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Determination of Pesticide Residues in Integrated Pest Management and Nonintegrated Pest Management Samples of Apple (*Malus pumila* Mill.)

Shashi Bala Singh,* Irani Mukherjee, Jaya Maisnam, Praveen Kumar, Madhuban Gopal, and Gita Kulshrestha

Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110012, India

Studies were undertaken to analyze the residues of commonly used pesticides viz. chlorpyrifos, endosulfan, dicofol, cypermethrin, fenvalerate, propargite, malathion, phorate, carbendazim, carbosulfan, thiamethoxam, and mancozeb in apple of integrated pest management (IPM) and non-IPM samples collected from the IPM and non-IPM fields of Shimla. We also present a method for the determination of these pesticides in apple samples. Residues of chlorpyrifos, endosulfan, dicofol, cypermethrin, fenvalerate, and propargite were analyzed by gas chromatography, while residues of carbendazim, carbosulfan, and thiamethoxam were analyzed by high-performance liquid chromatography. Residues of mancozeb were determined by a colorimetric method. Recoveries of all of the pesticides ranged from 61.30 to 95.46% at 0.1, 0.2, and 1.0 μg g^{-1} levels of fortification with relative standard deviations ranging between 0.8 and 8.7. Apples from IPM and non-IPM orchards were analyzed for these pesticides using a developed method. Except for carbendazim and chlorpyrifos, the residues of all of the pesticides analyzed were below detectable limits. Although residues of carbendazim and chlorpyrifos were below the prescribed limits of maximum residue levels in both IPM and non-IPM orchards, residues were lower in apples from IPM orchards.

KEYWORDS: Apple; IPM; non-IPM; pesticide residues; MRL

INTRODUCTION

Apple (*Malus pumila* Mill.), a deciduous fruit, is mainly cultivated in the North West Hills Region of India, which are comprised of the states of Jammu and Kashmir, Himachal Pradesh (H.P.), and Uttar Pradesh, and in the North Eastern Hills Region in the states of Arunachal Pradesh, Nagaland, Meghalaya, and Manipur (*1*). Presently, a small quantity of apple produced in India is exported, mainly to Bangladesh and Sri Lanka.

Apples are delicious, easy to carry for snacking, low in calories, and a natural mouth freshener. Apples are a source vitamin A, vitamin C, calcium, phosphorus, iron, a good amount of potassium, and both soluble and insoluble fiber (*2*). Soluble fiber such as pectin actually helps to prevent cholesterol buildup in the lining of blood vessel walls, thus reducing the incident of atherosclerosis and heart disease. The insoluble fiber in apples provides bulk in the intestinal tract, holding water to cleanse and move food quickly through the digestive system. It is a good to eat apples with their skins. Almost half of the vitamin C content is just underneath the skin. Eating the skin also increases the insoluble fiber content. Most of an apple's fragrance cells are also concentrated in the skin, and as they ripen, the skin cells develop more aroma and flavor. As they are eaten raw and with the skin, the pesticide residue analysis in apple becomes more appropriate.

*To whom correspondence should be addressed. Tel: 91-011-25841390. E-mail: shashi.sbsagch@gmail.com.

Although there has been a 5−6-fold increase in apple production during the last 50 years, the productivity level is still very low (5.56 t/ha). Apple cultivation has received greater attention by the growers. In apple, about a dozen pests cause serious damage to the crops. The most important ones are San Jose scale, woolly apple aphid, root borer, blossom thrips, codling moth, and European red mite. Some of the diseases of apple are collar rot and white root rot diseases, apple scab, and powdery mildew. To sustain the quality and productivity of the apple crop, the use of pesticides has become indispensable in modern plant protection. To minimize the economic losses caused by diseases and the insect pests, farmers use fungicides and insecticides (*3*) such as malathion, chlorpyrifos, endosulfan, mancozeb, dicofol, carbendazim, and phorate at a rate of 0.5−1.5 kg ha^{-1}. Very recently, thiamethoxam has also been used. About 7% of the pesticides used in the country is consumed on fruits (*4*). Apples alone consume one-third of the total pesticides in agriculture/horticulture in H.P. In apple cultivation, the input of pesticides is inevitable due to attack of various pests in its growth period. Insect pest management (IPM) involves the judiciously combined application of more than one component such as synthetic insecticides/fungicides/bactericides, botanicals, biocontrol agents, or mechanical or cultural practices. Moreover, if synthetic pesticides are used, they are always need-based and obviously at the recommended rates of application. However, in non-IPM, many times, farmers may apply an excessive dose of pesticides by their own knowledge after observing the pest attack without knowing

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the detailed properties of the pesticide. This can result in undesirable residues. Second, all of the orchards at one location are not the supervised trials or IPM trials. More than 95% of orchards are considered non-IPM orchards in which the pest control is decided by the individual farmers. So, in this regard, it becomes essential to determine and compare the residue pattern of two such modules. As the fruits make the Indian diet more nutritive and are consumed fresh, they may carry toxic quantities of pesticide residues. The agreement on the application of Sanitary and Phytosanitary measures under the WTO regime has set the basic rules for food safety (*5*). Under such circumstances, even the pesticide residues in export-oriented crops will have to be kept below permissible levels to avoid resistance from importers abroad. Keeping in view such factors, IPM modules have been developed for apple and validated at a farmer's orchard. This study was designed to determine the pesticide residues in IPM and non-IPM samples of apples collected from Shimla.

MATERIALS AND METHODS

Chemicals. *Pesticide Standards*. Required analytical grade standard pesticides {chlorpyrifos (*O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate), endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine 3-oxide), cypermethrin [(*RS*)-α-cyano-3-phenoxybenzyl (1*RS*)-*cis*-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate], fenvalerate [(*RS*)-α-cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate], dicofol [2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol], propargite [2-(4-*tert*-butylphenoxy)cyclohexyl prop-2-ynyl sulfite], malathion [*S*-1,2-bis(ethoxycarbonyl)ethyl *O*,*O*-dimethyl phosphorodithioate], phorate (*O*,*O*-diethyl *S*-ethylthiomethyl phosphorodithioate), carbendazim [methyl *N*-(1*H*-benzoimidazol-2-yl)carbamate], thiamethoxam [(*EZ*)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine], carbosulfan [2,3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio)methylcarbamate], and mancozeb [manganese ethylenebis (dithocarbamate) (polymeric) complex with zinc salt] were supplied by various pesticide manufacturing companies based in India.

Solvents. Analytical grade solvents were glass distilled before use. All of the glassware was rinsed with acetone and dried in an oven at around 350 °C prior to use. For high-performance liquid chromatography (HPLC) mobile phase, gradient grade acetonitrile and HPLC grade water (Merck India Ltd.) were filtered through 0.2 and 0.45 μm membranes, respectively, before use.

Sorbents/Drying Agents. Neutral alumina was procured from Merck India Ltd. and activated by heating at 450 °C for 4 h before use. Anhydrous sodium sulfate, analytical reagent grade, was procured from Merck India Ltd., washed with acetone, and activated by heating at 450 °C for 4 h before use.

Instruments. *GC Instrument*. A Schimadzu 17A, gas chromatographic (GC) system equipped with an electron capture detector (ECD-Ni^{63}) and mega bore column (OV-1, 25 mm × 0.53 μm i.d.) was used throughout the study.

HPLC Instrument. A Hewlett-Packard HPLC instrument (series 1100) equipped with a degasser, quaternary pump, and diode array detector coupled with rheodyne injection system and a computer (model Vectra) was used. The stationary phase consisted of Lichrosphere on a RP-18 packed stainless steel column (250 mm × 4 mm i.d.). A chromatogram was recorded on a Window NT based HP Chemstation program.

UV Spectrophotometer. An Analytikjena UV/vis spectrophotometer, model Specord 200, was used. The absorbance was recorded on a Window 95-based WinASPECT, version 1.5 program.

Mixer Grinder. A 1.5 L capacity mixer with grinder (model Remi) was used for comminuting the apple fruits.

Preparation of Standard Solutions. An accurately weighed 10 mg amount of an individual analytical grade pesticide (chlorpyrifos, endosulfan, propargite, dicofol, cypermethrin, fenvelerate, malathion, and phorate) was dissolved in a 10 mL volumetric flask using hexane to prepare the standard stock solution to 1000 μg mL^{-1}. The standard solution of each pesticide was serially diluted to a lower concentration of 100 μg mL^{-1}. For multiresidue analysis of five pesticides by GC, a mixture of standard pesticide solution "A" was prepared by taking a 1 mL solution of each compatible (chlorpyrifos, endosulfan, dicofol, cypermethrin, and fenvalerate) pesticide (100 μg mL^{-1}) in a 10 mL volumetric flask and making the volume up to the mark with hexane. The standard mixture contained 10 μg mL^{-1} of each pesticide. This was further diluted serially with hexane to obtain 1.0, 0.1, and 0.01 μg mL^{-1} of solution to determine the limit of detection (LOD) of the instrument. Propargite, malathion, and phorate were prepared separately. For HPLC solutions of carbendazim, carbosulfan, and thiamethoxam (1000 μg mL^{-1}), each was prepared in acetonitrile and serially diluted to 100, 10, 5, 1, and 0.1 μg mL^{-1}. A standard solution of mancozeb (1000 μg mL^{-1}) was prepared in ethanol and serially diluted.

Analytical Procedures. *GC*. A mixture of five pesticides (chlorpyrifos, endosulfan, dicofol, cypermethrin, and fenvalerate) was analyzed under specific operational conditions of a temperature programming of oven −220 °C (5 min) to 20 °C/min to 280 °C (3 min), with a total run time 13 min. The carrier gas was high-purity nitrogen with a flow rate of 30 mL min^{-1}. The injector and detector temperatures were maintained at 280 and 300 °C, respectively. Propargite was also analyzed under the above conditions but individually. Malathion and phorate were analyzed under isothermal conditions at 180 and 160 °C oven temperatures, respectively. A 3 μL aliquot of standards of individual pesticides and mixture A and propargite, malathion, and phorate were injected into the GC using described conditions. The retention time along with the peak area of each pesticide was recorded.

HPLC. Acetonitrile:water (1:1) was used as the mobile phase with a flow rate of 1 mL min^{-1}. All of the samples were filtered through a 0.2 μm membrane (Millipore) using a Millipore filtration syringe system, and a 20 μL (loop capacity) volume was injected in HPLC. Absorbances were recorded on 286, 254, and 230 nm (wavelength) for carbendazim, thiamethoxam, and carbosulfan, respectively.

UV Spectrophotometery. Mancozeb was analyzed using a colorimetric method, and the absorbance was measured at a 435 nm wavelength.

Instrumental Detection Limit (IDL). The IDL is defined as the minimum concentration of pure pesticide that can be detected reliably by a GC or HPLC system under the standardized conditions of analysis. Standard solutions of pesticides and mixture were injected 10 times consecutively in GC and HPLC, respectively, and the IDL was calculated using the following formulas.

$$\text{IDL } (\mu \text{ g mL}^{-1}) = \text{SD} \times \text{St}_{95}$$

where SD = standard deviation and St_{95} = 2.262 (Student's *t* at the 95% confidence level). The calculated concentrations were finally verified by actually injecting the standard solution of same concentration of each pesticide into GC and HPLC.

Extraction and Cleanup. Precisely weighed apple samples were chopped thoroughly and blended with organic solvent or solvent mixture (100 + 100 + 50 mL) at a high speed for 2−3 min to extract the pesticides. The extract was further cleaned up prior to analysis by GC or HPLC.

GC Analysis. For mixture A of pesticides (chlorpyrifos, endosulfan, dicofol, cypermethrin, and fenvalerate) and propargite to be analyzed on GC, a precisely weighed apple sample (100 g) was extracted with 100 mL of solvent mixture (acetone:cyclohexane:ethyl acetate, 2:1:1). The mixture was allowed to stand for some time until a clear supernatant was obtained. The extract was filtered by suction filtration using a Buckner funnel and Whatman filter paper #1. The extraction process was repeated twice more using the same solvent mixture (100 + 50 mL). The combined filtrate was concentrated (2 mL) on a vacuum rotavapor at 45−50 °C. The residual solution was diluted with 20% NaCl solution (150 mL) and partitioned three times with dichloromethane (100 + 70 + 50 mL). The aqueous phase was discarded. The organic phase was dried by passing it through anhydrous Na_2SO_4 (10 g) and evaporating it to dryness using a vacuum rotavapor at 35−40 °C. The contents were redissolved in 5 mL of acetone:hexane (1:1) and loaded on a prewashed (hexane) glass column packed with neutral alumina (5 g) sandwiched between acetone-washed anhydrous Na_2SO_4 (2 g). The column was eluted using 150 mL of acetone:hexane (1:9). The collected eluate was concentrated and finally dried under vacuum on a rotavapor at 40−44 °C. The residue was reconstituted in 10 mL of acetone−hexane (1:9) for GC analysis. Malathion and phorate

were extracted with acetone (100 + 100 + 50 mL) and processed in a similar way.

HPLC Analysis. For pesticides to be analyzed on HPLC viz. carbendazim and carbosulfan, the apple sample (100 g) was extracted three times with acetone (100 + 100 + 50 mL). The sample was allowed to stand for some time until a clear supernatant was formed. Then, the extract was filtered by suction filtration. The combined filtrate was evaporated to minimum on a vacuum rotavapor at 45−50 °C. The extract was diluted with 20% NaCl solution (150 mL), the pH of the aqueous layer was made acidic using hydrochloric acid (0.1 N), and coextractives were removed by partitioning with *n*-hexane (50 + 50 + 30 mL). The hexane layer was discarded; the aqueous layer was made alkaline by adding 1 N NaOH and partitioned three times with dichloromethane (100 + 70 + 50 mL). The organic layer was dried by passing through anhydrous Na_2SO_4 (approximately 10 g) and evaporated to dryness using a vacuum rotavapor at 35−40 °C. Finally, the residue was redissolved in acetonitrile for HPLC analysis. Thiamethoxam was extracted with acetone (100 + 100 + 50 mL). The filtered extract was concentrated on a rotavapor, and the residue was diluted with 20% aqueous NaCl solution (150 mL). The mixture was extracted with hexane (50 + 50 + 30 mL) followed by dichloromethane (100 + 70 + 50 mL). The hexane layer was discarded, and the dichloromethane layer was dried over anhydrous Na_2SO_4 (10 g) and evaporated on a vacuum rotavapor at 35−40 °C. For HPLC analysis, the residue was dissolved in acetonitrile.

Colorimetric Analysis. For mancozeb estimation, dilute HCl along with stannous chloride was taken with fortified apple to evolve carbon disulfide (*6*), which was absorbed in two traps holding (a) 10% sodium hydroxide with benzene−water to trap hydrogen sulfide and other volatiles and (b) cupric acetate monohydrate and diethanolamine. After the volume was made up with ethanol, the absorbance was taken at 435 nm.

A calibration curve was made using pure carbon disulfide (2.5−50 μg in 25 mL of ethanol). Both untreated and fortified apple samples were analyzed for mancozeb. The LOD was 0.5 $\mu g\ g^{-1}$. Finally, apple samples from both IPM and non-IPM villages were analyzed following the procedure described above.

Recovery Experiment. For the recovery of five GC compatible pesticides (chloropyriphos, endosulfan, dicofol, cypermethrin, and fenvalerate), chopped apple samples (100 g) were fortified at a level of 0.1, 0.2, and 1.0 $\mu g\ g^{-1}$ and kept at room temperature for 4 h. The samples were extracted with acetone:cyclohexane:ethyl acetate (2:1:1) and then cleaned up with activated neutral alumina as described in the above section. Propargite was extracted by the same method but singly. Malathion and phorate each were extracted with acetone separately.

For HPLC compatible pesticides (carbosulfan, carbendazim, and imidacloprid), the apple sample (100 g) was fortified at a level of 0.1, 0.2, and 1.0 $\mu g\ g^{-1}$ and processed for extraction and cleaned up as described above. All of the experiments were carried out in triplicate along with unfortified controls. The decision for analysis of these pesticides was taken on the basis of a general survey from non-IPM farmers.

Estimated Method Detection Limit (EMDL). The EMDL is defined as the appropriate minimum concentration of pesticide that can be determined from a particular matrix by a particular method, depending upon the IDL of the instrument and the recovery of a pesticide by the described method, and it can differ from matrix to matrix.

The EMDL for each pesticide for apple matrix was calculated after checking the IDL and recovery as follows:

$$\text{EMDL}\ (\mu g g^{-1}) = \text{IDL} \times V \times 100/M \times \%R$$

where IDL = instrumental detection limit, V = final volume made, M = mass of the sample taken, and $\%R$ = percent recovery. The EMDL for each pesticide from the apple matrix by the described method is depicted in **Table 2**.

Collection and Storage of Apple Samples. IPM and non-IPM samples were collected with the help of Y. S. Parmar University of Horticulture and Forestry, regional station Mashobra, Shimla (*7*). Approximately 1 kg samples were collected from six to seven sampling points from individual treatment/replicates and kept in a refrigerator pending analysis.

Table 1. Mode of Analysis, Retention Time (RT), and IDL of Various Pesticides Used for Analysis of Apple Samples

sample no.	pesticides	mode of analysis	RT (min)	IDL ($\mu g\ mL^{-1}$)
1	chlorpyrifos	GC	3.023	0.008
2	phorate	GC	3.827	0.007
3	malathion	GC	5.244	0.007
4	endosulfan-α	GC	4.673	0.010
5	endosulfan-β	GC	6.200	0.010
6	propargite	GC	6.525	0.010
7	dicofol	GC	8.503	0.050
8	cypermethrin	GC	10.576	0.030
9	fenvalerate	GC	11.572	0.020
11	carbendazim	HPLC	3.803	0.050
12	carbosulfan	HPLC	4.801	0.020
13	thiamethoxam	HPLC	6.646	0.010
14	mancozeb	spectrophotometry		

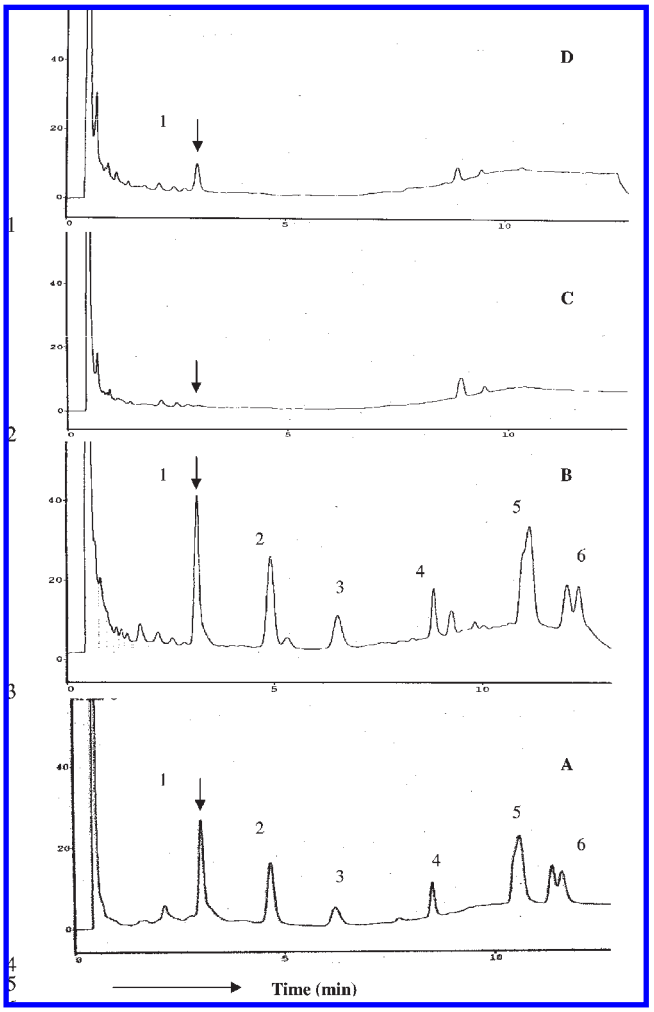

Figure 1. GC chromatograms showing recovery of various pesticides from apple: (**A**) standard mixture (1, chlorpyrifos; 2, α-endosulfan; 3, β-endosulfan; 4, dicofol; 5, cypermethrin; and 6, fenvalerate), 0.01 $\mu g\ mL^{-1}$; (**B**) fortified apple; (**C**) control apple; and (**D**) apple sample showing chlorpyrifos.

IPM Module. *Dorment Season Module: with effect from (w.e.f.) October to March*

1. Use of 5% urea (nonchemical method to promote decomposition and build up of antagonists against apple scab and premature leaf blotch).
2. Use of oils and soil-drenching methods against insect pests, viz. San Jose scale, wooly apple aphid, and the eggs of the red spider mite.

Table 2. Percent Recovery and EMDL of Pesticides from Apple

sample no.	pesticide	level of fortification (μg g^{-1})	amount recovered ± SD[a] (μg g^{-1})	recovery (%)	average % recovery (RSD[b])	EMDL (μg g^{-1})
1	chlorpyrifos	0.1	0.075 ± 0.006	75.0	80.3 (5.1)	0.005
		0.2	0.156 ± 0.005	78.0		
		1.0	0.880 ± 0.046	88.0		
2	phorate	0.1	0.069 ± 0.005	69.0	71 (1.3)	0.003
		0.2	0.150 ± 0.009	71.5		
		1.0	0.725 ± 0.070	72.5		
3	malathion	0.1	0.060 ± 0.012	60.0	61.3 (1.1)	0.004
		0.2	0.122 ± 0.006	61.0		
		1.0	0.632 ± 0.052	63.2		
4	endosulfan-α	0.1	0.086 ± 0.025	86.0	91.2 (4.26)	0.004
		0.2	0.180 ± 0.015	90.0		
		1.0	0.976 ± 0.043	97.6		
5	endosulfan-β	0.1	0.0824 ± 0.030	82.4	95.46 (8.7)	0.005
		0.2	0.100 ± 0.015	100.0		
		1.0	0.890 ± 0.116	104.0		
6	propargite	0.1	0.0838 ± 0.008	83.8	85.0 (1.5)	0.012
		0.2	0.140 ± 0.004	84.0		
		1.0	0.815 ± 0.086	87.3		
7	dicofol	0.1	0.093 ± 0.008	93.0	94.46 (1.28)	0.005
		0.2	0.162 ± 0.010	94.0		
		1.0	0.841 ± 0.010	96.4		
8	cypermethrin	0.1	0.0882 ± 0.010	88.2	89.26 (0.88)	0.006
		0.2	0.145 ± 0.002	90.6		
		1.0	0.750 ± 0.004	89.0		
9	fenvalerate	0.1	0.081 ± 0.012	81.0	81.8 (0.8)	0.006
		0.2	0.166 ± 0.005	83.0		
		1.0	0.814 ± 0.040	81.4		
11	carbendazim	0.1	0.0714 ± 0.005	71.4	74.86 (4.22)	0.009
		0.2	0.144 ± 0.002	72.0		
		1.0	0.812 ± 0.013	81.2		
12	carbosulfan	0.1	0.0738 ± 0.006	73.8	79.93 (4.08)	0.012
		0.2	0.164 ± 0.005	82.0		
		1.0	0.840 ± 0.024	84.0		
13	thiamethoxam	0.1	0.0802 ± 0.001	80.2	83.06 (3.28)	0.06
		0.2	0.162 ± 0.002	81.0		
		1.0	0.880 ± 0.018	88.0		
14	mancozeb	0.2	0.1388 ± 0.08	69.4	72.2 (2.13)	0.35
		1.0	0.750 ± 0.1	75.0		

[a] Average of three replicates; SD, standard deviation. [b] Mean of three concentrations; RSD, relative standard deviation.

3. Soil solarization and drenching with chemical pesticides against root rot and root borer of apple to break the life cycle of pathogens and over wintering stages of insect pest populations.

Spring Season Module: w.e.f. April to June

1. Protective sprays of fungicides against apple scab, premature leaf fall, and powdery mildew.
2. Applying bioagents against powdery mildew of apple at pink bud to blooming stages to avoid chemicals and their harmful effects on bees and other pollinating agents.
3. Encouragement of natural predators against San Jose scale, apple wooly aphid, and red spider mites to reduce the secondary inoculums and insect pest populations and need-based use of chemicals.

Summer Season Module: w.e.f. July to September

1. Monitoring the spray schedule, that is, protective, curative, and eradicative fungicides, against apple scab and premature leaf fall blotch.
2. Monitoring the spray schedule, that is, contact insecticides and nonchemical summer oils, against San Jose scale, wooly apple aphid, and red spider mite.

IPM vs Non-IPM Interventions. For mites, dicofol/propargite was used at the petal fall stage (3rd week of April) by both IPM and non-IPM farmers. For the control of powdery mildew, blossom disease, and thrips, IPM farmers used carbendazim, neemarin, and boric acid. The usual practice by non-IPM farmers was to use carbendazim, chlorpyrifos, wettable sulfur, and boric acid. The parasitoid *Aphelinus mali* is used for the control of woolly apple aphid populations in mid- and low-valley areas. However, in upper areas of the valley, need-based pesticides (thiamethoxam, carbosulfan, and chlorpyrifos) were applied. For thrips, the insecticide spray (endosulfan/chlorpyrifos/thiamethoxam) was given at the pink bud stage. *Bacillus thuringiensis* and malathion were used in IPM orchards as compared to endosulfan, synthetic pyretheroids (cypermethrin and fenvalerate), and malathion in non-IPM orchards against caterpillars. For root rot, IPM and non-IPM orchardists used *Trichoderma*, a Bordeaux mixture, and carbendazim. For root borers, chlorpyrifos was used in non-IPM orchards. IPM farmers used light traps to hold up the adults of the root borer. Four sprays of mancozeb and carbendazim were used at the fruit development stage (2nd week of May to 1st week of August).

Quantification of Residues. After extraction and cleanup, the samples were analyzed by GC/HPLC/colorimetric method for quantification of various pesticides. A standard mixture of five pesticides (chlorpyrifos, endosulfan, dicofol, cypermethrin, and fenvalerate) and three pesticides individually, that is, propargite, malathion, and phorate, respectively, were injected in GC. Three pesticides (carbosulfan, carbendazim, and thiamethoxam) were individually injected in HPLC under the standardized conditions. Mancozeb was estimated by colorimetry. The residue of each pesticide was calculated by using a calibration curve.

RESULTS AND DISCUSSION

IDL, Recovery, and EMDL. All of the pesticides gave a separate sharp peak under the described condition of GC and HPLC.

Their retention times and IDL are given in **Table 1**. In GC, chlorpyrifos, dicofol, propargite, and cypermethrin gave single sharp peaks; however, propargite was analyzed singly. Endosulfan gave two peaks because of two isomers (α- and β-endosulfan). Fenvelerate gave a bifurcated peak because of the stereoisomers, and thus, a sum of the total area was taken for calculation. The GC chromatogram of the standard pesticide mixture A is shown in **Figure 1**. The calibration curve was found to be linear from 0.01 to 1.0 μg mL^{-1}. The lowest concentration of pesticide solution that gave a reliable response with a signal-to-noise ratio of 5:1 was considered to be the LOD. The LOD of pesticides was found to be comparable (0.007−0.05 μg mL^{-1}) with the calculated IDL values.

Table 3. Status of Pesticides Residues in IPM and Non-IPM Samples of Apple[a]

		residues in apple sample[b] (μg g^{-1})					
		non-IPM sample			IPM sample		
sample no.	pesticide	T_1	T_2	T_3	T_1	T_2	T_3
1	dicofol	ND	ND	ND	ND	ND	ND
2	chlorpyrifos	0.04	0.05	0.03	0.008	0.006	0.01
3	malathion	ND	ND	ND	ND	ND	ND
4	phorate	ND	ND	ND	ND	ND	ND
5	propargite	ND	ND	ND	ND	ND	ND
6	endosulfan-α	ND	ND	ND	ND	ND	ND
8	endosulfan-β	ND	ND	ND	ND	ND	ND
9	cypermethrin	ND	ND	ND	ND	ND	ND
10	fenvalerate	ND	ND	ND	ND	ND	ND
11	carbendazim	0.63	0.66	0.60	0.009	0.009	0.010
12	carbosulfan	ND	ND	ND	ND	ND	ND
13	thiamethoxam	ND	ND	ND	ND	ND	ND
14	mancozeb	ND	ND	ND	ND	ND	ND

[a] MRL values (ppm): dicofol, 0.02; chlorpyrifos, 0.5; malathion, 0.5; phorate, 0.05; propargite, 3.0; endosulfan, 0.05; cypermethrin, 1.0; fenvalerate, 0.02; carbendazim, 2; carbosulfan, 0.05; thiamethoxam, 0.2; and mancozeb, 5.0. [b] Average of three replicates; ND, below detectable limits per EMDLs indicated in **Table 2**.

A standard solution of carbendazim, carbosulfan, and thiamethoxam was analyzed by HPLC. The IDLs for carbendazim, carbosulfan, and thiamethoxam were found to be 0.05, 0.02, and 0.01 μg mL^{-1}, respectively. The retention times of these pesticides are given in **Table 1**.

The extraction of pesticide residues from fruits by polar solvents acetone or acetonitrile is a usual approach of most multiresidue methods (*7*−*19*). In our earlier studies, it was found that acetone and acetonitrile extracted a large amount of undesirable polar coextractives, and recoveries were around 70−78% for GC compatible pesticides (*19*). A modified extraction was standardized (acetone:cyclohexane:ethyl acetate in the ratio 2:1:1) to analyze the IPM and non-IPM mango samples, and good recovery was obtained (*20*). The same method was tried for six pesticides in the case of apple samples, and no interfering peaks were found in the fortified samples (**Figure 1**). The recovery of chlorpyrifos, dicofol, cypermethrin, fenvelerate, propargite, and endosulfan from fortified apple samples ranged between 80.3 and 95.5%. The recoveries of malathion and phorate were found to be 61.3 and 71%, respectively. The recoveries of carbendazim, carbosulfan, and thiamethoxam were 74.6, 79.9, and 83.06%, respectively (**Table 2**). The recovery of mancozeb was 72.2% and was analyzed by a colorimetric method. Much lower values of calculated EMDLs than the prescribed MRL values (0.02−5.0 ppm) made the method useful in the estimation of residues for monitoring purposes.

Status of Pesticide Residue in IPM and Non-IPM Apple Samples. Parasitoid *A. mali* successfully controlled woolly apple aphid populations in mid- and low-valley areas. However, in

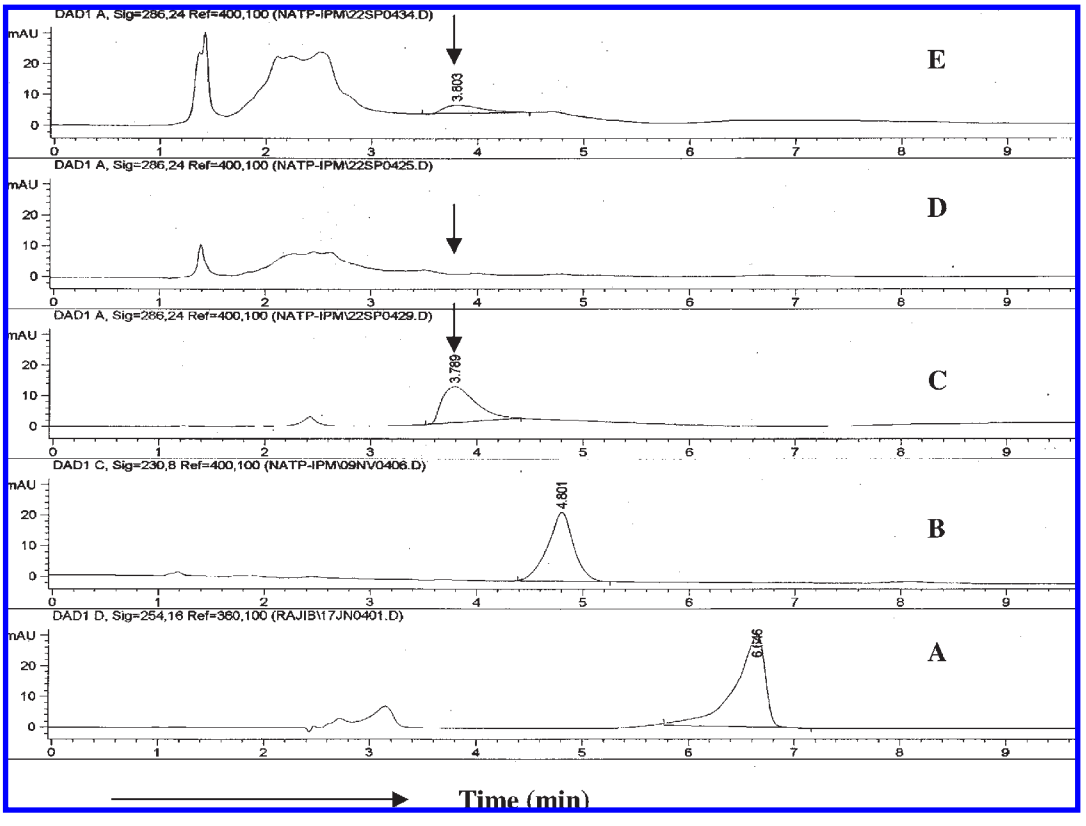

Figure 2. HPLC chromatograms showing carbendazim in apple sample: (**A**) standard thiamethoxam, (**B**) carfosulfan, (**C**) carbendazim, (**D**) control apple, and (**E**) apple sample showing carbendazim.

upper areas of the valley, need-based pesticidal application (thiamethoxam, carbosulfan, and chlorpyrifos) provided excellent control. IPM orchards were validated for *B. thuringiensis* as compared to endosulfan and malathion in non-IPM orchards against caterpillars. *Trichoderma* and a Bordeaux mixture gave good control for root rot in IPM orchards, while non-IPM used carbendazim, which was also quite successful.

For root borers, chlorpyrifos was used in non-IPM orchards. IPM farmers used light traps to hold up the adults of the root borer, further killed the adults manually, or dipped them in a pesticide solution or kerosene oil during rainy seasons. Thus, a further generation of the root borer was stopped as the adults after mating would have laid about 300 eggs, whose further hatching would have led to a damaging stage.

For pesticide residue determination, apple samples from IPM and non-IPM orchards of the farmers were extracted, cleaned up, and analyzed by the standardized method by GC and HPLC. Mancozeb was estimated by a spectrophotometric method taking absorbance at 435 nm. IPM samples were collected from the supervised field trials (*21*) where need-based pesticides were applied, while non-IPM samples were collected from those orchard farms in which pesticides were applied according to the farmers own knowledge gained from agricultural practices. All of the samples were analyzed for 13 pesticides, namely, chlorpyrifos, dicofol, malathion, phorate, cypermethrin, fenvelerate, propargite, endosulfan (α and β), carbendazim, carbosulfan, thiamethoxam, and mancozeb. The status of pesticide residues in various samples is given in **Table 3**. Results revealed that chlorpyrifos (**Figure 1**) and carbendazim (**Figure 2**) residues were present in the samples but below the prescribed limits of MRL. The study also showed that the quantity of residues of both chlorpyrifos and carbendazim were less in IPM samples than non-IPM samples of apple (**Table 3**). All remaining pesticides were below detectable limits.

In this era of awareness about contamination in environmental samples and because of the implementation of nontariff barriers, there is always an urgent need for a method to analyze the food samples well below the prescribed limits of contaminants. The described method provides a tool for the same. Two pesticides, that is, chlorpyrifos, an insecticide, and carbendazim, a fungicide, are the two chemicals that are very often used to combat termite and the fungal infection in fruits often during transportation and storage. Although chlorpyrifos is also very location specific, being a termiticide, carbendazim is used all over the world. The residues of both of these pesticides must be checked with special care as both of the pesticides are prone to persist for a longer period. During the present investigation, the residues of chlorpyrifos obtained were 0.006−0.05 μg g^{-1}. The residues of carbendazim were 0.009−0.01 and 0.60−0.66 μg g^{-1} in IPM and non-IPM samples, respectively, much below the MRL value of carbendazim.(*22*, *23*)

Conclusion. A small difference was observed in both the IPM and the non-IPM samples of apples. The reason behind this may be due to the different agricultural practices that pesticides were applied according to the farmers' knowledge in non-IPM field, while in IPM fields, pests were controlled by practicing IPM knowledge. It was concluded that most of the pesticides in the apple samples were nondetectable or within the permissible range of MRLs prescribed by codex/EU/UK and will not be harmful to public health. The standardized modified extraction method of GC compatible multipesticides for apple may be useful for the routine analysis of this fruit, which provides the EMDLs well below the MRLs of respective pesticides.

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