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Extraction of Multi-Class Pesticide Residues in Mango Fruits (*Mangiferae indica* L.): Application of Pesticide Residues in Monitoring of Mangoes

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Mango (*Mangiferae indica* L.) is an important export horticultural crop of India. Various insects and diseases infest the fruit especially in the fruiting season. The common insect pests are mango leafhopper, mealy bug, leaf webber, inflorescence midge and fruit fly. The major loss of about 60% is due to leafhopper and mango leafhopper. These pests infest the mango at the floral and bud stage to fruit setting, tender leaves and small fruits. The crop protection measures undertaken to control pest infestation involves spray of insecticides along with biological and cultural practices.

India produces 65% of the world's mango crop, 9,000,000 MT, but with very little export. Although, Asia accounts for 75% world production, its dominance does not translate into international trade. The presence of pesticide remnants in mango lowers the export quality of mango fruits in the international market. To increase foreign trade, under the WTO regime, it is imperative to produce pesticide free mangoes.

The insecticides commonly applied by the farmers are endosulfan, parathion methyl, chlorpyrifos, cypermethrin and fenvalerate. This paper presents a method for the estimation of the multi-class pesticides in mango and their recovery.

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Materials and Method

Solvents like acetone, dichloromethane, hexane (analytical grade), were distilled before use. Adsorbents neutral alumina and Florisil were activated before use. Pesticide standards of methyl parathion, endosulfan, chlorpyrifos, cypermethrin and fenvalerate were of analytical grade quality. Gas Chromatogram instrumen-Shimadzu GC-17A fitted with an auto-sampler and ECD detector was used for analysis. Other minor equipments required were rotary evaporator and Waring blender, etc.

Individual stock standard solutions (1,000 μg/mL) of parathion methyl, endosulfan, chlorpyrifos, cypermethrin and fenvalerate were prepared in hexane (analytical grade). Working standard solutions of each pesticide was prepared by serial dilutions to 100 μg/mL, which was further diluted to 0.1–10 μg/mL as per detector response. All the stock solutions were stored at 4°C. Appropriate aliquot of individual pesticides solutions were taken and a mixture of five pesticides of 100 μg mL^{-1} concentration were prepared. Mixture solutions of lower concentrations were prepared by serial dilutions using hexane.

Limit of detection (LOD) of each pesticide, individually and in a mixture was determined by injecting standard solutions of different concentration levels in duplicate in the GLC. The lowest concentration of the pesticide that gave peak area five times that of background level was considered as LOD.

Mango fruit samples without peel (20 g, cut into small pieces) were fortified with the standard mixture at 0.5 μg/mL levels in ten sets each set in triplicate. One control was set-aside for each of the sets. The spiked mango samples were extracted in a Waring blender-Remi make with acetone 50 ml for 2–3 min. The solvent was

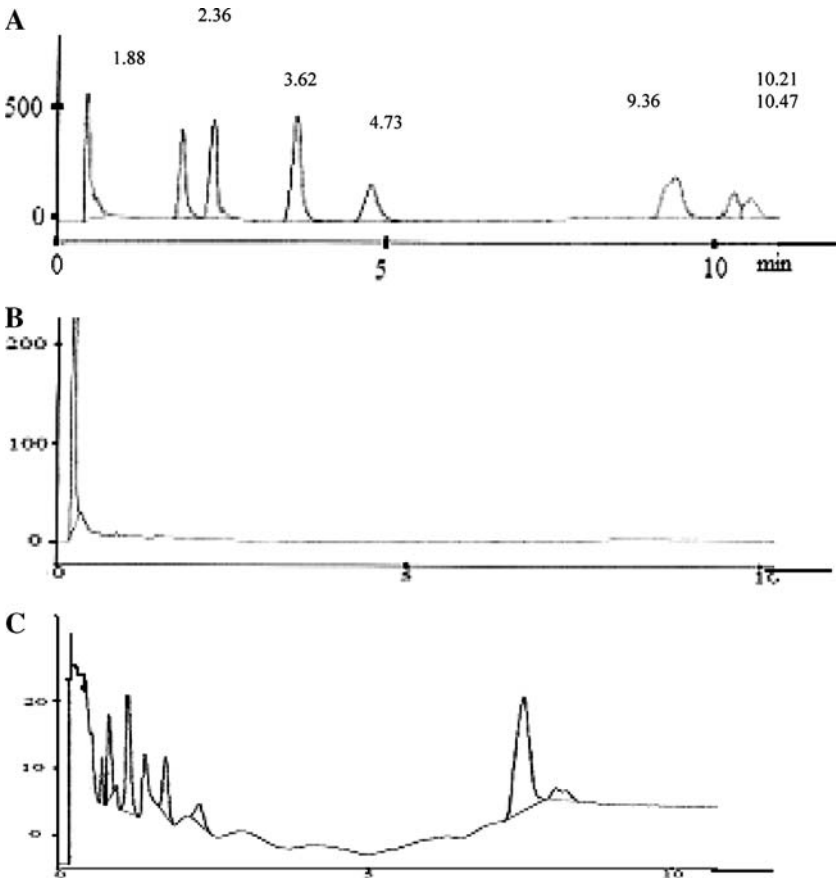

Fig. 1 GLC profile of standard mixture of pesticide: **A** methyl parathion (Rt-1.88 min), chlorpyrifos (Rt-2.36 min), α-endosulfan (Rt-3.62 min), β-endosulfan (Rt- 4.73 min), cypermethrin (Rt-9.36 min), fenvalerate (Rt-10.21 and 10.47 min). **B** Untreated mango sample. **C** Fortified mango sample

filtered through a Buchner funnel. The fruit residue was subjected to extraction with acetone 50 mL two more times. Similar process of extraction was carried out in triplicate with acetonitrile and ethyl acetate–hexane (8:2), separately.

The extracts from were evaporated under vacuum to about 5 ml and then transferred to a separatory funnel (500 ml). Saline water (2%, w/v, 150 ml) was added to it and the extract was exchanged into dichloromethane layer by liquid–liquid partitioning (3 × 50 ml). The extract was passed through a layer of sodium sulfate 5 gm and again evaporated to dryness in rota-vapor.

Glass columns (30 × 1.5 × 1.5 cm i.d.) were packed with anhydrous sodium sulfate (2 g) + neutral alumina (5 g) + Florisil (1 g) + anhydrous sodium sulfate (2 g). The column was prewashed with hexane. The concentrated extract was dissolved in 10 ml hexane–acetone (9:1) and subjected to column cleanup. The column was eluted with dichloromethane-acetone (8:2, 125 mL). Another set of column was packed as above and column eluted with ethyl acetate.

Similar glass columns were packed with neutral alumina (5 g) and Florisil (2 g) separately. After loading the sample the columns were separately eluted with dichloromethane-acetone (8:2, 125 mL) and ethyl acetate.

The pesticides were analyzed by GLC fitted with an electron capture detector. The column used was BP-5 (30m × 0.52 μm × 3 μ) and the oven temperature programmed from 220°C (6-min-hold time) @ 20°C/min 280°C (5-min-hold time). The injector and detector temperatures were set at 280 and 300°C, respectively.

Result and Discussion

The GC conditions were optimized to obtain distinct separate peaks of the five pesticides. The retention times of the methyl parathion, chlorpyrifos and cypermethrin and were 1.88, 2.36, and 9.36 min, respectively. Endosulfan separated giving two peaks at retention times 3.62 and 4.73 min for α-endosulfan and β-endosulfan and fenvalerate isomers eluted at 10.21 and 10.47 min, respectively (Fig. 1).

The fortified mango samples were extracted with different solvents to standardize the methodology. The solvents acetone and acetonitrile gave high recovery ranging from 78.6 to 92.2%, while mixed solvent like ethyl acetate–hexane gave percent recovery in the range 76.3–86.2 (Table 1).

Acetonitrile was found to be the most efficient single component solvent; that gave the best results among the

Table 1 Testing efficiency of solvents in extraction process for mango fortified at 0.5 mg/kg

Solvent	%Recovery ± SD				
	Methyl parathion	Chlorpyrifos	Endosulfan ($\alpha + \beta$)	Cypermethrin	Fenvalerate
Acetone	81.3 ± 1.12	87.6 ± 2.31	89.3 ± 2.31	78.6 ± 2.31	78.6 ± 6.21
Acetonitrile	88.2 ± 0.95	89.2 ± 1.21	92.2 ± 4.23	87.6 ± 3.11	83.3 ± 2.21
Ethyl acetate-hexane	79.3 ± 3.84	84.1 ± 2.54	86.2 ± 5.61	77.2 ± 4.52	76.3 ± 6.1

Table 2 Efficiency of recovery using different adsorbents

Solvent	%Recovery ± SD				
	Methyl parathion	Chlorpyrifos	Endosulfan ($\alpha + \beta$)	Cypermethrin	Fenvalerate
Alumina neutral	79.3 ± 3.22	85.3 ± 4.21	81.6 ± 3.54	78.6 ± 5.21	78.6 ± 4.31
Alumina + Florisil	88.2 ± 2.12	96.4 ± 3.22	98.2 ± 5.66	84.6 ± 2.35	80.3 ± 2.21
Florisil	74.3 ± 5.31	79.2 ± 2.13	76.1 ± 7.21	77.2 ± 8.66	73.3 ± 7.65

Table 3 Efficiency of recovery using different eluting solvents

Solvent	%Recovery ± SD				
	Methyl parathion	Chlorpyrifos	Endosulfan ($\alpha + \beta$)	Cypermethrin	Fenvalerate
Dichloromethane-acetone (8:2)	87.3 ± 1.13	95.3 ± 0.91	96.6 ± 0.32	90.6 ± 0.61	88.6 ± 0.84
Ethyl acetate	86.7 ± 1.52	89.1 ± 1.11	92.6 ± 1.71	83.5 ± 1.23	81.4 ± 1.41

solvents tested, recording percent recovery of 92.2 for endosulfan ($\alpha + \beta$), 89.2, 88.2, 87.6 and 83.3 for chlorpyrifos, methyl parathion, cypermethrin and fenvalerate, respectively. The extract from the above was subjected to column clean up over alumina-neutral, neutral alumina + Florisil and Florisil alone. The results indicated that Florisil alone was not effective as a clean-up reagent for the removal of the co-extractives (Table 2).

The nature of the adsorbent used for cleanup was found after comparison of recoveries. Higher percent recovery was recorded with the use of alumina + Florisil mixture, recording 98.2% recovery for endosulfan ($\alpha + \beta$), followed by 96.4% for chlorpyrifos and 88.2, 84.6 and 80.3%, respectively for methyl parathion, cypermethrin and fenvalerate. However, for fruits like apple, dry slurry with Florisil was able to yield efficient recoveries (Nakamura et al. 1993). Reports of column clean up using mini columns as used in this method were used for multi-residue analysis in vegetables (Ripley et al. 2001).

The column eluting solvent played a significant role in improving the percent recovery, a mixture of dichloromethane–acetone (8:2) gave higher recoveries in the range of 88.6–96.6% as compared to ethyl acetate, which recorded percent recoveries in the range of 81.4–92.6 (Table 3). Similar results have been reported in various vegetables[3]. Lower percent recoveries obtained with ethyl acetate may be attributed to the fact that complete removal of ethyl acetate before analysis by GLC proved to be tedious due to the presence of trace amount of acetic acid present in it.

The standardized method was validated by extraction of fortified mango samples with acetone, exchanging the concentrate into dichloromethane by liquid–liquid partitioning, and column clean up over neutral alumina + Florisil and eluting the column with dichloromethane–acetone. The results are given in Table 4, indicating that endosulfan ($\alpha + \beta$) gave highest recovery of 97.5%, with an RSD of 0.87%, followed by chlorpyrifos (96.2%), cypermethrin (88.8%), methyl parathion (88.6%) and fenvalerate (86.2%), respectively. There are reports of vegetables samples analysed using NPD detector (Ueno et al. 2003).

To validate the procedure developed for the estimation of pesticides in ripe mango fruits, monitoring of pesticides incurred samples was evaluated. Experiments were conducted in farmers' orchards to develop IPM package for pesticide free mango fruits under the NATP-World Bank project. Schedules prescribed under Integrated Pest Management were followed from the nursery stage.

The practices adhered by the local farmers in their mango orchards were taken as the non-IPM samples. The locations of the orchards adopted for IPM package were in Mahliahabad district near Lucknow, Uttar Pradesh. The

Table 4 Percent recovery of pesticides from mango

Matrix	Active ingredient	Fortification	Recovery ± SD	%RSD	Minimum detectable quantity MDQ μg	Limit of detection LOD μg
Mango	Methyl parathion	0.5	88.6 ± 3.21	2.95	0.02	0.03
Mango	Chlorpyrifos	0.5	96.2 ± 1.56	1.32	0.01	0.02
Mango	Endosulfan ($\alpha + \beta$)	0.5	97.5 ± 1.04	0.87	0.03	0.02
Mango	Cypermethrin	0.5	88.8 ± 7.00	6.43	0.03	0.04
Mango	Fenvalerate	0.5	86.2 ± 9.29	8.80	0.03	0.05

Table 5 Monitoring of pesticides in incurred samples

Pesticide	Average residues (mg/kg) in IPM mango			Average residues (mg/kg) in non-IPM mango		
Chlorpyrifos	ND	ND	ND	ND	ND	ND
Methyl parathion	ND	ND	ND	ND	ND	ND
α-endosulfan	ND	ND	0.04	ND	ND	ND
β-endosulfan	ND	ND	0.05	ND	ND	ND
Endosulfan sulfate	ND	ND	ND	ND	ND	ND
Cypermethrin	ND	ND	ND	ND	ND	ND
Fenvalerate	ND	ND	ND	ND	ND	ND

pesticide schedule was followed and the harvest time samples extracted, cleaned up and analyzed by GLC. The ripe mango fruit samples at were extracted for the presence of pesticides residues following the above protocol. The results are given in Table 5.

Mango fruit has many varieties and cultivars the water content and texture of mango fruit may vary depending on the varieties and cultivars, as well as on the maturity of fruit. The water content plays a crucial role in the recovery efficiency. This method can be applied successfully applied to assess the pesticide residues in monitoring of mango fruits (Kadenczki et al. 1992). The developed method is simple and specific for determination of mentioned five pesticides in mango fruit.

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