*Section A-Research paper*

# Decontamination of spirotetramat residues in capsicum fruits under open field condition in Tamil Nadu

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## ABSTRACT

The study aimed to quantify the effectiveness of different decontamination treatments in the removal of spirotetramat residues from treated capsicum fruits. The insecticide, spirotetramat was sprayed during 50 per cent fruit production stage on capsicum plants at recommended (X) and double recommended dose (2X). Capsicum fruit samples were collected after 48 hours of spraying and analyzed by validated QuEChERS method using LC-MS (Liquid chromatography-mass spectrometry). Among all the decontamination treatments, cooking of capsicum fruits for 10 minutes minimized higher spirotetramat residues (73.18 - 80.43%). The next best treatment was noted with combined treatments (tap water washing + treating in 2 per cent solution of NaCl/lemon/tamarind for one minute) with 55.99 - 66.28 per cent sprayed under both doses. Whereas, tap water washing for one minute minimized lower quantity of spirotetramat residues (21.83-25.78%).

**Keywords:** Capsicum, Decontamination, spirotetramat, LC-MS

## 1. Introduction

Vegetables are important nutraceutical sources for a balanced human diet. Capsicum (*Capsicum annuum* (L.) var. grossum Sendt.) belongs to the Solanaceae family with worldwide production of 5,40,000 metric tons (FAOSTAT, 2021). In India, capsicum is grown under 0.37 lakh ha area with a production of 0.56 MT (Indiastat, 2022). Major capsicum growing districts of Tamil Nadu include Krishnagiri (214 ha), The Nilgiris (19 ha) with a production of 0.51 lakh tonnes (DH and PC, 2020). The crop suffers severe yield loss by thrips, mites, aphids, whitefly, and fruit borers in commercial cultivation. Thrips and mite infestation together account for yield loss up to 50 per cent (Pathipati *et al*., 2017). Reports showed that 13-14 per cent of total pesticide

usage was on vegetables in the country. Among all vegetable crops, the highest pesticide consumption was in chilli (5.13%) (Debi and Choudhury, 2018). From the survey it was revealed that the insecticide spirotetramat was widely used in managing thrips and mite populations. The insecticide spirotetramat (cis-3-(2,5-dimethlyphenyl)-8-methoxy-2-oxo-1-azaspiro [4.5] dec-3-en-4-yl- ethyl carbonate) is a tetramic acid derivative with systemic and translaminar property that acts as lipid biosynthesis inhibitor against broad range of sucking pests like thrips, whiteflies, aphids, psyllids and scales (Li *et al*., 2022).

Thus, it is necessary to understand the efficiency of different household decontamination practices in reducing spirotetramat residues from treated fruits. Published information on the decontamination of spirotetramat residues in capsicum fruits is lacking, despite its higher usage by capsicum farmers to control sucking pests in India. Thus, there is a need for evaluating the decontamination efficiency of different household practices for the benefit of end users. The physicochemical properties of spirotetramat are solubility in water (29.9 mg/L at 20° C), vapor pressure ($4.2\times10^{-11}$ Hg at 20° C) and Octanol-Water Partition Coefficient ($3.24 \text{ X } 10^{2}$ at 21° C) (Pesticide Properties Data Base, 2022).

## 2. Materials and methods

### 2.1. Chemical reagents and standards

The commercial spray formulation of spirotetramat 15.31 % w/w OD was obtained from local pesticide shop, Coimbatore, Tamil Nadu, India. The sorbents, GCB (Graphitized Carbon Black), PSA (Primary Secondary Amine) were supplied by Agilent Technologies, USA. The mobile phase solvent, methanol of LC-MS grade was taken from Fisher Chemical, USA. The salts, anhydrous NaCl (> 99% purity), $Na_2SO_4$ (>99% purity) and $MgSO_4$ (>99.5% purity) of analytical grade were purchased from Merck, India. Before usage, $MgSO_4$ was heated at 400°C in muffle furnace for four hours and stored in desiccator. A lab-scale millipore unit (18.2 M Ω) purified water was used throughout the analysis. The laboratory analysis was conducted at the Pesticide Toxicology Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore.

### 2.2. Preparation of standards

The individual stock solution (400 mg/L) of spirotetramat was made using LC-MS grade methanol by adding 10.10 mg of analytical standard to 25 ml class A volumetric flask. To prepare the secondary standard solutions (40 mg/L), 2.5ml stock solution (400 mg/ L) was transferred to 25 ml flask and the remaining volume has been made with LC-MS grade methanol. Serial dilutions (0.0025 - 0.25 mg/L) were made from the intermediate standard solution. Matrix match

standards were made up of 0.01, 0.025, 0.05, 0.075, 0.1 and 0.25 mg $L^{-1}$. The deep freezer (-20°C) was used to store all of the standard solutions.

**2.3. Method validation**

For method development and its validation, the listed parameters *viz*., accuracy, linearity, sensitivity, matrix effect, precision and uncertainty were considered (SANTE, 2021). The linearity was studied by injecting eight different concentrations (0.0025 to 0.1µg $mL^{-1}$) of spirotetramat standard solutions with six replicates. The matrix match standard linearity curves were calculated in range between 0.01 - 0.25 mg $kg^{-1}$. For estimating LOD (Limit of Detection) & LOQ (Limit of Quantification), standard solution at six levels (0.01-0.25 mg $kg^{-1}$) of spirotetramat was used.

The LOD & LOQ were determined by the following formula,

$$LOD = 3 \times (\text{Standard Deviation} / \text{Slope})$$

$$LOQ = 10 \times (\text{Standard Deviation} / \text{Slope})$$

Six levels of standards (0.01, 0.025, 0.05, 0.075, 0.1 and 0.25 mg $kg^{-1}$) were spiked into 10 g sample of homogenized untreated capsicum fruit with six replications for recovery analysis. After an hour of spiking the samples were processed for residue analysis. The per cent recovery was determined by the formula,

$$\text{Recovery (\%)} = \text{Residue quantified in fortified sample} / \text{Fortified level} \times 100$$

The matrix effect was calculated by contrasting the results of matrix match standards with the solvent standards. The uncertainty of the method was calculated by the formula given by Ellison and Williams (2002),

$$\text{R } U_{comb} = \sqrt{\begin{matrix}(RUrec)^2 + (RUvolsampleandtemp)^2 + (RUpurrefstd)^2 \\ + (\text{R Uvol ref std})^2 + (RUmrefstd)^2 + (RUmofsample)^2\end{matrix}}$$

where R $U_{comb}$ is the relative combined uncertainty

R $u_{rec}$ is the relative uncertainty from repeated observations (recovery)

$RU_{vol\ sample\ and\ temp}$ is the relative uncertainty from sample volume and temperature

R $U_{purof\ ref\ std}$ is the relative uncertainty from purity of the reference standard used

R $U_{volof\ ref\ std}$ is the relative uncertainty from volume of reference standard

R $u_{mof\ ref\ std}$ is relative uncertainty from mass of reference standard

**2.4. Field Experiment**

The field study for decontamination was carried out at Kadambur village (11.37º N latitude, 77.16 º E longitude), Erode, Tamil Nadu, India to find an effective way for removing the insecticide residues from treated capsicum fruits. The insecticide was sprayed during 50 per cent

fruiting stage in the farmer's field at single (X) and double dose (2X), respectively. As there is no recommendation of insecticide in capsicum crop (CIB&RC, 2022), the insecticide recommended against sucking pests of chilli crop were chosen in the experiment. Spirotetramat was sprayed at the single dose (60 (X) g a.i. $ha^{-1}$) and double the recommended dose (120 (2X) g a.i. $ha^{-1}$) for managing thrips. After 48 hours of spraying one kilograms of capsicum fruits were randomly collected from each treated and control plots (sprayed with water).

The capsicum fruit samples were subjected to decontamination treatments for one minute *viz*., washing with running tap water, salt solution (2 %), tamarind solution (2 %), lemon solution (2%) lukewarm water ($40^0$C) and cooking for ten minutes. Combined treatments for one minute *viz*., tap water washing + tamarind solution (2%), tap water washing + salt solution (2%) and tap water washing + lemon solution (2%) have been also evaluated. Following decontamination, the fruits were homogenized with a high-capacity blender (Robot Coupe, Blixer 6, France) and processed for residue analysis.

**2.5. Extraction and clean-up**

The Modified QuEChERS (Easy, Quick, Rugged Effective, Cheap and Safe) method was employed to extract the residues from capsicum fruit (Anastassiades *et al*., 2003). Ten-gram sample of capsicum fruit was added to 50ml polypropylene centrifuge tube with 20ml acetonitrile and vortexed for one minute for proper mixing. Then, 1 g NaCl and 4 g of anhydrous $MgSO_4$ were added to the tube, vortexed and centrifuged for ten minutes at 6000 rpm. The moisture has been eliminated by passing the top layer of supernatant (10 ml) through anhydrous $Na_2SO_4$ (4 g). Six milliliters of supernatant were transferred into a 15ml centrifuge tube comprising 10 mg of GCB, 200 mg of PSA and 900 mg of $MgSO_4$, vortexed for one minute and centrifuged at 3000 rpm for ten minutes. After centrifugation, 4 ml of supernatant was pipetted to a glass test tube and evaporated in turbovap LV ($35^0$ C) with a moderate nitrogen flow until near dryness. Then the residues were redissolved with 1 milliliter of methanol (LC-MS grade) and filtered by PTFE syringe filter (0.2µm) and finally transferred into 1.5 milliliter autosampler glass vials for LC-MS estimation.

**2.6. LC-MS instrument**

Shimadzu 2020 series version 5.6 coupled with reverse phase C18 (Shimadzu-shim-pack) column of dimension 250 mm length x 4.6 mm was utilized. Mobile phase solvent of ultra-pure water with 2mM $NH_4HCO_2$ and acidified with 0.05 per cent formic acid (solvent A); methanol with 2mM $NH_4HCO_2$ and acidified with 0.05 per cent formic acid (solvent B) with a flow rate of 30:70 V/V were used in the experiment. The mobile phases were discharged with the isocratic flow (0.5 ml/min.) at a pressure of 48 $kg/cm^2$ by the LC-MS pump. Positive electrospray

ionization (ESI+) with an interface mode of 0.1 $\mu A^o$ was used to ionize the samples in SIM (Selective Ion Monitoring) at m/z 274 (spirotetramat). The optimized parameters were capillary voltage (3.5 kV), desolvation line temperature ($25^0$C), heat block ($200^0$C), column oven ($40^0$C), nebulizer gas ($N_2$- 99.99%), drying gas (15 L/min), flow (1.5 L/min), 15000-sec scan speed and injection volume (10 µl).

**2.7. Data analysis**

The final quantification of insecticides was estimated by the following equation,

$$\text{Residue (mg kg}^{-1}) = \frac{A_1 \times C \times I_1 \times F}{A_2 \times W \times I_2}$$

here, $A_1$ - Sample peak area; $A_2$- Standard peak area; C - indicates concentration of standard solution (mg $kg^{-1}$); W - indicates weight of sample (g); $I_1$ and $I_2$ - indicates injected volume of standard and sample (µl); F - sample's final volume (ml).

**3.0. Results and Discussion**

**3.1. Method validation**

The method validation parameters were evaluated using SANTE guidelines, the technique optimization findings were satisfactory (SANTE, 2021). The linearity calibration curve of spirotetramat was established with eight different concentrations (0.0025 to 0.1 $mg^{-1}$) with six replicates. A good correlation coefficient of spirotetramat standard and matrix match standard (fruit) was obtained with $r^2 > 0.99$. The LOD and LOQ of spirotetramat were 0.0025 and 0.01 mg $kg^{-1}$. In capsicum fruit matrix, the recovery of spirotetramat was 93.47 to 99.62 per cent with RSD value lesser than six per cent, respectively. The matrix effect of spirotetramat (-6.06-9.77%) in capsicum fruit which was under the permissible limit of 20 per cent. The uncertainty measured was 5 per cent, which is less than 20 per cent threshold globally (SANTE, 2021).

**3.2. Decontamination of spirotetramat residues in capsicum fruits**

The average initial deposit of spirotetramat residues after 48 hours of spraying at 60 and 120 g a.i $ha^{-1}$ was 0.65 and 1.02 mg $kg^{-1}$ in treated capsicum fruit samples respectively. Among the decontamination methods, cooking for 10 minutes was highly effective in removing spirotetramat residues (73.18 - 80.43%) under both doses. Followed by cooking, the combined treatments *viz*., tap water washing + treating in 2 per cent solution of NaCl/ lemon/ tamarind removed 57.60 - 62.41 per cent (X dose) and 55.99 - 66.28 per cent (2X dose) of spirotetramat residues. Capsicum fruits treated alone with 2 per cent NaCl/ lemon/ tamarind solution removed 40.16 - 52.30 per cent (X dose) and 38.85 - 48.08 per cent (2X dose) of spirotetramat. Lukewarm water treatment reduced 30.46 to 34.52 per cent spirotetramat residues when treated at both doses. Washing with

tap water alone eliminated 21.83-25.78 per cent of spirotetramat residues sprayed at X and 2X doses (Table.1) (Figure. 1).

The rate of pesticide residue breakdown primarily depends on their physicochemical properties. The insecticide spirotetramat has low vapour pressure ($4.2\times10^{-11}$ Hg) with lower solubility in water (USEPA, 2008). Jankowska *et al*. (2020) reported that tap water washing minimized 32-41 per cent of spirotetramat residues sprayed at 45 g a.i. $ha^{-1}$ in basil, pepper mint and sage leaves. The reduction efficiency of pesticide residues with sodium chloride solution may be due to its strong electrolyte property that interacts with the pesticide residues and minimized the residue concentration (Rasolonjatovo *et al*., 2017). The presence of higher citric acid content in lemon juice solution acts as a chelating agent with pesticide residues and that helps in reducing residues over the treated fruits (Mariappan and Kaithamalai, 2020). The acidic nature (pH-1.88) and presence of secondary metabolites like furan derivatives (44.4 %) and carboxylic acid components (38.2 %) in tamarind solution might have undergone the residue degradation of the insecticide (Nowowi *et al*., 2016). Shakoori *et al*. (2018) reported that cooking reduces pesticide concentrations by volatilization, thermal degradation and hydrolysis.

From the experimental findings, cooking (73.18-80.43%) for ten minutes was highly effective than other treatments in lowering the pesticide residues of spirotetramat residues on treated capsicum fruits.

**4.0. Conclusion**

The decontamination treatments minimized 21.83-80.43 per cent of spirotetramat residues under both doses. Cooking capsicum fruits for 10 minutes was highly efficient in residue reduction than any other washing treatment. Whereas, washing with tap water for 1 minute was found to be least effective than all other decontamination treatments.

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**References:**

Anastassiades, M., Lehotay, S.J., Stajnbaher, D. and Schenck, F.J., (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *Journal of AOAC international*, 86(2), pp.412-431.

Debi, S. and Choudhury P.P., (2018). Pesticide use and residue management in vegetables. Technical Bulletin No 74, ICAR-IIHR, Hesaraghatta, Bangalore-560089, pp.40.

DH and PC., (2020). Department of Horticulture and Plantation Crops, Government of Tamil Nadu, India. Retrieved from https://www.tnhorticulture.tn.gov.in/stateprofile.

Ellison, S.L.R. and Williams, A., (2012). Quantifying uncertainty in analytical measurement, 3rd edition. Teddington, UK, Eurachem. pp.141.

FAOSTAT: Food and Agriculture Organization of the United Nations., (2021). http://faostat.fao.org. Accessed on 18.02.2023.

Indiastat., (2022). https://www-indiastat-com.elibrarytnau.remotexs.in/table/total-vegetables/state-wise-area-production-productivity-total-vege/1413992. Accessed on 28.02.2023.

Jankowska, M., Kaczynski, P. and Łozowicka, B., (2020). Metabolic profile and behavior of clethodim and spirotetramat in herbs during plant growth and processing under controlled conditions. *Scientific Reports*, 10(1), pp.1-10.

Li, K., Chen, W., Xiang, W., Chen, T., Zhang, M., Ning, Y. and Chen, A., (2022). Determination, residue analysis and risk assessment of thiacloprid and spirotetramat in cowpeas under field conditions. *Scientific Reports*, 12(1), pp.3470.

Pathipati V.L., Singh T.V.K., Vemuri, S.B., Reddy, R.V.S.K., Bharathi, N.B., (2017). Dissipation dynamics of flubendiamide on capsicum in open field and poly house conditions. *International Journal of Forestry and Horticulture*, 3(1), pp.30-35.

Pesticide Properties Data Base., (2022). Pesticide Properties Data Base. Agriculture and Environment Research Unit. Hertfordshire, UK. http:// sitem.herts.ac.uk/aeru/ppdb /en/Reports /397.htm. Accessed 17.03.2023.

SANTE., (2021). Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed. SANTE/12682/2019. https://food.ec.europa.eu/system/files/20 22 02/pesticides_mrl_guidelines_wrkdoc_2021-113 12.pdf.

**Table. 1. Effect of various decontamination techniques on spirotetramat residues at recommended and double recommended dose on capsicum fruit after 48 hours of spraying**

| S. No | Treatments | Spirotetramat 60 g a.i ha$^{-1}$ (X dose) | | Spirotetramat 120 g a.i ha$^{-1}$ (2X Dose) | |
|---|---|---|---|---|---|
| | | Residues (mg/kg) | Reduction (%) | Residues (mg/kg) | Reduction (%) |
| T0 | Untreated (control) | 0.65 | - | 1.02 | - |
| T1 | Washing in tap water | 0.48 | 25.78 | 0.80 | 21.83 |
| T2 | Washing in lukewarm water | 0.43 | 34.52 | 0.71 | 30.46 |
| T3 | Washing in salt solution (2%) | 0.31 | 52.30 | 0.53 | 48.08 |
| T4 | Washing in lemon juice (2%) | 0.33 | 49.94 | 0.59 | 42.02 |
| T5 | Washing in tamarind solution (2%) | 0.39 | 40.16 | 0.62 | 38.85 |
| T6 | Cooking | 0.18 | 73.18 | 0.20 | 80.43 |
| T7 | Tap water washing + salt solution (2%) | 0.23 | 62.41 | 0.34 | 66.28 |
| T8 | Tap water washing + lemon juice (2%) | 0.25 | 61.70 | 0.41 | 59.58 |
| T9 | Tap water washing + tamarind solution (2%) | 0.28 | 57.60 | 0.45 | 55.99 |

**Figure. 1. Decontamination efficiency of different treatments in minimizing spirotetramat residues in capsicum fruits collected after 48 hours of spraying at recommended and double recommended dose**