose)			MPI (mg/ person/ day)	
	Mean residue* ($\mu g\ g^{-1}$)	Dissipation %	TMRC (μg/ person)	Mean residue* ($\mu g\ g^{-1}$)	Dissipation %	TMRC (μg/ person)	Adult male (65kg)	Adult female (55kg)
0	0.42	-	0.08	0.65	-	0.13	3.90	3.30
1	0.30	29.15	0.06	0.43	34.43	0.09		
3	0.09	78.42	0.02	0.26	59.38	0.05		
5	BLQ	-	-	0.07	89.68	0.01		
7	BLQ	-	-	BLQ	-	-		
Mature fruit	BLQ	-	-	BLQ	-	-		
Soil	BLQ	-	-	BLQ	-	-		
R^2	0.987	-	-	-	0.926	-		
$t_{½}$ (days)	1.31	-	-	-	1.64	-		
Safe waiting period	4.48	5.75	-			-		
Regression equation	Y= 2.692-0.268x	-	-	Y= 2.874-0.219x	-	-		

*Mean of three replications, BLQ- below limit of quantification values of average residue, to estimate the regression. The regression equation estimated the half – life period as 1.21 (X dose) and 1.64 days (2X dose). This shows the faster degradation behavior of the insecticide even at a higher dose. Since no MRL is available for tetraniliprole, safe waiting period calculation was done using LOQ and arrived as 4.48 days for single dose and 5.75 days for double doses. The harvest time samples *viz*., mature fruit and soil collected at 20 days after treatment were found to be BLQ (< 0.05 µg g^{-1}). The residue of the metabolite, Chinazolinon was not detected in any of the tomato fruit or soil samples collected and analyzed.

In a field study conducted at Solan, the half life period of tetraniliprole in tomato was reported as 2.70 and 3.49 days with the safe waiting period of 11.77 and 14.86 days (Kaushik et al., 2019). Plant character (type, form, growth, plant surface, crop stage, kind of fruit surface and texture) and environmental factors (humidity, temperature and rain) influences the initial deposit and persistence of pesticide (Ebling, 1963). In the present study, the mean maximum temperature recorded during the experimental period was 36.2^{0}C which might have favored the faster degradation of tetraniliprole residue. Volatilization is one factor favoring degradation and influenced by higher temperature plays a vital role in dissipation of pesticide from crops which shares 90% of the total loss of applied dose (Tepper, 2017). The safety of tetraniliprole residues was studied by comparing the TMRC values with the MPI. The TMRC value of tetraniliprole, on the day of spraying (within 2 hrs) was determined as 0.01 µg $person^{-1}$ at recommended and 0.02 µg $person^{-1}$ at double the recommended dose. TMRC values were far less than the MPI value for both adult male and female (3.90 and 3.30 mg/ person/ day) (Table 2).

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