Bull Environ Contam Toxicol (2011) 87:444–447
DOI 10.1007/s00128-011-0362-4

Residual Fate of the Fungicide Tetraconazole (4% EW) in Mango

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Received: 11 March 2011 / Accepted: 1 July 2011 / Published online: 27 July 2011

Abstract A field trial was carried out to understand the persistence behaviour of tetraconazole in mango and also in the soil of mango orchard following five applications @ 50 g a.i./ha (T_1) and 100 g a.i./ha (T_2). The initial deposits were found to be 0.23 and 0.38 μg/g for T_1 and T_2 doses. The theoretical maximum residue contribution (TMRC) of tetraconazole in dietary exposure appeared to be toxicologically safe for consumption as compared with maximum permissible intake (MPI). The half-life values of tetraconazole in mango were in the range of 4–5 days. The harvest samples of mango and soil were free from tetraconazole residues.

Significance of the study: The above entitled research work was conducted in Deptt of Agril Chemicals, BCKV. A field trial was carried out to understand the persistence behaviour of tetraconazole in mango and also to find out the occurrence of residues in harvest samples of mango and in the soil of mango orchard. The theoretical maximum residue contribution (TMRC) of tetraconazole in dietary exposure was evaluated as compared with maximum permissible intake (MPI). Mango (*Mangifera indica*) is the leading fruit crop of India and considered to be the king of fruits. The ripe fruits as well as the green fruits are consumed in a number of ways. Tetraconazole is recommended for use in Mango to control Powdery Mildew disease. Dissipation pattern of the compound and corresponding safety evaluation is necessary.

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Keywords Persistence · Tetraconazole · Mango · Soil · TMRC · MPI

Mango (*Mangifera indica*) is the leading fruit crop of India and considered to be the king of fruits. Its popularity is because of its delicious taste, excellent flavour and attractive fragrance. It is also rich in vitamin A and C. Mango is grown in an area of 1.23 million ha with an annual production of 10.99 million tonnes, which accounts for 57.18 per cent of the total world production (Negi 2000). Although India is the largest producer of mango but in terms of productivity, it ranks sixth. The low productivity is mainly due to the associated disease problem. Mango is affected by a number of diseases at all stages of its development (Prakash 2004). Among the different diseases of mango, powdery mildew caused by *Oidium mangiferae* Berthet is widely distributed and can be a major foliar disease of field grown mango trees (Reuveni et al. 1998). Occurrence of mildew has been recorded in India, Pakistan, Ceylon, South Africa, Rhodesia, Palestine, Brazil, Jamaica, Australia and the USA. In India, the disease is particularly destructive in Uttar Pradesh, Maharashtra and Karnataka. For controlling the disease, tetraconazole [(RS)-2-(2, 4-dichlorophenyl)-3-(1H.-1,2,4-triazol-1-yl)-propyl-1,1,2-tetrafluoroethyl ether, $C_{13}H_{11}Cl_2F_4N_3O$] is used. It is a broad spectrum systemic triazole fungicide (Khalfallah et al. 1998). It has been registered in various European countries. This fungicide is a steroid demethylation inhibitor, acting mainly on the vegetative stages of fungi by blocking the mycelial growth either inside or on the surface of the host plant. Tetraconazole is effective in controlling a broad spectrum of diseases such as powdery mildew and scab on fruits, powdery mildew on vines and cucumbers, powdery mildew and rust on vegetables and powdery

mildew, brown rust, *Septoria* and *Rhynchosporium spp.* on cereals (Menkissoglu et al. 1998). Literatures are available on the analysis of tetraconazole residues in different matrices by the manufacturing company Isagro Riceria and also by Khalfallah et al. (1998) and Menkissoglu et al. (1998). The objective of the present research programme was to investigate the dissipation behavior of tetraconazole in mango along with the residue content at harvest in mango fruits as well as in the soils of mango orchard for food safety assessment.

Materials and Methods

The trial was carried out at the Mango orchard of Farmers Training Centre, Bidhan Chandra Krishi Viswavidyalaya (BCKV) located at Kalyani, West Bengal, India. The orchard was 15 years old and planted with mango (variety-Amrapali). The spacing between both plant to plant and row to row was 6 m. A total of 27 plants were selected for the experiment. For each replication of the two treatment doses and control plots, three plants were taken. An aqueous emulsion of a 4% w/v tetraconazole formulation (EW) was applied at the rates of 50 g a.i./ha (5 g a.i./100 l water) and 100 g a.i./ha (10 g a.i./100 l water), which correspond to recommended (T_1) and double the recommended doses (T_2), respectively. The application was done by a Knapsack sprayer. Fifteen liters of emulsion were sprayed to each plant. The first application was made, when the plants were at the flowering bud initiation stage. The next applications were followed at 14 days interval. Nine plants (3 plants/replication) were kept untouched as control plants.

During the experimental period (from first spraying to last sampling), the maximum and minimum daily temperature ranged from 25.8° to 43.6°C and 16.8° to 29.6°C, respectively. The average relative humidity (%) was recorded in the range of 54–93 during the experimental period. There was no rainfall recorded during the study period (9th March to 29th June, 2005).

Samples, for both treated and control were collected randomly from different heights of the plants. About 0.5–1 kg mango samples of approximately the same size were collected. Sampling at the 0th day was performed 2 h after the last application and the subsequent samples were taken 1, 3, 7, 10, 15 and 20 days after the last application. The harvest samples were collected 55 days after the last application. Field samples were put in plastic bags and transported in ice box to the laboratory within 2 h from the time of collection. At harvest about 1 kg of soil sample was taken from different points of each replicated plot.

Mango samples were chopped and blended in a remi automix blender. From the fully homogenized sample, 50 g representative sample was drawn and extracted further. Soil samples were reduced to 100 g through quartering technique and subjected to extraction.

After usual quartering, a representative mango samples (50 g) were taken in a 250 mL conical flask. Acetone (100 mL) was added to each conical flask and kept for overnight. Then the samples were blended in a Remi automix blender for 2 min and the content was filtered through Buchner funnel and washed with 50 mL of acetone.

The soil samples were transferred to 500 mL conical flask prior to shaking. Solvent mixture of Methanol: water:: 9: 1 (200 mL, v/v) was added to the soil sample and the pesticide was extracted by continuous shaking for 2 h using a mechanical shaker. The extract was then filtered through Buchner funnel and washed with 50 mL of methanol.

The extracts from mango and soil were concentrated in the rotary vacuum evaporator and were partitioned thrice with dichloromethane (100, 50, 50 mL) after addition of 100 mL aqueous saturated NaCl solution. The organic layers were collected by passing through anhydrous Na_2SO_4 and concentrated to a minimum volume in the rotary vacuum evaporator.

The concentrated dichloromethane fraction was transferred to a glass column packed with silica gel (15 g), activated charcoal (0.5 g) and anhydrous sodium sulfate (5 g) and for soil samples the composition was silica gel (20 g) and anhydrous sodium sulfate (5 g). In both cases Hexane: acetone:: 1:1 (150 mL, v/v) mixture was used as eluant after discarding 200 mL of hexane.

Tetraconazole was anlyzed by GC Hewlett Packard (USA) model 5890A coupled with ECD (Ni^{63}) attached to Chemito 5000 data processor. DB 1701 column (30 m × 0.53 mm, 1.5 μm) was used with carrier gas flow rate of 50 mL/min. The oven temperature was maintained at 210°C for 10 min. After each analysis, oven temperature was raised to 260°C and kept for 3 min. Detector temperature and Injector temperature was kept at 340° and 275°C, respectively. Tetraconazole was eluted at an RT of 6.02 min.

Results and Discussion

The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated according to the peak-to-peak noise method as proposed by US EPA (Corley 2003). The LOD and LOQ were found to be 0.001 and 0.003 μg g^{-1}, respectively. To validate the method, recovery experiments were carried out at two fortification levels (1 and 10 LOQ). Five replications were taken at each fortification level along with two control samples. The average recovery percentages for mango were ranged

Table 1 Recovery study of mango and soil samples spiked with analytical standard of tetraconazole

Substrate	Amount fortified (μg/g)	Amount recovered (μg/g)*	% Recovery	RSD
Mango	0.003	0.0026	86.7	15.1
	0.030	0.0262	87.3	12.5
Soil	0.003	0.0025	82.0	12.1
	0.030	0.0250	83.3	12.6

RSD Relative standard deviation

* Average of five replicates

Table 2 Residues of tetraconazole on different days following fifth application in Mango

Days	Residues in μg/g (Mean ± SD)		% Dissipation	
	T_1 (50 g a.i./ha)	T_2 (100 g a.i./ha)	T_1 (50 g a.i./ha)	T_2 (100 g a.i./ha)
0	0.23 ± 0.040	0.38 ± 0.021	–	–
1	0.20 ± 0.030	0.31 ± 0.017	13.04	18.42
3	0.15 ± 0.006	0.23 ± 0.010	34.78	39.47
7	0.09 ± 0.006	0.15 ± 0.006	60.87	60.53
10	0.05 ± 0.006	0.10 ± 0.010	78.26	73.68
15	0.02 ± 0.006	0.05 ± 0.017	91.30	86.84
20	BQL	BQL		

BQL Below quantification limit (0.003 μg/g)

between 86% and 87% with relative standard deviations (RSD) from 12.5 to 15.1, while for soil the average recovery percentages were in between 82% and 83% and the relative standard deviations (RSD) varied from 12.1 to 12.6 (Table 1). Control samples were free from tetraconazole residues.

The results regarding the residue of tetraconazole in mango after 5th application have been summarized in Table 2, which shows the average initial deposit of 0.23 μg/g for T_1 and for T_2 it was 0.38 μg/g. The residues were found to dissipate by about 13%–18% by 1 day, 35%–39% by 3 days, 60% by 7 days, which further increased to 74%–78% by 10 days and 87%–91% by 15 days (Table 2). At 20 days after spraying no residue was detected (i.e. below the quantification limit) in the mango samples. The harvest samples of mango and soil were also free from residues of tetraconazole. No residue could be detected in the samples of mango of control plots during the entire study.

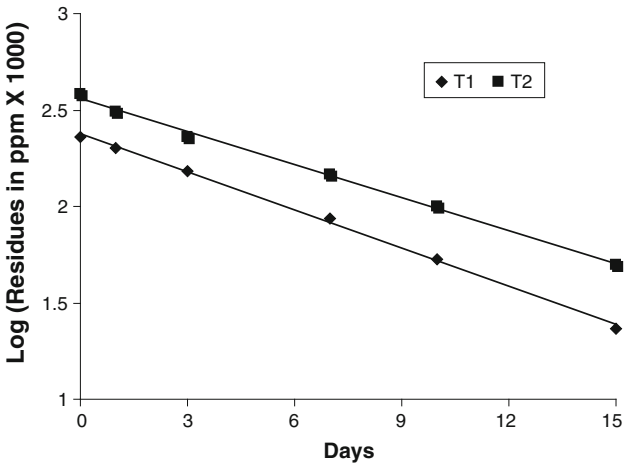

Fig. 1 Linear plot for first order kinetics of tetraconazole residues in mango

The log values of the mean residues ($\times 10^3$) for the two treatments were plotted against different days of observation to interpret statistically (Fig. 1). The declining nature of the residues followed first order kinetics for both the treatments.

The regression equations and half-life values for tetraconazole residues with different treatments were computed statistically as depicted in Table 3. The half-life values ($T_{1/2}$) of Tetraconazole in mango were found to be 4.5 days for T_1 and 5.2 days for T_2. The values are comparable to those observed earlier in the range of 5.1–5.7 days in sugar beet roots or foliage (Menkissoglu et al. 1998) and 6.3 days in grapes (Cabras and Angioni 2000).

MRL value of tetraconazole in mango has not yet been set by India or by international organizations like WHO/FAO. Therefore maximum permissible intake (MPI) for the pesticide was calculated based on the acceptable daily intake (ADI) values of 0.004 mg/kg (APVMA 2005) and compared with theoretical maximum residue contribution (TMRC) values (Table 4). The calculated TMRC values of tetraconazole for both the treatments were much lower than the MPI value from the 0th day sampling. The calculations

Table 3 Regression equation and half-life values of tetraconazole in mango

Treatment	Regression equation	Regression coefficient (R^2)	Half-life, $T_{1/2}$ (days)
T_1	Y = 2.3757–0.0659 X	0.9981	4.57
T_2	Y = 2.5594–0.0571 X	0.9971	5.27

Table 4 Theoretical maximum residue contribution (TMRC) of tetraconazole in mango

MPI[a] (mg/person/day)	Treatment	TMRC[b] (mg/person/day)					
		0 day	1 day	3 day	7 day	10 day	15 day
0.24	T_1	0.0575	0.0500	0.0375	0.0225	0.0125	0.0050
	T_2	0.0950	0.0775	0.0575	0.0375	0.0250	0.0125

[a] MPI (mg/person/day) = ADI (0.004 mg/kg) × Average body weight (60 kg)

[b] TMRC = Residues × Average daily consumption (250 g)

Residues are safe when TMRC < MPI

show that even the initial deposits of tetraconazole on mango were toxicologically safe for consumption. However, the need for establishment of MRL value in Mango by the competent authority is emphasized for food safety evaluation of tetraconazole in mango.

This study found that the harvest samples of mango and soil were free from tetraconazole residues.

Acknowledgments The reference standards, tetraconazole formulations and financial assistance provided by Isagro (Asia) Agrochemicals Pvt Ltd. are duly acknowledged. Facilities provided by BCKV for conducting the experiment are also acknowledged.

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