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Relative uptake of the fungicide carbendazim by selected fruits and vegetables and keeping quality of apple and tomato after dip treatment

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Abstracts

Uptake of fungicide carbendazim by eight fruits and vegetables on dipping in carbendazim aqueous suspension, and its effects on keeping quality of tomato and apple were investigated. The uptake of carbendazim varied significantly (p<0.01), ranging from 68.97±2.89 to 813.64±11.46 µg (mean 342.13 µg) among the fruits and vegetables. The lowest uptake was in apple followed by banana, orange, tomato, okra, grape (golden), grape (blue), sapota and carrot. The dip treatment was more effective for storage life extension of tomato than apple and at ambient (32±2°C) than at low temperature (7±2°C). Cumulative physiological loss in weight, physical appearance and spoilage in tomato and apple, and lycopene content, titratable acidity, ascorbic acid content and moisture content in tomato were also analysed during the course of storage.

Key words: Carbendazim uptake, fruits and vegetables, dip treatment, keeping quality

Introduction

Benomyl (IUPAC: Methyl 1 (Butyl carbamoyl) benzimidazole 2 yl carbamate) and carbendazim or MBC (IUPAC: Methyl benzimidazole 2 yl carbamate), two important benzimidazole class of fungicides, are applied predominantly on fruits and vegetables in pre- as well as post-harvest stages (Ben-Yeshoshua and Cohen, 1981; Mohammed and Sealy, 1988; Gupta and Mukherjee, 1980; Awasthi and Sharma, 1997). Benomyl and other benzimidazole fungicides, as such or after hydrolysis to MBC inside the plant body, leave stable residues, having long term toxicity to growing cells and inhibitory effect on DNA, RNA and protein synthesis by inhibiting cell division (Charles, 1987).

Monitoring of farm gate samples in India, of late, has shown carry-over pesticide residues to an extent of 40% in fruits (Agnihotri, 1999) and 60% in vegetables (Ahuja *et al.*, 1998; Awasthi and Ahuja, 1997), showing contamination from pre-harvest application of pesticides including benomyl and MBC. On the other hand, benomyl and MBC are applied post-harvest to control fungal decay in a number of fruits and vegetables, and hence to extend their shelf-life (Waskar *et al.*, 1999a, 1999b; Amadioha, 1996, 1998; Sanderson, 1999; Nwufo *et al.*, 1994; Jahangir *et al.*, 1994; Abu-Baker and Abdul-Karim, 1994; Papadopoulou-Mourkidou, 1991; Goulart *et al.*, 1992; Rathu *et al.*, 1989; Sharma *et al.*, 1989). Due to high persistence of carbendazim, its residues have been reported in many fruits and vegetables (USDA, 1993; Awasthi 1998; Awasthi and Ahuja, 1997; Awasthi and Sharma, 1997; Baldi *et al.*, 1981, 1982; Bencivenga and *et al.*, 1982; Cano and Plaza, 1987; Hamil and Harper, 1982; Hargreaves, 1983; Kiigemagi *et al.*, 1991; Meloni and Pirisi, 1984; Monico-Pifarrae, 1987; Nagayama *et al.*, 1983).

But there is no systematic study carried out on uptake of MBC residues due to dip treatment of fruits and vegetables. In the present study, an attempt has been made to study the extent of uptake of MBC residue by eight common fruits and vegetables dipped in MBC suspension under laboratory conditions. Effect of dip treatment using MBC suspension, on extension of storage life of tomato and apple was investigated.

Materials and methods

Selection of fruits and vegetables: Eight common fruits and vegetables *e.g.* tomato (*Lycopersicon esculentum* L.), apple (*Malus pumila*), carrot (*Daucus carrota* L.), okra (*Abelmoschus esculentus* Moench.), orange (*Citrus sinensis* Osbeck.), grapes (*Vitis vinifera* L.) of golden yellow and blue colour, sapota (*Achras zapota* L.) and banana (*Musa paradisiaca* L.), which predominantly get the application of benomyl or MBC in pre-harvest and occasionally in post-harvest stages, were selected for the present study. Fresh, fully mature firm medium ripe tomato (S-15 variety), freshly harvested carrot and tender okra, fully mature and hard-ripe sapota and fully mature medium ripe banana obtained as farmgate samples were selected, whereas firm and full apple (Golden Delicious), ripe orange and fully mature ripe grapes purchased from local market were used.

Dip treatment: Whole fruit or vegetable without any structural damage or injury was weighed (~1500 g) and immersed completely for 1 hr at ambient temperature in 2000 ml aqueous suspension of 750 ppm Bavistin 50% WP. (*i.e.*, 375 ppm of carbendazim as *a.i.*), taken in a stainless steel vessel covered with a lid. After 1 hr, the fruit or the vegetable was freed of the suspension and air-dried in laboratory condition till there was no trace of visible water on the surface. The weight of the fruit or the vegetable and the volume of the suspension before and after dip treatment were recorded to calculate percent gain in weight by the treated fruits or the vegetables and loss in volume of MBC suspension.

Analysis of fruits and vegetables for MBC: The fruits or the vegetables prior and after dip treatment were analysed for MBC residue by modifying the method of Rangaswamy *et al.* (1987). The difference in the carbendazim residue contents was considered as the residue uptake, and expressed as µg per 100 g sample, µg per 100 g MBC and µg per 100 ml MBC suspension.

Studies on storage life: The spiked and the non-spiked tomato and apple samples were stored at 32±2°C (ambient) and 7±2°C (low) to observe cumulative physiological loss in weight (CPLW), skin shrivelling and fungal spoilage. The period in days just prior to appearance of fungal spoilage was considered as the storage life. Percent spoilage of tomato and apples was calculated by expressing the ratio of the weight of the spoiled tomato or apple to the weight of tomato or apple taken for storage study in percentage. The CPLW was determined by the difference in weight between the fruits or vegetables of the first day and that of the any respective storage day to get the CPLW of the particular day.

Chemical quality analysis: Chemical quality parameters such as moisture content, titratable acidity, ascorbic acid and lycopene contents of the fresh as well as the stored samples of the treated and the control tomatoes were analysed by the standard oven dry (AOAC, 1990), titrimetric (AOAC, 1990) and spectrophotometric (Beerh and Siddappa, 1959) methods, respectively.

Statistical analysis: All the data were subjected to calculation of mean±standard deviation of a number replicates and subsequent one-way analysis of variance (ANOVA) for significant differences among them (Snedecor and Cochran, 1989)

Results and discussion

Uptake of MBC residue: Mean weights of fruits and vegetables samples before and after dip treatment ranged from 1500.5±0.2 to 1519.6±2.4 g (mean 1508.67g) and 1509.1±2.1 to 1536.1±2.5g (1523.38g), respectively (Table 1). Gain in weight by the samples due to dip treatment varied from 0.41±0.02 to 1.55±0.09% (mean 0.97%) depending upon the type of fruits and vegetables (Table 1). On the other hand, mean volumes of MBC suspension before and after dip treatment were 2000±0.00 ml (mean 2000 ml) and 1942.7±2.6 to 1979.2±1.2 ml (mean 1962.82 ml), respectively (Table 1). Decrease in volume of MBC suspension used for dip treatment ranged from 1.04±0.05 to 2.86±0.15% (mean 1.86%) depending upon the type of fruits and vegetables (Table 1).

Percentage weight gain during dip treatment was considered as actual uptake of MBC suspension by the fruits and vegetables, whereas percentage decrease in volume of MBC suspension included the loss due to actual uptake of MBC suspension as well as the loss due to evaporation or spillage, etc. at the time of handling the produce. Hence, the percentage weight gain represented the uptake of MBC residue more appropriately than the percentage volume loss. The percentage weight gain values were 39.42 to 72.95% (mean 51.87%) lower than the percentage loss in volume. The fruits and vegetables like tomato, grapes, apple with waxy smooth skin showed less gain in weight than the ones like carrot, sapota, banana, okra, etc. with rough skin. Because uptake of residues was due to penetration of residue through skin or peel.

Quantity of MBC uptake (ppm) was found to vary significantly ($p<0.01$) among the fruits and vegetables as the case may be, and ranged from 68.97±2.89 to 813.64±11.46 µg (mean 342.13 µg) (Table 1). Uptake of MBC residue expressed as µg per100g sample, µg per100 µg MBC and µg per100 ml suspension was also found to vary significantly ($p<0.01$) from one fruit or vegetable to others (Table 1). However, uptake of MBC residue (expressed in all three different forms) was the lowest in apple followed by (in an increasing order), banana, orange, tomato, okra, grape (golden), grape (blue), sapota and carrot. Lower uptake in grape was due to poor penetration of suspension through peels. During spiking the residue might have deposited on the surface, leading to slight absorption by outer waxy layers and subsequently inner cuticles. A similar observation was made on absorption of field-applied pesticides on standing crops (Cabras *et al.*, 1998). In a detailed study on mechanism of pesticide absorption on plant body, enough evidence was presented to show that the driving force for pesticide penetration into plant body depended on formulation, lipophilicity and concentration of the active ingredient (Baur *et al.*, 1997; Marzouk *et al.*, 1998).

Physical change or fungal spoilage: Shrivelling of skin started on 5th and 10th day in case of the spiked tomato and 4th and 8th day in case of the non-spiked one at AT and LT, respectively (Table 2). On the other hand, initiation of fungal spoilage was observed on 9th and 18th day for the spiked tomato and 6th and 14th day for the non-spiked one at AT and LT, respectively (Table 2). It showed that initiation of fungal spoilage in tomato was delayed by the MBC treatment to a greater extent at LT than that at AT. Moreover, percentage spoilage was found to be significantly ($p<0.01$) less in the spiked tomato than the non-spiked one on 10th and 22nd day of storage at AT and LT, respectively (Table 2).

Storage life: Although skin shrivelling set in earlier than initiation of fungal decay, storage life of tomatoes and apples were determined by initiation of fungal spoilage and the appearance of black spot on skin as an indication, respectively. Both the spiked and the non-spiked apples showed significantly ($p<0.01$) longer storage life than the spiked and the non-spiked tomato, respectively at AT as well as LT (Table 2). Furthermore, the spiked tomato were found to have a storage life of 9 and 18 days at RT and LT respectively, which were significantly ($p<0.01$) greater than the respective storage life of the non-spiked tomato (Table 2). It showed that dip treatment with MBC extended the storage life of tomato significantly ($p<0.01$) at both the storage temperatures. Use of benomyl or MBC as a post-harvest treatment for extension of storage life of a number of fruits and vegetables was reported earlier (Abu-Baker and Abdul-Karim, 1994; Amadioha, 1996, 1998; Goulart *et al.*, 1992; Jahangir *et al.*, 1994; Nwufo *et al.*, 1994; Papadopoulou-Mourkidou, 1991; Rathu *et al.*, 1989; Sanderson, 1999; Sharma *et al.*, 1989; Waskar *et al.*, 1999a, 1999b).

Cumulative physiological loss in weight (CPLW): Percentage CPLW was found to be significantly ($p<0.01$) greater in tomatoes than in apples at both the storage temperatures. Values of CPLW were more at AT than at LT for both the spiked and the non-spiked tomatoes and apples on the same days of storage (Table 2). Physiological loss in weight in fruits and vegetables is due to respiration and evapo-transpiration, which in turn depends on atmospheric temperature and humidity and number of lenticels or stomata per unit area of the fruits and vegetables. Furthermore, the treated samples of both tomatoes and apples showed significantly ($p<0.01$) lower % CPLW than the corresponding non-treated sample during the course of storage at both the temperatures (Table 2).

Moisture content (wet weight basis): Percent moisture content of the treated and the control tomatoes was 95.03±0.49, which decreased significantly ($p<0.01$) after 4 and 8 days of storage at

Table 1. Uptake of MBC residue by different fruits and vegetables

Samples	Weight of Sample (g)			Volume of MBC suspension (375 ppm) ml			Concentration of MBC uptake (ppm)	Amount of MBC used mg	Uptake of MBC by various fruits and vegetables			Average recovery extraction (%)
	Before spiking	After spiking	Gain in weight (%)	Before spiking	After spiking (%)	Gain in weight			µg/100 sample	µg/100 µg MBC	µg/100 ml MBC solution	
Tomato	1503.1±1.6	1509.1±2.1	0.41±0.02^{a}	2000±0.00	1979.2±1.2	1.04±0.05^{a}	375±0.00	276.55±4.65^{d}	18.40±0.31^{d}	0.0369±0.0006^{d}	13.82±0.23^{d}	
Apple	1519.6±2.4	1531.7±2.9	0.79±0.03^{cd}	2000±0.00	1961.4±1.9	1.93±0.09^{d}	375±0.00	68.97±2.89^{a}	4.54±0.19^{a}	0.0092±0.0004^{a}	3.45±0.14^{a}	
Carrot	1507.8±0.8	1531.2±1.3	1.55±0.09^{f}	2000±0.00	1946.2±2.3	2.69±0.12^{e}	375±0.00	813.64±11.46^{i}	53.96±0.76^{i}	0.1085±0.0015^{i}	40.68±0.57^{i}	
Okra	1500.8±0.5	1518.6±1.0	1.19±0.11^{e}	2000±0.00	1962.6±1.7	1.87±0.09^{d}	375±0.00	303.44±4.03^{e}	20.22±0.27^{e}	0.0405±0.0005^{e}	15.19±0.20^{e}	
Orange	1514.2±2.2	1528.0±2.7	0.91±0.10^{o}	2000±0.00	1960.3±3.0	1.98±0.13^{d}	375±0.00	189.41±3.44^{c}	12.51±0.23^{c}	0.0253±0.0005^{c}	9.47±0.17^{c}	79.39
Grapes$^{\&}$	1501.6±0.2	1510.8±0.6	0.61±0.04^{b}	2000±0.00	1974.4±1.5	1.28±0.08^{b}	375±0.00	328.63±3.09^{f}	21.89±0.21^{f}	0.0438±0.0004^{f}	16.43±0.15^{f}	
Grapes$^{\&\&}$	1500.5±0.2	1511.2±0.5	0.71±0.04^{c}	2000±0.00	1970.5±1.4	1.47±0.09^{bc}	375±0.00	342.55±3.41^{g}	22.83±0.23^{g}	0.0457±0.0005^{g}	17.13±0.17^{g}	
Sapota	1511.9±1.5	1533.7±1.9	1.44±0.09^{f}	2000±0.00	1942.7±2.6	2.86±0.15^{e}	375±0.00	671.93±9.91^{h}	44.44±0.66^{h}	0.0896±0.0013^{h}	33.60±0.50^{h}	
Banana	1518.5±2.4	1536.1±2.5	1.16±0.06^{e}	2000±0.00	1968.1±1.1	1.59±0.06^{c}	375±0.00	84.06±3.12^{b}	5.54±0.21^{b}	0.0112±0.0004^{b}	4.20±0.16^{b}	
Mean	1508.67	1523.38	0.97	2000	1962.82	1.86	375	342.13	20.70	0.0456	17.11	

Mean ± Standard Deviation values with different superscripts a, b, c,….. differ significantly (p<0.01). &=pale to green, &&=blue black

Table 2. Effect of MBC treatment, storage period and temperature on CPLW(%), physical change or spoilage and storage life of tomato and apple

Sample	Storage temp*	Percent CPLW on storage at different temperature on different days										Physical change and fungal spoilage	Storage life(Day)**
		2	4	6	8	12	16	20	24	28	32		
Spiked tomato	AT	3.28±0.07^{c}	5.76±0.03^{i}	8.59±0.07^{k}	10.95±0.14^{q}	ND	ND	ND	ND	ND	ND	Skin shrivelling started on 5th day; spoilage started on 9th day and 38.5 % (weight basis) spoiled on 10th day.	9^{B}
	LT	1.33±0.00^{a}	2.31±0.06^{g}	3.14±0.03^{c}	4.02±0.04^{p}	5.04±0.05^{t}	5.87±0.02^{u}	6.41±0.04^{j}	ND	ND	ND	Skin shrivelling started on 10th day; fungal spoilage started on 18th day; 8.8 % (weight basis) spoiled on 22nd day.	18^{E}
Non-spiked tomato	AT	3.84±0.02^{d}	6.69±0.06^{j}	9.31±0.06^{l}	11.78±0.19^{r}	ND	ND	ND	ND	ND	ND	Skin shrivelling started on 4th day; fungal spoilage started on 6th day; 82.1 % (weight basis) spoiled on 10th day.	6^{A}
	LT	1.98±0.05^{b}	2.81±0.03^{i}	3.71±0.07^{d}	4.63±0.06^{o}	5.99±0.04^{u}	6.79±0.04^{j}	7.31±0.08^{y}	ND	ND	ND	Skin shrivelling started on 8th day; fungal spoilage started on 14th day; 89.6 % (weight basis) spoiled on 22nd day.	14^{C}
Spiked apple	AT	ND	1.98±0.01^{b}	ND	3.76 ±0.05^{d}	4.47±0.07^{o}	6.02±0.16^{u}	8.30±0.11^{k}	ND	ND	ND	Skin shrivelling started on 10th day; black spot appeared on 16th day.	16^{D}
	LT	ND	0.69±0.00^{e}	ND	1.34±0.02^{a}	1.61±0.02^{s}	2.41±0.03^{v}	2.78±0.01^{i}	3.10±0.02^{c}	3.37±0.05^{c}	3.72±0.05^{d}	Skin shrivelling started on 30th day; black spot appeared on 35th day.	35^{G}
Non-spiked apple	AT	ND	2.13±0.04^{g}	ND	4.17±0.02^{n}	5.11±0.00^{t}	7.86±0.06^{x}	9.37±0.13^{l}	ND	ND	ND	Skin shrivelling started on 10th day; black spot appeared on 14th day.	14^{C}
	LT	ND	0.78±0.02^{f}	ND	1.48±0.00^{m}	1.98±0.04^{b}	2.55±0.05^{w}	2.94±0.01^{z}	3.24±0.04^{c}	3.68±0.04^{d}	4.03±0.02^{p}	Skin shrivelling started on 30th day; black spot appeared on 32nd day.	32^{F}

AT - Ambient temperature (32±2°C), LT - Low temperature (7±2°C), ND - Not done*Mean ± Standard Deviation values with different superscripts a, b, c,….. differ significantly (p<0.01).
**Mean values with different superscripts A, B, C,…..vary significantly (p<0.01).

Table 3. Effect of MBC treatment, storage period and temperature on percent moisture content and acidity of tomato

Samples	Storage Temperature	Storage period (days) 0	4	8	12	16	24
Percent moisture content (wet weight basis)							
Spiked tomato	AT	95.03±0.49^{Aa}	92.06±0.26^{Bbc}	91.14±0.31^{CFc}	91.02±0.20^{Cc}	ND	ND
	LT	95.03±0.49^{Aa}	93.65±0.40^{Dabc}	92.77±0.43^{Deb}	92.41±0.23^{EHb}	92.19±0.27^{EHb}	92.02±0.31^{EHbc}
Non-spiked tomato	AT	95.03±0.49^{Aa}	91.88±0.29^{EFbc}	90.79±0.28^{Gc}	90.63±0.34^{Gc}	ND	ND
	LT	95.03±0.49^{Aa}	93.42±0.38^{Dab}	92.69±0.24^{DEab}	92.26±0.19^{EHb}	91.94±0.27^{FHbc}	91.78±0.22^{Hbc}
Percent titratable acidity as citric acid (dry weight basis)							
Spiked tomato	AT	7.63±0.24^{Aa}	9.38±0.32^{Bbc}	10.07±0.42^{BCbc}	10.46±0.36^{Cc}	ND	ND
	LT	7.63±0.24^{Aa}	7.88±0.19^{Aa}	8.19±0.17^{ADFa}	8.59±0.21^{DEFa}	8.91±0.29^{BEFb}	9.45±0.25^{BFbc}
Non-spiked tomato	AT	7.63±0.24^{Aa}	9.56±0.24^{BCbc}	10.43±0.30^{Cc}	10.66±0.44^{Cc}	ND	ND
	LT	7.63±0.24^{Aa}	7.96±0.26^{ADa}	8.28±0.18^{DFa}	8.71±0.33^{BFGa}	9.06±0.20^{FBb}	9.52±0.32^{BGbc}

Table 4. Effect of MBC treatment, storage period and temperature on lycopene and ascorbic acid contents of tomato

Samples	Storage temp	Storage period (days) 0	4	8	12	16	24
Lycopene content (mg per 100 g dry weight)							
Spiked tomato	AT	34.41±3.22^{Aa}	85.77±4.66^{BHcd}	106.79±3.51^{Cef}	119.60±3.56^{DFfghi}	ND	ND
	LT	34.41±3.22^{Aa}	44.88±3.31^{Eab}	79.81±2.07^{Bc}	105.93±3.95^{Cef}	116.90±2.69^{Dfgh}	127.19±3.51^{Fghi}
Non-spiked tomato	AT	34.41±3.22^{Aa}	108.50±4.68^{Cdefg}	117.92±2.50^{Dfgh}	131.59±4.59^{Gh}	ND	ND
	LT	34.41±3.22^{Aa}	49.10±3.19^{Ea}	94.12±2.60^{Hde}	126.49±3.36^{FGgh}	129.53±2.55^{FGhi}	132.24±3.60^{Fgi}
Ascorbic acid content (mg per 100 g dry weight)							
Spiked tomato	AT	423.46±6.31^{Aa}	264.11±4.75^{Bc}	188.21±3.29^{Cf}	151.09±2.87^{Dg}	ND	ND
	LT	423.46±6.31^{Aa}	379.30±4.84^{Eb}	291.56±3.64^{Fe}	262.19±3.95^{Bc}	218.51±2.44^{Gh}	172.17±2.26^{Hi}
Non-spiked tomato	AT	423.46±6.31^{Aa}	239.69±3.78^{Id}	181.33±4.04^{Cf}	150.66±3.12^{Dg}	ND	ND
	LT	423.46±6.31^{Aa}	371.92±5.07^{Eb}	287.84±3.20^{Fe}	247.16±3.17^{Icd}	209.46±2.70^{Jh}	169.28±2.42^{Hi}

AT - Ambient temperature (32±2°C), LT - Lowl temperature (7±2°C)

ND - Not doneMean ± Standard Deviation values with different superscripts a, b, c,….at p<0.01 & A, B, C, ….at p<0.05 differ significantly

AT and LT, respectively. There was no significant difference in % moisture content of the treated tomato from the corresponding untreated control after any days of storage at both the storage temperatures (Table 3).

Titratable acidity as citric acid (g per 100 g dry weight): Titratable acidity of the treated as well as non-treated tomatoes was 7.63±0.24 g per 100 g dry weight, which increased significantly (p<0.01) after 4 and 16 days of storage at AT and LT, respectively (Table 3). This was supported by the fact that tomato is a climacteric fruit (Burton, 1982), where respiration increases first and then decreases at later stages during ripening (Wills *et al.,* 1981). Organic acids too increase during storage of such fruit resulting in increased titratable acidity. It showed no significant (p<0.01) variation from that of the untreated control during storage for any days studied at both temperatures in case of the treated tomato (Table 2).

Ascorbic acid content (mg per 100 g dry weight): Ascorbic acid content of both the treated and the untreated control tomatoes decreased significantly (p<0.01) even after 4 days of storage at AT as well as LT (Table 4). There was no significant variation in the ascorbic acid content of the treated and the untreated tomatoes on any day of storage, carried out at both the temperatures, except 4 days of storage at AT, wherein the samples showed significant (p<0.01) difference in the ascorbic acid content (Table 4).

Lycopene content (mg per 100 g dry weight): Lycopene content of both the treated and the untreated tomatoes was 34.41±3.22 mg per 100 g dry weight, which increased significantly (p<0.01) after 4 and 8 days of storage at AT and LT, respectively (Table 4). The increase in the lycopene content during storage of the treated and the untreated tomatoes was significantly (p<0.01) greater at AT than LT upto 8 days of storage, subsequent to which the difference was insignificant (Table 4). This finding was confirmed from the earlier studies, which showed that lycopene content of stored tomatoes at AT was higher than that at LT (Ajlouni *et al.*, 2001; Hamauzu *et al.*, 1998). The treated sample had non-significantly lower lycopene content than the untreated control during storage in most of the cases, except LT storage for 8 and 12 days, which was significant (p<0.01) (Table 4).

Uptake of MBC residue (µg per g sample) was lowest in apple followed by banana, orange, tomato, okra, grape (golden) grape (blue black) and sapota. Low level of uptake of MBC residue in some fruits and vegetables was probably due to presence of waxy layer or characteristic features of peels. Dip treatment with MBC suspension was able to prolong to a greater extent the storage life of tomato than that of apple and at ambient temperature than that at chill temperature. The dip treatment of tomato decreased percent cumulative physiological loss in weight significantly during storage. Therefore, post-harvest controlled MBC treatment of selected fruits and vegetables would result in significant increase in their shelf-life at room temperature, and could be beneficial as the residues remain below tolerance limits.

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