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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 oftetraconazole (DOMARK 3.8 % EW w/w) was applied at the rate of 40 ga.i.ha^{-1} (recommended dose i.e. T_1) and 80 g a.i. ha^{-1} (double the recommended dose i.e. T_2) along with untreated control. The average recovery of tetraconazole was100.3% in plant and 97 % in fruit matrix.The calculated half-life ($t_{1/2}$) value of tetrazonazole 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*:** Hail-life, LC-MS/MS, persistence, tetraconazole, watermelon

Watermelon (*Citrullus lanatus*), is a vining annual plant belonging to the family *cucurbitaceae* grown for it's 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, Himalchal 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 approximately4,00,000 tonnes of watermelon has been produced in India during 2012.However loss in productivity due fungal diseases is one of the major constrains 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 theSterol 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, misfunctioning of the cell membranes and fungal death as a final consequence (Tetraconazole technical bulletin,ISAGRO). Although there are no report on efficacy as well as residue study in any crop in India but some publications are available with other azole 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.

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MATERIALS AND METHODS

Certified reference materialof 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 cyclohexanewas 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 analyticalgrade from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Magnesium sulfate and sodium sulfate were heated at 400-450 ºC 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^{0}56' E 88^{0}31'), Mondouri, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal. The fungicide

was applied thriceon the watermelon plant at the rate of 40 g a.i. ha^{-1} (T_1, recommended dose) and 80 g a.i. ha^{-1} (T_2, 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 application 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 wasimmediately 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 $MgSO_4$ were added to it and mixed on avortex (Spinix) mixer for 2 minutes followed by rotospin for 15 minutes at 50 r.p.m. The sample was then centrifuged using ahigh-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 cliklok 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 andfiltered with syringe filter using 2 µm nylon 6,6 membrane filter paper (Ultipor $N_{66}^{®}$, 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 tetraconazoleat 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-quadruple 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 C_{18} column(Water's XTerra MS C_{18}, 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 conformation 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, $R^2 > 0.99$ (Fig. 1). Average recovery of tetraconazolewas found to be 97 % in fruit and 100.3 % in water melon plant.The limit of detection (LOD) and limit of quantification (LOQ) of tetraconazole in both matrixes 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 µg 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 µg g^{-1} in T_1 and 0.040 µg g^{-1}in T_2after 10 days of application. No residue of tetraconazolewas 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 from1.36 to 1.55 days. The earlier observation(Alam *et al.* 2011)revealed the half-life values of teraconazole 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.

Compound name: Tetraconazole
Correlation coefficient: r = 0.997620, r^2 = 0.995245
Calibration curve: 25.1954' x + 43.4805
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None

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

Table 1: Residues of tetraconazole in watermelon plant

Dose	Day(s)	Residues in ppm (µg g^{-1}) Mean (c) ± S.D.	% Dissipation	Regression equation	Coefficient of Determination (R^2)	Half-life (Days)
T_1	0	3.180 ± 0.044	-	$y = 0.222x + 3.143$	0.92	1.36
	1	0.631 ± 0.015	80.17			
	3	0.192 ± 0.011	93.96			
	5	0.063 ± 0.002	98.02			
	10	0.013 ± 0.002	99.60			
	15	ND	-			
T_2	0	5.901 ± 0.222	-	$y = 0.194x + 3.377$	0.89	1.55
	1	1.009 ± 0.324	82.90			
	3	0.399 ± 0.017	93.24			
	5	0.162 ± 0.027	97.25			
	10	0.040 ± 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 beconcluded that tetraconazole (3.8 % EW w/w) can be applied on watermelonat the recommended dose in accordance with good agricultural practices andthe harvested fruits may safely beused for human consumption.

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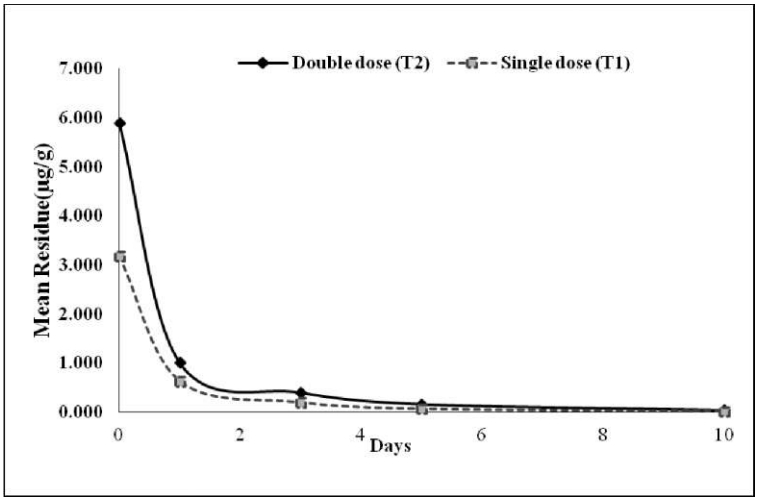

Fig. 2: Dissipation behaviour of tetraconazole in watermelon plant

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