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Dissipation of carbendazim in mango after pre- and post-harvest treatments

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A B S T R A C T

Dissipation of carbendazim was evaluated in mango fruit after pre as well as post harvest applications. Two pre harvest sprays of carbendazim @ 0.05 and 0.1 per cent were given to mango fruit (cv. Chausa) at 10 days interval in such a way that last spray fell 12 days prior to harvest to control post harvest diseases viz., anthracnose (*Colletotrichum gloeosporioides*) and stem end rot (*Lasiodiplodia theobromae*). Carbendazim dissipated to 1.01 and 2.14 mg kg^{-1} in mature whole fruit after harvest (12 days after second spray) at 0.05 and 0.1 per cent concentrations, respectively, following first order rate kinetics. The corresponding values in fruit pulp after 12 days were 0.57 and 1.26 mg kg^{-1}. In another experiment, cold water dip treatment in carbendazim solution @ 0.05 and 0.1 percent was given for 10 min to a separate lot of fruit having no pre harvest spray. Dissipation of carbendazim in whole fruit again followed first order rate kinetics with 0.92 and 2.06 mg kg^{-1} of the fungicide recovered after 10 days of storage from 0.05 and 0.1 percent doses, respectively. The corresponding values in fruit pulp after same days of storage were 0.51 and 1.05 mg kg^{-1}. The residual half-life values of carbendazim in whole fruit were calculated as 4 and 3 days at both the concentrations from pre harvest spray and post harvest dip treatment, respectively. Pre and post harvest interval of 3 and 7 days were suggested from 0.05 and 0.1 percent concentrations. Carbendazim @ 0.05 percent was found safe and can be recommended as pre harvest spray or post harvest dip in cold water for the management of post harvest diseases of mango.

Keywords: Carbendazim, dissipation, mango, pre-harvest spray, post harvest dip

Introduction

Mango is consumed in India in fresh as well as processed forms. The commercial cultivation of mango requires frequent application of different contact and systemic pesticides throughout the fruiting season for controlling various insect-pests and diseases. The contamination of fruit with pesticide residues at bioconcentrations may be hazardous to consumers and render the fruits unsuitable for market. Nowadays commercial cultivars from Uttar Pradesh like Dashehari, Chausa and Langra are exported to various countries, which requires strict maintenance of pesticide residue level below their tolerance limits as per the standards set by Codex Alimentarius Commission. The growing concern for ensuring safety of fruits from pesticide contamination and protecting consumers have made pesticide residue analysis one of the most important aspects.

Carbendazim (methyl-2-benzimidazole carbamate), a systemic fungicide, is commonly used to control post harvest diseases of mango

like anthracnose (*Colletotrichum gloeosporioides*) and stem end rot (*Lasiodiplodia theobromae*) through either pre harvest spray (Prakash & Pandey 2000) or post harvest spray and dip in hot fungicidal solution (Sharma & Badiyala 1994; Bhattacherjee *et al.* 2009). Awasthi & Sharma (1997) reported that carbendazim residues persisted throughout the ripening period of mango during storage either at room temperature (25 ± 3^{0}C) or at low temperature (15 ± 1^{0}C) after post harvest dip in cold water at 500 ppm. Awasthi *et al.* (1998) suggested that longer persistence of carbendazim in mango after post harvest dip could be due to the slow rate of residue decay where residues were found above the prescribed maximum residue limit (2 mg kg^{-1}) on fully ripened fruits. High performance liquid chromatographic (HPLC) technique was found most suitable for residue analysis of carbendazim in mango (Sharma & Awasthi 1999; Bhattacherjee *et al.* 2009). Banerjee *et al.* (2009) employed HPLC coupled with tandem mass spectrometry for multiresidue analysis of 87 pesticides including carbendazim in mango. Carbendazim residues could also be analyzed by HPLC in water (Chiba & Singh 1986), cow milk, urine, feces and animal tissues (Kirkland 1973), fresh fruits and vegetables like grape, chilli, tomato and onion (Mohapatra *et al.* 1998). HPLC after solid phase extraction was also found suitable for residue analysis of carbendazim in processed products like orange, apple and grape juices (Young *et al.* 2001). Literature on analysis of carbendazim residues in commercial mango cultivars of Central India, especially Uttar Pradesh, is very scanty. Since both pre harvest spray and post harvest dip treatment of carbendazim on mature mango fruit may result in its uptake during consumption, the present investigation was carried out to evaluate the level of dissipation of carbendazim in mature mango fruits during harvest as well as in ripe fruit during storage under ambient conditions.

Materials and Methods

In first experiment, carbendazim (Bavistin 50 WP) was sprayed twice at the rate of 0.05 and 0.1 percent concentrations to mature mango fruit of cv. Chausa 22 days before harvest at 10 days interval. The experiment was undertaken with three replications for each treatment. Fruit samples (one fruit as one replication) were withdrawn for residue study after 0 (1h after second spray), 2, 5, 7, 10 and 12 days.

In second experiment, post harvest dip treatment in cold carbendazim solution (0.05 and 0.1 percent doses) was given for 10 min to a fresh lot of mature fruit (cv. Chausa), which did not receive any spray. Control fruit were dipped in cold water only. Fruits were surface dried after dip treatment and stored in corrugated fiber board (CFB) boxes under ambient conditions (35 ± 2^{0}C, 80-90% RH) for ripening and observations on disease development. Samples were withdrawn in triplicate after 0 (1h after dipping), 2, 4, 6, 8 and 10 days of storage for residue analysis. Residue study could not be carried out after 10 days of storage for over ripe, senescence, unmarketable and shrinking fruit.

The method of analysis, which was followed in this study, was earlier advocated by Awasthi & Sharma (1997) for residue analysis of

carbendazim in ripe mango fruits of cv. Totapuri. Fifty gram samples of each of whole fruit (peel + pulp) and pulp were separately extracted in a Virtishear (Virtis, USA) vertical shaker with 120ml ethyl acetate and filtered through Buchner funnel. The samples were re-extracted with 100ml ethyl acetate, filtered and combined filtrates were evaporated to nearly 5ml in a rotary vacuum evaporator. This 5ml extract was dissolved in 30ml of 0.5N sulfuric acid and washed with 50ml chloroform in a separating funnel. The procedure was repeated for two more times and chloroform layers were discarded each time after phase separation and the pH of the final acidic aqueous layer was adjusted between 8.5 and 9.0 with 5N sodium hydroxide solution. The alkaline aqueous solution was then extracted with dichloromethane (2 x 40ml), dried by passing through anhydrous sodium sulphate and combined dichloromethane extracts were completely evaporated in a rotary evaporator under vacuum. The residues were immediately dissolved in 5ml HPLC grade acetonitrile and analyzed by HPLC.

A Shimadzu make HPLC (model LC10 ATVP) coupled with a photodiode array (PDA) detector and a reverse phase Phenomenex Luna 5 100Å C18 column (250 x 4.6 mm i.d.) as stationary phase was used in this study. The mobile phase was 10% v/v phosphate buffer (pH 7.0) in water and acetonitrile (55 : 45) run isocratically at a flow-rate of 1.0 ml min^{-1}. Twenty microlitre (20 l) sample was injected each time through a rheodyne injector having 20l loop. Phosphate buffer was prepared by mixing solutions of 0.067M disodium hydrogen phosphate and 0.067M potassium dihydrogen orthophosphate at a ratio of 3 : 2 (v/v) so that the pH of the buffer solution was maintained around 7.0. PDA detector wavelength was set at 286nm. Before injection samples were filtered through a membrane filter (Millipore, 0.45mm thickness and 13mm diameter) held in a filter holder attached to a glass syringe.

A stock solution of 1000 mg l^{-1} concentration was prepared by dissolving 50.71 mg of technical grade carbendazim (98.6% pure, BASF, Mumbai) in minimum quantity of 0.1N hydrochloric acid and making up the volume to 50ml with acetonitrile. A calibration curve for standard solutions of carbendazim in acetonitrile was found linear in the range of 0.1 to 10 mg l^{-1}. The limit of quantification was determined to be 0.1 mg l^{-1} 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^{-1} varied between 93.3 – 99.1 per cent and 81.6 – 82.7 percent, 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 fruit sample with MPI. The prescribed acceptable daily intake (ADI) of carbendazim is 0.03 mg kg^{-1} body weight day^{-1} and its prescribed maximum residue limit (MRL) is 2.0 mg kg^{-1} in edible portion of mango fruit in India (Sharma 2007). The MPI was worked out as 0.48 mg $child^{-1}$ day^{-1} by multiplying the ADI

with the average body weight of an Indian child of 16 kg, because children are more susceptible to pesticide toxicity.

Results

Carbendazim dissipated to 1.01 and 2.14 mg kg^{-1} in mature whole fruits after harvest from its initial residue levels of 2.48 and 5.28 mg kg^{-1} just after second spraying at 0.05 and 0.1 percent doses, resulting in around 60 percent loss after 12 days (Table 1). The corresponding values in fruit pulp were 0.57 and 1.26 mg kg^{-1}, which were considerably below MRL value. Initially carbendazim concentration in fruit pulp increased because of slow absorption and reached highest at 5th day (1.71 and 3.52 mg kg^{-1} at 0.05 and 0.1%), then it decreased gradually. Almost 50 per cent carbendazim was retained in fruit after 10 days of second spray from both the doses. The rate of dissipation followed first-order kinetics only in whole fruit with exponential regression equations being $y = 2.998e^{-0.1762x}$ and $y = 6.301e^{-0.1732x}$ for 0.05 and 0.1 percent doses, respectively. The residual half-life values of carbendazim in whole fruit were calculated as 4 days at both the doses, which showed that degradation rate was almost same at both the doses. The pre harvest interval (PHI) was suggested to be 2.5 and 7 days for 0.05 and 0.1 percent doses, respectively.

The dissipation pattern of carbendazim, when applied as post harvest dip in cold water, also followed first-order rate kinetics in ripe mango fruit during storage with 0.92 and 2.06 mg kg^{-1} of the fungicide detected in fruits after 10 days of storage from 0.05 and 0.1 percent concentrations. However, very low residue level of 0.51 and 1.05 mg kg^{-1} was recovered from fruit pulp treated with 0.05 and 0.1 percent carbendazim, respectively, after 10 days of storage (Table 2). More than 50 percent carbendazim was retained in fruits from both the concentrations during 6th day of storage. The reduction of residues in whole fruit after 10 days of storage was 70.61 and 66.06 percent at lower and higher concentrations of the fungicide. The exponential regression equations in whole fruit were $y = 4.238e^{-0.2406x}$ and $y = 8.256e^{-0.2113x}$ for 0.05 and 0.1 per cent doses, respectively. In pulp, carbendazim residue attained maximum concentration after 4th day of storage, i.e., 1.46 and 3.12 mg kg^{-1} from 0.05 and 0.1 percent concentrations, respectively, and thereafter, degraded gradually (Table 2). The residual half-life values in whole fruit were 7 days at both the concentrations, while post harvest interval (PHI) was suggested as 3 and 7 days for 0.05 and 0.1 percent doses, respectively. Neither anthracnose nor stem end rot was observed in fruits treated with fungicide and stored up to 10 days in CFB boxes

Discussion

The TMRC values calculated for residues corresponding to each sampling date were found below the MPI level at both the concentrations and at both pre and post harvest applications rendering carbendazim a safe chemical to be used in mango. Similar trends of residue dissipation in mango fruit and pulp were reported in cv. Dashehari by Bhattacherjee *et al.* (2009) where carbendazim dissipated with a half-life of 7 and 6.5 days at both the concentrations (0.05 and 0.1%) from pre harvest spray or post harvest dip treatment in hot water. They suggested 2 and 3 days as pre

and post harvest intervals. However, Awasthi & Sharma (1997) reported that carbendazim dissipated with a longer half-life of 19 days in whole mango fruit during storage at room temperature (25 ± 3^{0}C) after post harvest dip treatment in cold water at 500 ppm concentration and a waiting period of 16 days was suggested. While Awasthi *et al.* (1998) suggested 26 days as waiting period for post harvest treatment with carbendazim in mango because of the slow rate of residue decay during storage. The slower rate of degradation of carbendazim residue under South Indian condition might be attributed to the lower temperature during storage.

Table 1.

Dissipation of carbendazim residues in mango fruits after pre harvest spray

Period (days)	Concentration (%)	Residues (mg kg $^{-1}$)		Percent of initial residue in fruits
		whole fruit	Pulp	
0	0.05	2.48	1.23	100.00
	0.1	5.28	2.51	100.00
3	0.05	2.07	1.47	83.47
	0.1	4.33	2.78	82.01
5	0.05	1.79	1.71	72.18
	0.1	3.81	3.52	72.16
7	0.05	1.56	1.33	62.90
	0.1	3.24	2.83	61.36
10	0.05	1.24	0.91	50.00
	0.1	2.73	1.95	51.70
12	0.05	1.01	0.57	40.73
	0.1	2.14	1.26	40.53
DT_{50} (d)	0.05	4.0	–	–
	0.1	4.0	–	–
PHI (d)	0.05	2.5	–	–
	0.1	7.0	–	–
MRL (mg kg^{-1})	–	–	2.00	–
Exponential regression equation	0.05	$y = 2.998e^{-0.1762x}$	–	–
	0.1	$y = 6.301e^{-0.1732x}$	–	–
R^2 value	0.05	0.99	–	–
	0.1	0.99	–	–

Table 2.

Dissipation of carbendazim residues in mango fruits after post harvest dip in cold water

Period (days)	Concentration (%)	Residues (mg kg $^{-1}$)		Percent of initial residue in fruits
		whole fruit	Pulp	
0	0.05	3.13	0.78	100.00
	0.1	6.07	1.83	100.00
2	0.05	2.63	1.17	84.02
	0.1	5.39	2.33	88.79
4	0.05	2.21	1.46	70.61
	0.1	4.79	3.12	78.91
6	0.05	1.64	1.24	52.39
	0.1	3.91	2.51	64.41
8	0.05	1.35	0.83	43.13
	0.1	2.97	1.62	40.93
10	0.05	0.92	0.51	29.39
	0.1	2.06	1.05	33.94
DT_{50} (d)	0.05	3.0	–	–
	0.1	3.0	–	–
PHI (d)	0.05	3.0	–	–
	0.1	7.0	–	–
MRL (mg kg^{-1})	–	–	2.00	–
Exponential regression equation	0.05	$y = 4.238e^{-0.2406x}$	–	–
	0.1	$y = 8.256e^{-0.2113x}$	–	–
R^2 value	0.05	0.98	–	–
	0.1	0.95	–	–

Carbendazim at 0.1 percent concentration could control both the post harvest diseases (anthracnose and stem end rot). But its residue level did not dissipate below its MRL value of 2 mg kg^{-1} during 12 days after harvest and 10 days after post harvest dip. Hence, it was not found suitable for recommendation. Whereas, 0.05 percent dose of carbendazim was found safe, because of less pre and post harvest intervals of 2.5 and 3 days, and could also control anthracnose and stem end rot. Therefore, it can be recommended as pre harvest spray or post harvest dip in cold water for controlling the post harvest diseases of mango.

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