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Persistence and dissipation of carbendazim residues in mango fruits after pre- and post-harvest applications

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Dissipation of carbendazim residues in mango fruits of cv. 'Dashehari' was studied following both pre-harvest foliar spray and post-harvest dip treatment in hot water. Carbendazim dissipated to 0.83 and 1.76 mg/kg in mature fruits during harvest after its spraying at 0.05 and 0.1% concentrations, while its residue was very low in fruit pulp (0.24 and 0.51 mg/kg). The residues of carbendazim in whole ripe fruits, applied as post-harvest dip in hot water (52±1°C) for 10 min at 0.05 and 0.1% concentrations, were 0.90 and 1.86 mg/kg after 10 days of storage at room temperature (32±2°C). The corresponding values for fruit pulp were 0.32 and 0.65 mg/kg after same period of storage. The residual half-life of carbendazim in whole fruits was approximately 7.0 and 6.5 days at 0.05 and 0.1% concentrations, respectively from pre-harvest spray as well as post-harvest dip treatment. The safe waiting periods of 2 and 3 days are suggested for 0.5% dose after pre-harvest spray and post-harvest dip treatment, respectively.

Keywords: Carbendazim, Residues, Mango, Pre-harvest spray, Post-harvest dip

Carbendazim (methyl-2-benzimidazole carbamate) is a systemic fungicide from benzimidazole group used for controlling post-harvest diseases of mango like anthracnose and stem end rot through pre-harvest foliar spray (Ullasa 1993, Prakash and Pandey 2000). Post-harvest dip of mature mango fruits in hot fungicidal solution immediately after harvest has also controlled these diseases (Laxminarayana et al 1974, Sharma and Badiyala 1994). Analyses of carbendazim residues in plants and soil by gas liquid chromatography (Rouchaud and Decallone 1974) and in water by spectrophotometric technique (Chiba 1977) have been tried earlier, but these methods need derivatization of fungicide which is a cumbersome and time consuming process. High performance liquid chromatographic (HPLC) methods have also been developed to analyse benomyl and carbendazim residues in water, cow milk, urine, feces and animal tissues (Kirkland 1973, Chiba and Singh 1986). HPLC method has also been found most simple and suitable for determining residues of carbendazim in apple, mango, grape, chilli, tomato and onion (Mohapatra et al 1998, Chiba and Veres 1980, Sharma and Awasthi 1999). Even in orange, apple and grape juices HPLC for carbendazim residues after solid phase extraction was found suitable with a limit of 20 µg/l (Young et al 2001). But suitability of HPLC to determine carbendazim residues in North Indian mango varieties has not yet been tested. Since pre-harvest spray and post-harvest dip treatment of carbendazim on mature mango fruits may result in its uptake and persistence during harvesting and storage, the present study was undertaken to evaluate the extent of carbendazim residues in mature mango fruits after harvest and its dissipation pattern in ripe fruits during storage at ambient temperature after dip treatment in hot carbendazim solution.

Materials and methods

Two pre-harvest sprays of carbendazim (Bavistin® 50 WP) at the rate of 0.05 and 0.1% (recommended and double the recommended dose) were applied to mature mango fruits of cv. 'Dashehari' 15 days prior to harvest. The experiment was conducted in randomized block design and each treatment replicated thrice. The samples were withdrawn 1 h after spraying and subsequently after 2, 4, 6, 9 and 15 days. Hot water (52±1°C) dip of carbendazim (0.05 and 0.1%) was given in water bath for 10 min to another lot of harvested mango fruits (cv. 'Dashehari') devoid of pre-harvest spray. Control fruits were dipped in hot water only. After dip treatment fruits were dried and stored in corrugated fiber board (CFB) boxes at ambient conditions (32±2°C, 72.6% RH) up to 10 days for ripening and observations. Samples were withdrawn in triplicate 1 h after dip treatment and subsequently at 2-day intervals up to 10 days.

Fruit samples [50 g whole fruit (peel + pulp) and 50 g pulp only] of mango were homogenized and extracted in a Virtishear (Virtis, USA) vertical shaker with 120 ml ethyl acetate and filtered through Buchner funnel. The solid residue was re-extracted with 100 ml ethyl acetate and combined extracts were evaporated to near dryness in a rotary vacuum evaporator. The residue was dissolved in 0.5N H_2SO_4 (3 x 30 ml) and washed thrice with 50 ml chloroform at each time after passing through glass wool into a separating funnel. The chloroform layers were discarded after phase separation. The pH of the acidic aqueous layer was then adjusted between 8.5 and 9.0 with 5N NaOH solution and extracted with 2 x 40 ml dichloromethane. The combined dichloromethane extracts were dried by passing through anhydrous Na_2SO_4 and completely evaporated in a rotary vacuum evaporator (Awasthi and Sharma 1997). The residues were immediately dissolved in 5 ml HPLC grade acetonitrile and analysed by HPLC.

A Shimadzu make HPLC (model LC10 ATVP) was equipped with a photodiode array detector and a reverse phase Phenomenex® Luna C-18 column (250 x 4.6 mm i.d., 5 m film thickness) as stationary phase. The mobile phase consisted of 10% v/v pH 7.0 phosphate buffer in water and acetonitrile (55:45) run isocratically at a flow-rate of 1.0 ml/min. Each time 20 µl sample was injected through a rheodyne injector. Phosphate

buffer was prepared by mixing solutions of 0.067 M Na_2HPO_4 and 0.067 M KH_2PO_4 at a ratio of 3:2 (v/v) so that the pH of the buffer solution was maintained around 7.0. The detector wavelength was set at 286 nm. The sample extracts were filtered through a membrane filter (Millipore, 0.45 mm thickness and 13 mm dia.) held in a filter holder attached to a glass syringe before injection. Carbendazim was separated at the retention time of 4.18±0.05 min.

A stock solution of carbendazim (1000 ppm) was prepared by dissolving 50.71 mg of technical grade carbendazim (98.6% pure) in minimum quantity of 0.1N HCl and making up the volume to 50 ml with acetonitrile. A calibration curve for standard solutions of carbendazim in acetonitrile was found linear in the range of 0.1 to 10 mg/kg. The limit of quantification was determined to be 0.1 mg/kg by considering a signal to noise ratio of 10:1. The recovery of carbendazim residues from mango fruits and pulp fortified at 0.1 and 0.2 mg/kg ranged between 93.3-99.1% and 81.6-81.7%, respectively. The residue data was subjected to statistical analysis (Hoskins 1961) for calculating the residue decay in terms of half-life (DT_{50} in day) values and safety constants in terms of Maximum Permissible Intake (MPI) and Theoretical Maximum Residue Contribution (TMRC) through comparison of dietary exposure of everyday sample with MPI. The prescribed acceptable daily intake (ADI) of carbendazim is 0.03 mg/kg body weight /day and its prescribed maximum residual limit (MRL) value is 2 mg/kg in mango in India (Sharma 2007). The MPI was found to be 0.48 mg/child/day by multiplying the ADI with the average body weight of an Indian child of 16 kg, because children are more susceptible to pesticide toxicity. The values of dietary exposure (TMRC) were calculated by multiplying the residue levels with average per capita daily consumption of 20 g of mango fruits.

Results and discussion

Carbendazim dissipated to 0.83 and 1.76 mg/kg in mature whole fruits during harvest from its initial residues of 2.4 and 4.8 mg/kg just after spraying at 0.05 and 0.1%, resulting in 65.2 and 63.4% loss after 15 days (Table 1). The rate of dissipation followed first-order kinetics in whole fruit (Fig. 1) but not in fruit pulp. Initially carbendazim concentration in fruit pulp increased and reached maximum after 2nd day (1.5 and 2.9 mg/kg at 0.05 and 0.1%), afterwards it decreased gradually. Very low residue level of 0.24 and 0.51 mg/kg was recovered after harvest from fruit pulp treated with 0.05 and 0.1% carbendazim, respectively. The half-life values of carbendazim in whole fruit were 7 days at both the doses, which showed that degradation rate was almost same in both the concentrations. The pre-harvest interval was suggested to be 2.5 and 4 days for 0.05 and 0.1% concentrations, respectively. The faster degradation of carbendazim in mango pulp and fruit may be attributed to the relatively higher temperature in the Northern region during May-June (>40°C) as compared to Southern region. It was also observed that temperature in fruit pulp was 2-3°C higher than surface temperature during maturity (unpublished data).

The dissipation pattern of carbendazim, when applied as hot water dip treatment, in mature mango fruits followed first-order rate kinetics during storage also with 0.91 and 1.86 mg/kg of the fungicide detected in fruits after 10 days of storage from 0.05 and 0.1% concentrations (Table 2). The corresponding values in fruit pulp were 0.32 and 0.65 mg/kg during the same period of storage. The reduction of residues in whole fruit was 67.6 and 68.1% at lower and higher concentrations, respectively and followed first-order kinetics (Fig. 2). In pulp the residues of carbendazim attained maximum concentration after 2nd day of storage, i.e., 1.54 and 3.29 mg/kg from 0.05 and 0.1% concentrations, respectively and

Table 1. Persistence of carbendazim residues in mango fruits after pre-harvest sprays

Period, days	Concn, %	Residues, mg/kg		PR in
		WF	Pulp	WF, %
0	0.05	2.40	1.13	-
	0.1	4.80	2.37	-
2	0.05	1.92	1.50	19.6
	0.1	3.95	2.91	17.6
4	0.05	1.70	1.16	29.2
	0.1	3.49	2.24	27.3
6	0.05	1.37	0.82	42.6
	0.1	2.85	1.75	40.6
9	0.05	1.04	0.52	56.5
	0.1	2.11	1.23	56.0
15	0.05	0.83	0.24	65.2
	0.1	1.76	0.51	63.4
DT_{50} (d)	0.05	7.0	-	-
	0.1	7.0	-	-
PHI (d)	0.05	2.5	-	-
	0.1	4.0	-	-

(n=3), PR: Progressive reduction, DT_{50} (d): DT_{50} in days, PHI (d): Post-harvest interval (days), WF: Whole fruit

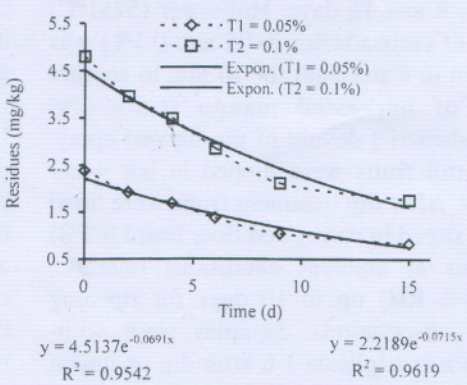

Fig. 1. First order exponential equation of degradation of carbendazim residue in whole mango fruits after pre-harvest spray (n=3)

Table 2. Dissipation of carbendazim residues in mango after post-harvest dip in hot water

Period, days	Concn, %	Residues, mg/kg		PR in
		WF	Pulp	WF, %
0	0.05	2.79	1.10	-
	0.1	5.84	2.48	-
2	0.05	2.22	1.54	20.3
	0.1	4.26	3.29	27.0
4	0.05	1.74	1.32	37.6
	0.1	3.59	2.66	38.5
6	0.05	1.39	0.84	50.3
	0.1	2.76	1.64	52.7
8	0.05	1.17	0.53	58.0
	0.1	2.34	0.99	59.8
15	0.05	0.91	0.32	67.6
	0.1	1.86	0.65	68.1
DT_{50} (d)	0.05	6.5	-	-
	0.1	6.5	-	-
PHI (d)	0.05	3.0	-	-
	0.1	5.0	-	-

(n=3), PR, DT_{50} (d), PHI (d), WF as in Table 1

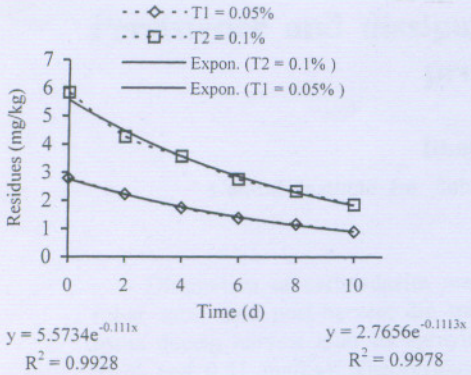

Fig. 2. First order exponential equation of dissipation of carbendazim residues in whole mango fruits after post-harvest dip in hot water (n=3)

afterwards it degraded gradually (Table 2). The residual half-life values in whole fruits were calculated as 6.5 days at both the concentrations, while post-harvest interval was suggested as 3 and 5 days for 0.05 and 0.1% doses, respectively. Since hot water treated fruits attained full ripening by 10th day of storage under ambient conditions, no further residue analysis was done in over ripe fruits. The TMRC values calculated for residues corresponding to each sampling date were below the MPI level in all cases at both the concentrations and at both pre- and post-harvest treatments. Similar trends of residue dissipation in mango fruit and pulp were noticed in cv. 'Totapuri' where carbendazim dissipated with a longer half-life of 19 days and waiting period of 16 days in the whole fruit during storage at room temperature (under South Indian conditions) after post-harvest dip treatment in cold water (Awasthi and Sharma 1997). Neither anthracnose nor stem end rot was observed in fruits stored up to 10 days in CFB boxes from both the treatments. However, few fruits treated with 0.05% concentration of carbendazim were found infected by *Aspergillus* rot.

Conclusion

In case of both pre-harvest spray and post-harvest dip, carbendazim residues came down below its minimum residue level of 2 mg/kg in mango after 9 days in field and 10 days in storage from 0.1% concentration (double dose) and hence not safe for recommendation, but its 0.05% concentration has found safe (due to waiting period of 2 and 3 days only) and can be recommended as pre-harvest spray as well as post-harvest dip in hot water for management of post harvest diseases of mango.

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