

Persistence behaviour of tetraconazole in watermelon

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ABSTRACT

A field study was conducted to observe the persistence behavior of tetraconazole on watermelon, at University Research Farm, Mondouri, Nadia, West Bengal season the season of 2013. The commercial formulation of tetraconazole (DOMARK 3.8 % EW w/w) was applied at the rate of 40 g a.i. ha⁻¹ (recommended dose i.e. T₁) and 80 g a.i. ha⁻¹ (double the recommended dose i.e. T₂) along with untreated control. The average recovery of tetraconazole was 100.3% in plant and 97% in fruit matrix. The calculated half-life (t_{1/2}) value of tetraconazole ranged 1.36 – 1.55 days. No residue of the fungicide was found in the watermelon fruit samples at harvest. This study revealed that watermelon fruit may safely be recommended for human consumption.

Keywords: Half-life, LC-MS/MS, persistence, tetraconazole, watermelon

Watermelon (*Citrullus lanatus*), is a vining annual plant belonging to the family *cucurbitaceae* grown for its freshly fruits. It is one of the major commercial crop, cultivated in tropical-subtropical climate and consumed throughout the world. India grows approximate 25 varieties of watermelon in number of states including Uttar Pradesh, Himachal Pradesh, Punjab, Haryana, West Bengal, Andhra Pradesh, Maharashtra etc. Over the years, the cultivated area and production of watermelon has been increasing in India. FAO estimates that approximately 4,00,000 tonnes of watermelon has been produced in India during 2012. However loss in productivity due fungal diseases is one of the major constraints in getting high yield in watermelon cultivation. Tetraconazole [(RS)-2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether] is a broad-spectrum, systemic, triazole fungicide effective against powdery mildew disease in cucurbits. It has protectant and curative properties. It acts on the vegetative form of fungi by blocking the growth of the pathogen mycelium, both outside and inside of the treated plant (Health Canada Pest Management Regulatory Agency). It belongs to the Sterol Biosynthesis Inhibitors (SBI) class and like all triazoles, it acts by inhibiting the metabolic pathway leading to fungal sterol production. This action is carried out by blocking the lanosterol demethylation reaction which leads to the inhibition of the ergosterol production, accumulation of non-functional products, malfunctioning of the cell membranes and fungal death as a final consequence (Tetraconazole technical bulletin, ISAGRO). Although there are no report on

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efficacy as well as residue study in any crop in India but some publications are available with otherazole derivatives especially in rice and maize (Bag 2009, Anand *et al.* 2013). The presence of pesticide residues in fruit in general and in watermelon in particular is an important concern for consumers, due to their possible long adverse health effects, especially for children. Therefore the present study was conducted to know the persistence behavior of tetraconazole in plant and residual fate in fruits at harvest.

MATERIALS AND METHODS

Certified reference material of Tetraconazole (purity 99.0 %) was purchased from Sigma Aldrich, Germany. Tetraconazole (DOMARK; 3.8 % EW w/w) formulation was obtained from Isagro (Asia) Agrochemicals Pvt. Ltd., India. Acetonitrile, ethyl acetate, methanol were of HPLC grade from JT Baker (Phillipsburg, NJ) and HPLC grade cyclohexane was purchased from Rankem (Ranbaxy, Fine Chemicals Limited) were used. Analytical grade sodium chloride, magnesium sulfate was purchased from Merck India Ltd (Mumbai, India). Anhydrous sodium sulfate used was analytical grade from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Magnesium sulfate and sodium sulfate were heated at 400-450 °C for 5 hours before use and stored in desiccator. Primary secondary amine (PSA; Varian, Harbor City, CA) and Graphitized carbon (GCB; United Chemical Technology, Bellefonte, PA) were used as SPE sorbent. The field study was conducted in watermelon in the year of 2013 at University research farm (N 22°56' E 88°31'), Mondouri, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal. The fungicide

was applied thrice on the watermelon plant at the rate of 40 g a.i. ha⁻¹ (T₁, recommended dose) and 80 g a.i. ha⁻¹ (T₂, double the recommended dose) by knapsack sprayer with flat pan nozzle along with untreated control. The experiment was conducted using Randomized Block Design (RBD) replicated thrice. First application of the fungicide was done 30 days after sowing followed by two more applications at an interval of 15 days.

Plant samples (approx. 250 g of leaves) were randomly collected from each plot at 0 (2 hrs), 1, 3, 5, 10 & 15 days after the last application. The samples were brought to the laboratory and immediately cut, mixed and blended to make a laboratory representative sample. Watermelon fruit samples were also collected in the same manner at harvest and taken to the laboratory where it was immediately cut into pieces and homogenized by polytron homogenizer (PT-MR-3100; Switzerland) for analysis. A stock solution (100 µg ml⁻¹) was prepared by accurately weighing 5.05 ± 0.01 mg of analytical standard in a 50 ml calibrated (class 'A') volumetric flask and dissolved in methanol. From this stock solution intermediate working standard solution of 10 µg ml⁻¹ was prepared by proper dilution with methanol. This working solution was further used for preparation on calibration standard ranged 0.005 – 1.0 µg ml⁻¹ by proper dilution with methanol.

Watermelon plant and fruit samples were extracted using QuEChERS method (Anastassiades *et al.* 2003) with some modifications. Representative plant sample (5 g) was taken in a 50 ml centrifuge tube and soaked in 10 ml millipore water (Milli-Q water purification system, Millipore Corp., Billerica, MA) for 5 minutes. Then 10 ml acetonitrile, 1.5 g NaCl and 6 g MgSO₄ were added to it and mixed on a vortex (Spinix) mixer for 2 minutes followed by rotospin for 15 minutes at 50 r.p.m. The sample was then centrifuged using a high-speed, refrigerated centrifuge (Avanti J-30I, Beckman Coulter, Fullerton, CA) at 10,000 r.p.m. for 5 minutes. In case of fruit 10 g of homogenized sample was taken in a 50 ml centrifuge tube, 10 ml ethyl acetate – cyclohexane mixture (9:1) and 1.5 g NaCl were added to it and extracted in the same manner as stated above.

After that 2 ml supernatant was taken and evaporated to dryness using a low volume concentrator (Turbo Vap LV from Caliper Life Science, Hopkinton, MA). The residue was reconstituted with methanol and transferred into a 2 ml micro centrifuge tube (Tarsons Products Pvt. Ltd., Kolkata, India) containing previously weighted

PSA and GCB, 25 mg each. The tube was vortexed for 45 seconds and then centrifuged at 6000 r.p.m for 5 minutes. Finally 1 ml supernatant was taken and filtered with syringe filter using 2 µm nylon 6,6 membrane filter paper (Ultipor N₆₆, PALL Life science, PALL corp., Mumbai, India). The cleaned up extract was collected in a glass vial and subjected to analysis by LC-MS/MS. To determine the efficiency of the analysis method, a recovery experiment was carried out by fortifying the fresh untreated plant and fruit samples with tetraconazole at the concentration levels of 0.01, 0.05 and 0.5 µg g⁻¹.

For detection and quantification of tetraconazole residue a Liquid chromatograph (Alliance 2695 separation module; Waters, Milford, MA, USA) coupled with a Micromass (Manchester, U.K.) QuattroMicro triple-quadrupole spectrometer equipped with an electrospray ionization (ESI) probe was used. The separation was carried out by injecting an aliquot of 20 µl via an autosampler on a reversed phase C₁₈ column (Water's XTerra MS C₁₈, USA: 3.5 µm; 2.1 x 50 mm). The mobile phase was composed of (A) water, 5 mM ammonium acetate and 0.1% acetic acid and (B) methanol, 5 mM ammonium acetate and 0.1% acetic acid and programmed in gradient mode: 0.0 – 2.0 min – 5.0% B to 95 % B, 2.0-6.0 min – back to the initial condition of 5% B and holds up to 8 min. A constant flow rate of 0.3 ml min⁻¹ was maintained throughout the analysis. The retention time (RT) of tetraconazole was 5.45 min. The estimation of tetraconazole in LC-MS/MS was performed in positive mode by multiple reaction monitoring (MRM) with mass transition 371.67 > 158.80 for quantification and 371.67 > 69.44 for confirmation at collision energies 27.0 and 23.0 volt respectively. The ratio of the peak area of these two daughter ions was 0.87. The corresponding ratio in the samples was determined and confirmed in accordance with European Union guidelines (Anon., 2002).

RESULTS AND DISCUSSION

The calibration curve established in the range of 0.005 to 1.0 µg g⁻¹ showed a correlation coefficient, R² > 0.99 (Fig. 1). Average recovery of tetraconazole was found to be 97 % in fruit and 100.3 % in watermelon plant. The limit of detection (LOD) and limit of quantification (LOQ) of tetraconazole in both matrices were found to be 0.005 and 0.01 µg g⁻¹ respectively. Residual behavior of tetraconazole in watermelon plant is presented in table-1. The concentrations of the fungicide after 2 hrs (0 day) of

application were 3.180 and 5.901 $\mu\text{g g}^{-1}$ for single (T_1) and double dose (T_2) respectively. The residues were dissipated over 80% within the 24 hrs of application in both of the treatments (Figure 2). The residues dissipated gradually to 0.013 $\mu\text{g g}^{-1}$ in T_1 and 0.040 $\mu\text{g g}^{-1}$ in T_2 after 10 days of application. No residue of tetraconazole was found in the samples collected after 15 days of application irrespective of any doses. Watermelon fruit samples collected at harvest contained no detectable amount of tetraconazole residues. The dissipation of the fungicide exhibited first order kinetics. The half-life ($T_{1/2}$) of tetraconazole was calculated using Hoskins (1961) formula. The

half-life value of tetraconazole in watermelon ranged from 1.36 to 1.55 days. The earlier observation (Alam et al. 2011) revealed the half-life values of tetraconazole ranged between of 4.5 – 5.2 days in mango and 5.1 – 5.7 days in sugar beet (Menkissoglu et al., 1998), 6.3 days in grape (Cabras et al. 2000), 5.02 days in tomato (Abdellseid et al., 2014). This deviation in half-life values can be explained by the fact that the dissipation of the pesticide residues in crops depends on environmental condition, type of application, plant species, dosage, and interval between application, the relation between the treated surface and its weight and living state of the plant surface, in addition to harvest time.

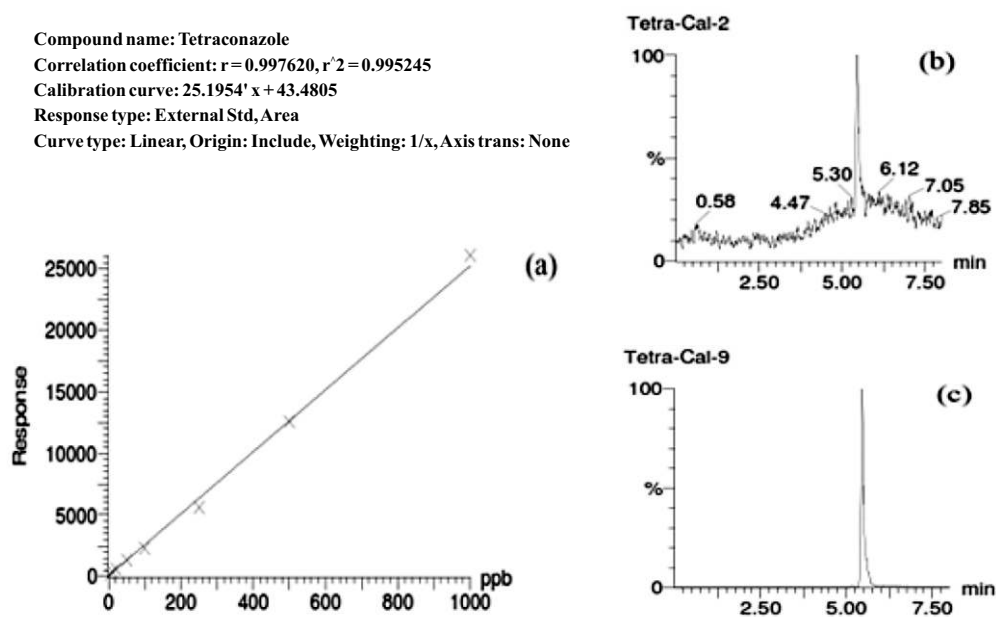


Fig. 1: (a) Calibration curve, ranged from 0.005 – 1.0 $\mu\text{g g}^{-1}$; (b) & (c) Chromatogram of tetraconazole analytical standard at the level of 0.005 $\mu\text{g g}^{-1}$ (LOD) and 1.0 $\mu\text{g g}^{-1}$ respectively

Table 1: Residues of tetraconazole in watermelon plant

Dose	Day(s)	Residues in ppm ($\mu\text{g g}^{-1}$) Mean (c) \pm S.D.	% Dissipation	Regression equation	Coefficient of Determination (R^2)	Half-life (Days)
T_1	0	3.180 \pm 0.044	-	$y = 0.222x + 3.143$	0.92	1.36
	1	0.631 \pm 0.015	80.17			
	3	0.192 \pm 0.011	93.96			
	5	0.063 \pm 0.002	98.02			
	10	0.013 \pm 0.002	99.60			
	15	ND	-			
T_2	0	5.901 \pm 0.222	-	$y = 0.194x + 3.377$	0.89	1.55
	1	1.009 \pm 0.324	82.90			
	3	0.399 \pm 0.017	93.24			
	5	0.162 \pm 0.027	97.25			
	10	0.040 \pm 0.004	99.32			
	15	ND	-			

*ND= Not detected

This study revealed that, tetraconazole dissipates rapidly in watermelon after application. As the harvested watermelon fruits contained no residue of tetraconazole, it can be concluded that tetraconazole (3.8 % EW w/w) can be applied on watermelon at the recommended dose in accordance with good agricultural practices and the harvested fruits may safely be used for human consumption.

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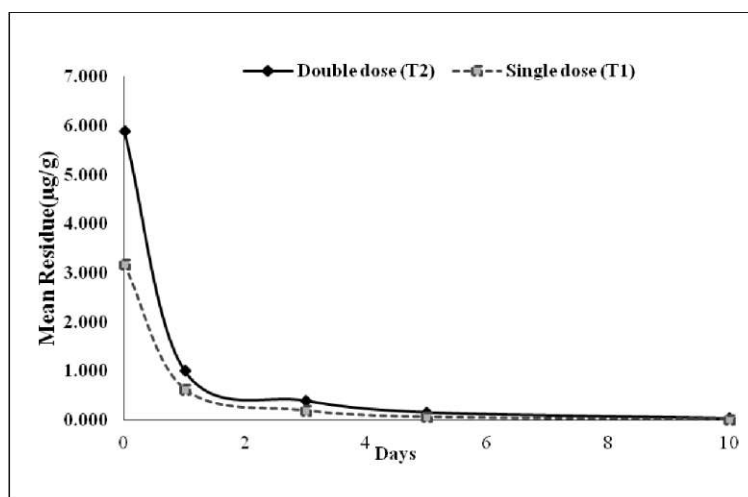


Fig. 2: Dissipation behaviour of tetraconazole in watermelon plant

REFERENCES

- Abdellseid, A. M. and Rahman, T. A. Abd El. 2014. *Residue and dissipation dynamics of tetraconazole in tomato fruit using QuEChERS methodology*. International Conference on Food, Biological and Medical Sciences (FBMS – 2014) Jan. 28-29, Bangkok (Thailand), pp. 20-22.
- Alam, S., Kole, R.K., and Bhattacharyya A. 2011. Residual Fate of the Fungicide Tetraconazole (4% EW) in Mango. *Bull. Environ Contam Toxicol*, **87**:444-47.
- Anand, Y. R., Begum, S., Dangmei, R., and Nath, P. S. 2013. Evaluation of trifloxystrobin 25% + tebuconazole 50% (Nativo 75 WG) against *Exserohilum turcicum* causing leafblight disease of maize. *J. Crop Weed*, **9**: 198-200.
- Anastassiades, M., Lehotay, S.J., Stajnbaher, D. and Schenck, F. 2003. Fast and easy multiresidue method employing extraction/partitioning and “Dispersive solid-phase extraction” for the determination of pesticide residues in produce. *J. AOAC International*, **86**:412-31.
- Anonymous. 2002. Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities* **L221**, 17 August 2002, pp. 8-36.
- Bag, M. K. 2009. Efficacy of a new fungicide ‘Trifloxystrobin 25% + Tebuconazole 50%’ 75WG against sheath blight (*Rhizoctonia solani* Kühn) of rice. *J. Crop Weed*, **5**: 221-23.
- Cabras, P. and Angioni, A. 2000. Pesticide residues in grapes, wine, and their processing products. *J. Agric. Food Chem.*, **48**: 967-73.
- Health Canada Pest Management Regulatory Agency. 2012. Tetraconazole technical fungicide, proposed registration decision, PRD2012-29, 16 November 2012, pp. 1-116.
- Hoskins W. M. 1961. Mathematical treatment of the rate of loss of pesticide residue. *FAO Plant Protection Bulletin* **9**:163-68.
- Menkissoglu, S.U., Xanthopoulou, N.J. and Ioannidis, P.M. 1998. Dissipation of the fungicide tetraconazole from field-sprayed sugar beets. *J. Agric. Food Chem.* **46**: 5342-46.