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Residues of Acephate and its Metabolite Methamidophos in/on Mango Fruit (*Mangifera indica* L.)

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Abstract Mango, the major fruit crop of India is affected by stone weevil, which can cause serious damage to the fruits. Acephate gives good control of mango stone weevil. Residues of acephate and its major metabolite, methamidophos were evaluated on mango fruits following repeated spray applications at the recommended dose (0.75 kg a.i. ha^{-1}) and double the recommended dose (1.5 kg a.i. ha^{-1}). Acephate residues mostly remained on the fruit peel which persisted up to 30 days. Movement of residues to the fruit pulp was detected after 1 day of application, increased to maximum of 0.14 and 0.26 mg kg^{-1} after 3 days and reached to below detectable level (BDL) after 20 days. Methamidophos, a metabolite of acephate, was detected from 3rd day onwards in both peel and pulp and persisted up to 15 days. The residues (acephate + methamidophos) dissipated with the half-life of 5 days in peel and pulp. A safe pre-harvest interval of 30 days is recommended for consumption of mango fruits following treatment of acephate at the recommended dose of 0.75 kg a.i. ha^{-1}.

Keywords Acephate · Methamidophos · Mango · Pre-harvest interval

Mango (*Mangifera indica*), is the leading fruit crop of India occupying 22% of the total area under fruit crops, comprising 1.2 million hectares (NABARD 2003). India ranks first among world's mango producing countries accounting for about 50% of the global mango production. Mango crop is affected by a number of pests and diseases, especially during the fruiting season. The major pests are hopper, mealy bug, inflorescence midge, fruit fly, scale insect, shoot borer, leaf webber, stone weevil etc. These insects infest the crop during flowering and fruiting periods causing severe damage to the crop. Mango stone weevil (MSW), *Sternochetus mangiferae* Fabricius (Coleoptera : Curculionidae), is a widely distributed direct monophagous pest of mango, causing substantial loss of fruits estimated to be between 5% and 80%, both as percentage infestation of harvested fruit and by the enhanced premature drop of infested fruit from the tree (Verghese 1998). The best way to manage MSW is by chemical treatment and acephate (O,S-dimethyl acetylphosphoramidothioate) is one such chemical which is used for control of stone weevil of mango (Verghese et al. 2005). Methamidophos (O,S-dimethyl phosphoramidothioate), a toxic metabolite of acephate, is a widely used organophosphorus insecticide with systemic properties and is effective against chewing and sucking insects. Methamidophos is much more toxic than acephate. The acute dermal toxicity for methamidophos DL_{50} in rats is 130 mg kg^{-1} which that of acephate DL_{50} is 2,000 mg kg^{-1} (Tomlin 1995). Acephate residue in vegetables too has been attracting much attention because of its metabolite, methamidophos, which may be hazardous to human health (Fen and Li 2003). This experiment was therefore carried out to evaluate the residue persistence of both acephate and methamidophos in/on mango and their residue level in mature mango fruits and in soil after harvest.

Materials and Methods

Acephate (purity, 96%) and methamidophos (purity, 98.4%) was procured from Sigma–Aldrich and its

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formulation Acephate 75 WP was obtained from market. All reagents and solvents used were of analytical grade. Standard solutions of acephate and methamidophos were prepared with acetone and suitably diluted to obtain the working standards.

The study was carried out at the Experimental Farm of Indian Institute of Horticultural Research, Bangalore, India, using a mango variety, Totapuri. For each treatment 5 trees were selected. Repeated spray application of acephate (Acephate 75 WP) was given at recommended (0.75 kg a.i. ha^{-1}) and double the recommended dose (1.5 kg a.i. ha^{-1}) to mango trees. Untreated control trees were sprayed with water. The spray volume used was 1,000 L ha^{-1}. The first foliar spray application was given at flowering stage, the second at lime size of the mango fruits. The third (last) application was given 1 month before harvest of mature mango fruits. The treatments were carried out in such a way that a set of trees received only one spray (at flowering stage only), another set received 2 sprays (at flowering and lime size of the fruits) and the third one received 3 sprays (at flowering, lime size and 1 month before harvest). Periodic analysis of mango samples was carried out at 0 (1 h), 1, 3, 5, 7, 10, 15, 20, 25 and 30 days (harvest) from trees which received 3 spray applications. Harvest time residues were evaluated in mature mango fruits and soil from all treatments, i.e. from trees which received only 1 spray, 2 sprays and also 3 sprays.

On every sampling day mango fruits from all the five trees (total 2.5 kg) were collected and brought to the laboratory in plastic covers. The fruits were peeled, the peel and pulp samples were cut into small pieces. The samples were mixed thoroughly and a representative 50 g pulp or 25 g peel sample (in triplicate) was taken for analysis. The samples were extracted with 50 mL of ethyl acetate by blending in a Waring blender. The extract was filtered through a Buchner funnel under vacuum and the process was repeated twice with 75 mL of solvent. The combined extracts were diluted with 30 mL water, the ethyl acetate layer was collected, dried over anhydrous sodium sulphate and concentrated to 5 mL. Clean-up of the samples were carried out with silica gel as the adsorbent. In a glass column silica gel (15 g) was loaded between two 1 cm layers of sodium sulphate and the column was pre-washed with ether. The concentrated extracts were passed through the chromatographic column and acephate along with methamidophos residues eluted with 100 mL of methanol + ether (1:9, v/v) mixture, which was concentrated to dryness and redissolved in 5 mL methyl isobutyl ketone for analysis by Gas Liquid Chromatography (GLC). At harvest mature mango fruits were collected from all the treatments and processed as described above. Soil samples were collected from all treatments (5 kg each), air-dried, passed through 2 mm sieve. A representative 100 g sample was processed in a similar manner as mango fruit without any cleanup. Recovery study of acephate and methamidophos in mango fruits was carried out before carrying out analysis of field samples. A 25 g portion of blended mango peel and 50 g pulp sample in 5 replicates were fortified with both acephate and methamidophos at 0.01, 0.02, 0.05 and 0.10 mg kg^{-1} levels. Soil samples (100 g) in 5 replicates were also fortified at 0.01, 0.02, 0.05 and 0.10 mg kg^{-1} levels of acephate and methamidophos.

The residues of acephate and its metabolite methamidophos in methyl isobutyl ketone were estimated by GLC (Varian 3800) equipped with a thermoionic specific detector (TSD) and fitted with a capillary column (fused silica 30 m × 0.25 mm i.d.). The column, injector and detector temperatures were maintained at 160, 280 and 300°C, respectively. The carrier gas (nitrogen) flow rate was 1 mL min^{-1}, hydrogen flow rate was 3.0 mL min^{-1} and air flow at 175 mL min^{-1}. Under these parameters, the retention time of methamidophos was 3.07 min and that of acephate was 6.26 min. The limit of quantification (LOQ) of the method for both acephate and methamidophos was 0.01 mg kg^{-1}. The residue data was subjected to statistical analysis according to Hoskins (1961) to compute the residual half-life ($t_{1/2}$) and safe pre-harvest interval (PHI).

Results and Discussion

Fortification of mango fruits with acephate and methamidophos showed that recovery of acephate was in the range of 81.55–85.40% from mango peel, 84.29–87.32% in mango pulp and 90.36–92.26% from soil; methamidophos recovery from mango peel was 82.66–84.72%, mango pulp 84.12–86.41% and from soil 89.75–91.44%. The LOQ of the method was 0.01 mg kg^{-1} (Table 1).

Acephate spray application was given to mango trees at flowering, lime size of the fruits and 1 month before harvest. Periodic analysis of mango fruits which received 3 applications of acephate 75 WP @ 0.75 and 1.5 kg a.i. ha^{-1} was carried out to estimate residues of acephate and methamidophos in raw mango fruits. Initial residue deposit of 0.68 and 1.109 mg kg^{-1} of acephate was recovered in mango peel from treatment at the recommended and double the recommended dose, respectively (Table 2). Dissipation of residues from the fruit surface was very fast in the initial stages, with more than 80% residues being lost in the initial 7 days from both the treatments. Further residue dissipation was very slow and it reached the LOQ level of 0.01 mg kg^{-1} by 30 days (harvest stage of mature mango fruits) from treatment at recommended dose and 0.014 mg kg^{-1} from double dose. In fruit pulp acephate residues were detected 1 day after the last application, reached a maximum of 0.14 and 0.26 mg kg^{-1} after 3 days and reached below detectable limit on 20 days and 25 days

Table 1 Recovery study of acephate and methamidophos residues in mango at various fortification levels

Fortified concentration (mg kg^{-1})	Average residues recovered (%) ± SD*					
	Acephate			Methamidophos		
	Mango peel	Mango pulp	Soil	Mango peel	Mango pulp	Soil
0.01	81.55 ± 3.07	84.29 ± 3.58	90.36 ± 2.64	82.66 ± 3.32	84.12 ± 4.03	89.75 ± 1.17
0.02	82.41 ± 1.38	84.54 ± 3.63	91.05 ± 2.41	83.52 ± 2.08	84.47 ± 1.96	89.94 ± 2.68
0.05	84.56 ± 1.44	85.66 ± 4.02	91.78 ± 1.73	83.46 ± 1.12	85.02 ± 4.52	90.56 ± 1.06
0.10	85.40 ± 2.62	87.32 ± 1.98	92.26 ± 1.85	84.72 ± 4.70	86.41 ± 4.18	91.44 ± 2.33

* Average of 5 replicates

Table 2 Residues of acephate and methamidophos in/on mango fruit peel and pulp following application of acephate 75 WP at 0.75 and 1.5 kg a.i. ha^{-1}

Days after application	Residues of acephate recovered (mg kg^{-1})*							
	Mango peel				Mango pulp			
	Acephate		Methamidophos		Acephate		Methamidophos	
	A1	A2	M1	M2	A1	A2	M1	M2
0 (1 h)	0.684	1.109	BDL	BDL	BDL	BDL	BDL	BDL
1	0.561 (18.0)	0.981 (11.5)	0.015	0.025	0.020	0.050	BDL	BDL
3	0.324 (52.6)	0.580 (47.7)	0.027	0.042	0.141	0.260	BDL	0.011
5	0.153 (77.63)	0.289 (73.9)	0.046	0.074	0.082	0.140	0.04	0.07
7	0.103 (84.9)	0.201 (81.8)	0.027	0.051	0.06	0.088	0.03	0.05
10	0.078 (88.6)	0.136 (87.7)	0.020	0.036	0.041	0.071	0.02	0.04
15	0.037 (94.6)	0.079 (92.8)	BDL	0.015	0.031	0.056	BDL	0.016
20	0.025 (96.3)	0.050 (95.5)	BDL	BDL	BDL	0.021	BDL	BDL
25	0.018 (97.3)	0.031 (97.2)	BDL	BDL	BDL	BDL	BDL	BDL
30 (harvest)	0.01 (98.3)	0.014 (98.7)	BDL	BDL	BDL	BDL	BDL	BDL

* Average of 3 replicates, BDL-Below detectable level, <0.01 mg kg^{-1}

A1—Acephate residues from treatment at 0.75 kg a.i. ha^{-1}

A2—Acephate residues from treatment at 1.5 kg a.i. ha^{-1}

M1—Methamidophos residues from treatment at 0.75 kg a.i. ha^{-1}

M2—Methamidophos residues from treatment at 1.5 kg a.i. ha^{-1}

Figures within the parenthesis are the percent dissipation of acephate residues

from treatment at recommended and double dosage, respectively. Acephate residues dissipated with the half-life of about 5 days in peel and pulp from both the treatments.

Methamidophos, the toxic metabolite of acephate, was detected in mango peel after 3 days, reached a maximum of 0.046 mg kg^{-1} after 5 days and reached BDL after 15 days from treatment at the recommended dose. The residues of methamidophos were 0.074 mg kg^{-1} after 5 days following treatment at double the recommended dose and the same were not detected in mango fruit (peel and pulp) 15 days after the last spray application was given (Table 2).

The pre-harvest interval or the time taken for the residues to reach the LOQ value of 0.01 mg kg^{-1} from treatment at recommended and double the recommended dose was 28 and 31 days, respectively. The residue levels of both acephate and methamidophos were evaluated in mature mango fruits which received either 1 or 2 spray applications. Mango fruits which received only 1 or 2 applications were free from residues of both acephate and methamidophos. These results showed that when 1 or 2 applications of acephate was given, mature mango fruits were free from any residues, but when 3 applications were given, the last being one month before harvest, some residues remained in the fruits at the time of harvest. Soil samples collected from tree basin from all the treatments at harvest were free from residues of both acephate and methamidophos.

In a study conducted earlier on behavior of acephate and methamidophos in apple fruits conversion of acephate to methamidophos and its movement to the fruit pulp was observed (Asensio et al. 1999). In the present study also a similar trend has been observed. Report on residue evaluation of acephate on citrus showed that significant systemic penetration occurred from the peel into the fruit flesh (Anonymous 1976). In the present study it was found that in the initial stages (up to 5 days) there was movement of acephate from peel to the pulp as well as formation of metabolite, methamidophos. Residues of acephate in pulp was maximum on the 3rd day and that of methamidophos on the 5th day. These results show that acephate residues penetrate into the fruit pulp and subsequently metabolize to methamidophos. Methamidophos formed 2.5% of acephate residues after 1 day of treatment, which increased to about 26% by 5th day of treatment at recommended and double the recommended dose. Further though acephate residues degraded faster, methamidophos residues formed nearly 25% of acephate residues until 15th day. After this period it reached below detectable level. Even in mango pulp methamidophos residue deposit was 50% of acephate residues from 5th to 10th day after which its concentration reduced. It was evident from the fact that 77.6% and 74.0% of the initial deposit of acephate has dissipated from the fruit peel by the 5th day from treatment at recommended and double the recommended dose, respectively. On the fruit peel metabolism of acephate was observed on the 1st day itself with some amount of residues remaining up to 30 days. These results show that when only 1 or 2 applications of acephate is given mango fruits at harvest are completely free from residues of acephate or methamidophos. Whereas if 3 applications are given, which may be required for successful control of mango stone weevil, residues remain on the fruits up to 30 days. Based on these results a 30 day pre-harvest interval is recommended for consumption of mango fruits.

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