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Direct Estimation of Carbaryl by Gas Liquid Chromatography with Nitrogen Phosphorus Detection

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Abstract A simple and efficient analytical method was standardized for the estimation of residues of carbaryl in various substrates comprising grape berries, kinnow pulps, kinnow rind and soil. The samples were refluxed using mixture of methanol: 0.5 N HCl (1:1 v/v); diluted with brine solution, partitioned into chloroform and dried over anhydrous sodium sulfate. Further the samples were treated with anhydrous magnesium sulfate and primary secondary amine. Final clear extracts were concentrated under vacuum and reconstituted the volume into acetone. The residues were estimated directly on gas liquid chromatograph equipped with nitrogen phosphorus detection system equipped with a capillary column packed with 5 % diphenyl 95 % dimethyl polysiloxane non-polar phase. A consistent recovery from 82 % to 97 % for carbaryl was observed when samples were spiked at levels ranging from 0.05 to 1.00 mg kg^{-1}. The limit of quantification of the method was worked out to be 0.05 mg kg^{-1} for grape berries, kinnow pulp, kinnow rind and soil.

Keywords Carbaryl · Grape berries · Kinnow pulp · Kinnow rind · Soil

Carbaryl ($C_{12}H_{11}NO_2$, 1-naphthyl-*N*-methyl carbamate), a carbamate insecticide is used as a broad spectrum contact and stomach insecticide with some systemic properties on crops and trees (Fig. 1). Its insecticidal properties were first described by H. L. Haynes and his associates and introduced in 1956. Carbaryl is available as granules, dispersions, and suspensions formulations. It is a white crystalline solid commonly sold under the brand name Sevin, a trademark of the Bayer CropScience, India. Union Carbide discovered carbaryl and introduced it commercially in 1958. Bayer purchased Aventis Crop Science in 2002, a company that included Union Carbide pesticide operations. It controls over 100 species of insects on fruit, cotton, forests and many other crops, as well as on poultry, livestock, and pets (Mathew et al. 1995; Bacchetta et al. 2008). It is effective against organochlorine resistant insects, cattle and poultry lice, ticks and mites of animals, crop pests. It is used for the control of insect pests in home garden and domestic situations, on ornamentals, lawns, fruit and vegetables and around public buildings. It is also used in public health and veterinary practices.

The insecticide carbaryl acts as an inhibitor of acetylcholinesterase (AChE), a key enzyme in the insect nervous system (O'Malley 1997), causing the accumulation of acetylcholine (Ach) in the synaptic cleft because it impairs the hydrolysis of Ach (Ecobichon 2001). The presence of traces of carbaryl in fruits and vegetables poses a potential hazard for consumers. European Union legislation has established maximum residue level of carbaryl in food as 0.05 mg kg^{-1} (Abad et al. 2001).

Earlier routine analysis method of carbaryl was based on chromatography methods after derivatization or spectrophotometer (Benson and Finocchiaro 1965) and colorimetric (Benson and Finocchiaro 1965; Sachan and Singh 1994; Deshmukh and Saramma 1971; Iwata et al. 1979; Chiba 1981; Singh et al. 1980; Galhotra et al. 1985; Rao and Ramasubbaiah 1988; Choudhary et al. 1988; Dikshit

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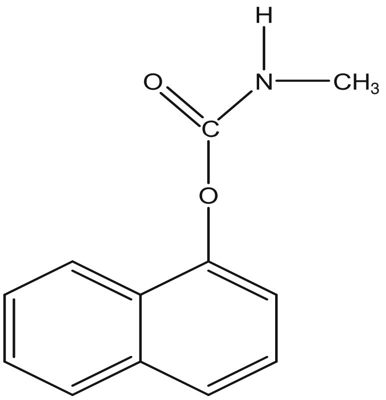

Fig. 1 Chemical structure of carbaryl

et al. 1988), thin layer chromatography (Yucel et al. 2006), high performance liquid chromatography (HPLC; Yucel et al. 2006; Gebreegzi et al. 2000; Biswas et al. 2010), enzyme-linked immunosorbent assay (ELISA) (Abad and Montoya 1995; Sun et al. 2010) which are characterized by high precision and sensitivity. Keeping in view its widespread use, it was planned to standardize an analytical methodology based on gas chromatography which may allow direct estimation of residues of carbaryl without any derivatization process in various substrates comprising grape berries, kinnow pulps, kinnow rind and soil.

Materials and Methods

Solvents like acetone, dichloromethane and hexane were procured from Merck, Darmstadt, Germany. Sodium chloride (ASC reagent grade ≥99.9 %) was also obtained from Merck, Darmstadt, Germany. The certified reference standard of carbaryl (purity 99.0 %) was supplied by Dr. Ehrenstorfer GmbH, Augsburg, Germany. Stock solution (1,000 µg mL^{-1}) was prepared by dissolving carbaryl standard in acetone and stored at 4°C. Series of standard dilutions were made for preparation of calibration curve and fortification studies (Fig. 2).

A representative 50 g sample of grape berries, kinnow pulp and kinnow rind were chopped and macerated. Similarly, a representative sieved 50 g soil sample was obtained after removal of extreneous matter. The samples were refluxed for 30 min using about 100 mL mixture of methanol: 0.5 N HCl (1:1, v/v). The extract was cooled and filtered into 1 L separatory funnel along with rinsings of methanol, diluted with 600 mL brine solution and partitioned the contents three times into 100, 50 and 50 mL chloroform. The combined organic layers were drained into 500 mL and dried over anhydrous sodium sulfate. The combined extract was concentrated to about 10 mL under vacuum rotary evaporator at <35°C.

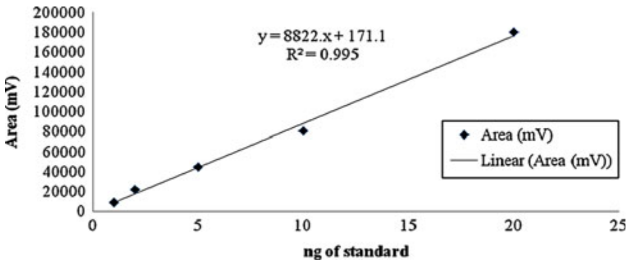

Fig. 2 Calibration curve of different concentrations of carbaryl

The chloroform extract was then subjected to cleanup by dispersive solid phase extraction (DSPE). An aliquot of 6 mL chloroform was taken in a test tube containing 0.15 ± 0.01 g PSA sorbent and 0.90 ± 0.01 g anhydrous $MgSO_4$ and the contents were thoroughly mixed on vortex spinix (Tarson®). Again the contents were centrifuged at 2,500–3,000 rpm for 1 min. Out of it 4 mL aliquot of extract was evaporated to dryness using rotary vacuum evaporator (Hediloph Laborata®) to near dryness at 35°C. Volume was made up to 2 mL using acetone and 2 µL of this final extract was injected on GLC for residue analysis.

The final analysis of the carbaryl insecticides was carried out on a gas liquid chromatograph (GLC; Perkin Elmer) equipped with nitrogen phosphorus detector (NPD). A capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) packed with 5 % diphenyl 95 % dimethyl polysiloxane non-polar phase was used for estimation of carbaryl insecticides. GC operating parameters were as follows: carrier gas flow rate: nitrogen flow rate: 30.0 mL min^{-1}, hydrogen flow rate: 2.0 mL min^{-1} and air flow rate: 100.0 mL min^{-1}, temperature: injection port: 280°C, detector: 310°C. The column temperature was initially maintained at 170°C for 5 min, then increased at the rate of 10°C min^{-1}, to 220°C, kept hold for 3 min and was finally increased at the rate of 5°C min^{-1} to 240°C and kept hold for 13 min. The peak of carbaryl was detected at 5.16 min.

Results and Discussion

Carbaryl is an insecticide for use in broad number of annual and perennial crops and belongs to the carbamate group. Residues of carbaryl were determined in a number of crops and soil on which its use is expected to be high in the future. The crops thus selected included grapes and kinnow which are affected by various insect pests.

As the quantitative determination of carbaryl in different agricultural commodities is directly related to the evaluation and interpretation of data, a reliable method is required which is reproducible and can be applicable to different commodities. The method was fully validated according to bio analytical method recommendations described in the

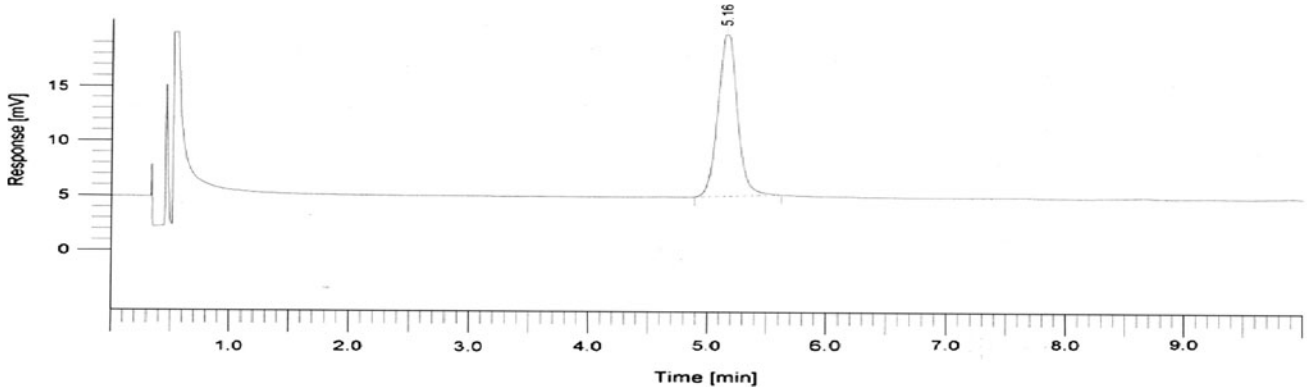

Fig. 3 Chromatogram of 2 ng carbaryl

Food and Drug Administration (FDA) guidelines in terms of selectivity, linearity, precision (repeatability), precision (reproducibility), and accuracy for both detection systems (Viswanathan et al. 2007).

Selectivity of the methods was assessed by comparing the GLC chromatogram of a set of six blank samples with that of six samples spiked at the limit of quantification (LOQ) level (0.05 µg mL^{-1} for GC detection). As shown in Fig. 3, no extraneous peak was detectable at the retention times of carbaryl.

The linearity of a method is a measure of range within which the results are directly, or by a well defined mathematical transformation, proportional to the concentration of analyte in samples within a given range (Francotte et al. 1996). The calibration curves with respect to carbaryl produces a linear relationship between detector response (y) and analyte concentration (x). The parameters obtained by the selected chromatographic conditions for carbaryl calibration correspond to: $y = 8822x + 171.1$, $R^2 = 0.995$ where $y =$ peak area, $x =$ carbaryl concentration (mg L^{-1}), and $R =$ correlation coefficient (Fig. 2).

In general, residues of carbaryl were determined by comparison of peak areas of the reference standards with that of the unknown or spiked samples run under identical working conditions of the instruments employed. The limit of detection (LOD in mg kg^{-1}) of analyte was determined as the lowest concentration giving response of three times the baseline noise defined from the analysis of three control samples and worked out to be 0.017 mg kg^{-1}. Limit of quantification (LOQ) for carbaryl was worked out on the

Table 1 Recovery studies of carbaryl on grape berries, kinnow rind, kinnow pulp and soil (n = 5)

Substrates	Level of fortification (mg kg^{-1})	Recovery (%)[a]	RSD$_r$, %
Grape berries	1.00	89.03	3.87
	0.50	85.23	2.66
	0.10	92.89	1.84
	0.05	86.82	3.62
Kinnow pulp	1.00	90.45	4.91
	0.50	93.84	3.11
	0.10	91.07	3.74
	0.05	86.33	4.70
Kinnow rind	1.00	85.63	2.05
	0.50	82.61	1.75
	0.10	90.28	4.51
	0.05	85.98	3.95
Soil	1.00	93.72	2.87
	0.50	96.85	4.23
	0.10	91.03	1.82
	0.05	93.77	2.54

[a] Each value is mean ± standard deviation of three replicate determinations

Table 2 Recovery and RSD values obtained from analyses of samples spiked with carbaryl

Sample	Day	Recovery (%)	RSD$_r$ (%)	RSD$_R$ (%)
Grape berries	1	86.82	3.62	3.84
	2	83.67	2.97	
	3	91.04	4.03	
Kinnow pulp	1	86.33	4.70	6.91
	2	89.51	3.98	
	3	88.60	3.11	
Kinnow rind	1	85.98	3.95	5.79
	2	91.18	4.83	
	3	87.59	2.70	
Soil	1	93.77	2.54	3.85
	2	91.69	2.69	
	3	95.71	3.61	

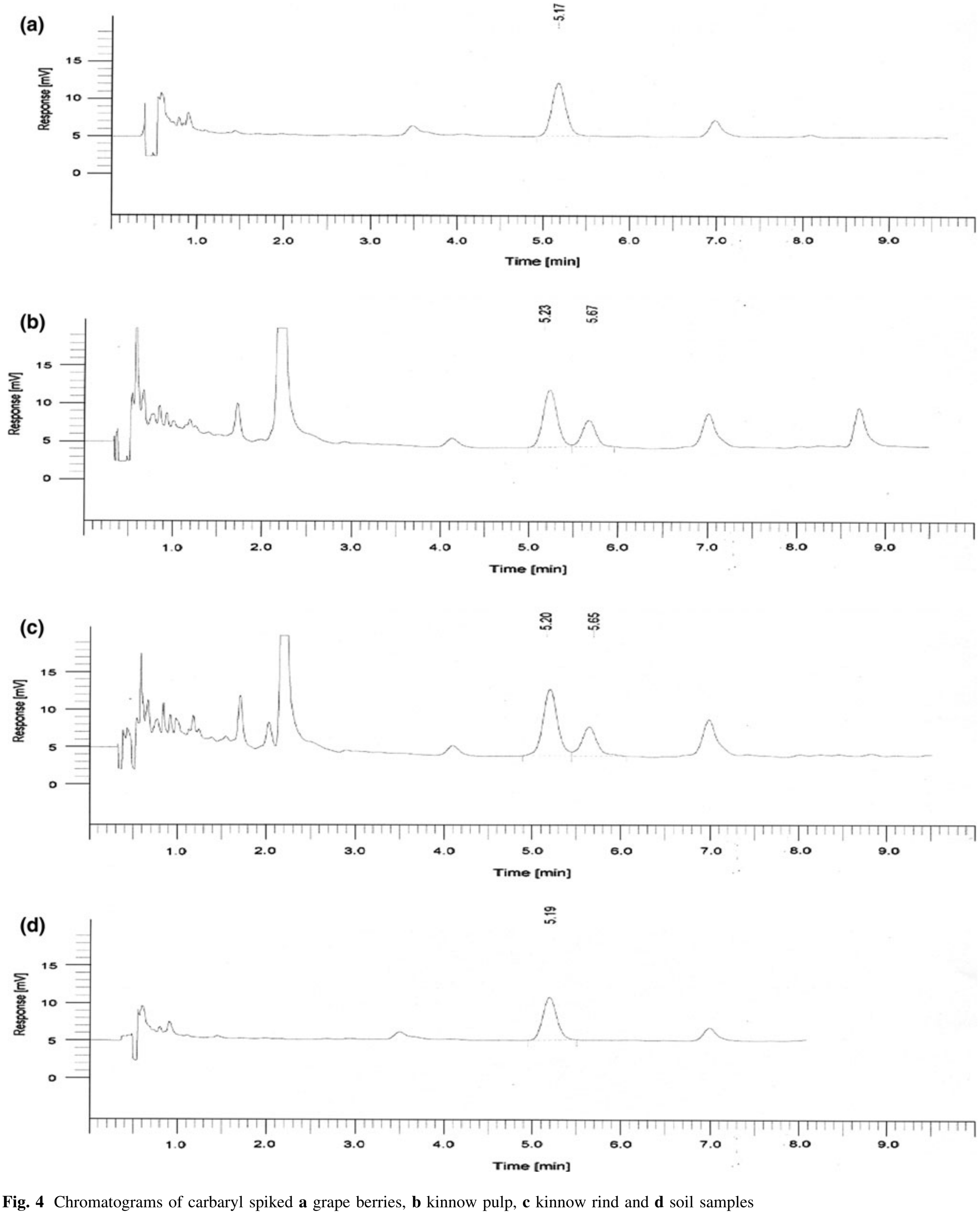

Fig. 4 Chromatograms of carbaryl spiked **a** grape berries, **b** kinnow pulp, **c** kinnow rind and **d** soil samples

basis of the response of the ng of standard working solution injected as well as the sample weight in mg injected so that the base line of the instrument remains stable and no noise is observed. As 1 ng of carbaryl produced more than 10 % deflection which can be measured and the instrument remained stable even after 20 mg of the substrate is injected, the LOQ for both the compounds was found to be 0.05 mg kg^{-1}.

Repeatability of the developed analysis method was determined by spiking carbaryl in different concentrations to different substrates. The within-batch recovery and relative standard deviation for repeatability (RSD_r) of spiked carbaryl in samples at the levels of 0.05, 0.10, 0.50 and 1.0 mg kg^{-1} for grape berries, kinnow pulp, kinnow rinf and soil are summarized in Table 1. The precision (repeatability) in different substrates were range from 1.75 % to 4.91 % for carbaryl. The results are good for all the concentration levels investigated and within specified probability (95 %).

The reproducibility of this analytical method was determined by analyzing spiked samples under various test conditions (different analysts and different days). The between-batch recoveries and relative standard deviation for reproducibility (RSD_R) investigated at several levels are given in Table 2. The precision (reproducibility) of carbaryl in different substrates ranged from 3.84 % to 7.92 % and all measurements are within 15 % at all concentrations.

The recovery tests were carried out on six replicates at each spike level. The average recoveries obtained for carbaryl at all concentrations and conditions investigated (Tables 1, 2) were determined as above 80 % in all the samples.

Per cent recoveries and standard deviations of carbaryl from grape berries, kinnow pulp, rind and soil are presented in Table 1. Initially, the representative samples of these crops were placed in methanol and partitioned in dichloromethane, but the recoveries were observed to be very poor, that is, to the extent of only 10 %. Later on, hexane, ethyl acetate and toluene were also tried, but the percent recoveries were again very poor. A representative 50 g sample of grape berries was spiked with carbaryl at 1.00, 0.50, 0.10 and 0.05 mg kg^{-1} level, extracted with methanol: 0.5 N HCl (1:1, v/v) and partitioned into chloroform (100, 50 and 50 mL). The results were astonishing as the percent recoveries ranged from 85.23 to 92.89. The same method was applied to other substrates like kinnow pulp, rind and soil the results were found to be excellent. The percent recoveries of carbaryl from different substrates spiked at 1.00, 0.50, 0.10 and 0.05 mg kg^{-1} are reported in Table 1, each value is the mean of three replicate determinations (Fig. 4). The results were encouraging and suggested that the method could be extended to more substrates. Moreover, this seems to be the first report regarding the direct estimation of carbaryl on GC according to a method that is simple, efficient, and easy to adopt in laboratories engaged in pesticide residue analysis.

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References

Abad A, Montoya A (1995) Application of a monoclonal antibody-based ELISA to the determination of carbaryl in apple and grape juices. Anal Chim Acta 311:365–370

Abad A, Moreno MJ, Pelegri R, Martinez MI, Sáez A, Gamón M, Montoya AJ (2001) Monoclonal enzyme immunoassay for the analysis of carbaryl in fruits and vegetables without sample cleanup. J Agric Food Chem 49:1707–1712

Bacchetta R, Mantecca P, Andrioletti M, Vismara C, Vailati G (2008) Axial-skeletal defects caused by carbaryl in *Xenopus laevis* embryos. Sci Total Environ 392:110–118

Benson WR, Finocchiaro JM (1965) Rapid procedure for carbaryl residues: modification of the official colorimetric method. J Assoc Off Anal Chem 48:676–679

Biswas AK, Kondaiah N, Anjaneyulu ASR, Rao GS, Singh RP (2010) A simple assay for analyzing residues of carbaryl insecticide in buffalo meat by liquid chromatography-photodiode array detection. Anal Methods 2:393–396

Chiba M (1981) A rapid method for analysis of carbaryl spray deposits on fruit tree foliage. J Agric Food Chem 29:118–121

Choudhary R, Singh KM, Singh RN (1988) Dissipation of carbaryl residues in *Sesamum indicum* Linn. Indian J Ent 50:1–4

Deshmukh SN, Saramma PV (1971) Estimation of carbaryl residues in maize and cauliflower. Indian J Ent 33:338–341

Dikshit AK, Mishra SS, Lal L (1988) Persistence of endosulfan and carbaryl residues in potatoes. Indian J Ent 50:429–432

Ecobichon DJ (2001) Carbamate Insecticides. In: Krieger R (ed) Handbook of pesticide toxicology, vol 2. Academic Press, San Diego, pp 1087–1106

Francotte E, Davatz A, Richart P (1996) Development and validation of chiral high-performance liquid chromatograpic methods for the quantification of valsartan and of the tosylate of valinebenzyl ester. J Chrom B 686:77–83

Galhotra R, Garg PK, Halve AK, Pandey SY, Sivasankaran K (1985) Carbaryl and lindane residues in potato crop. J Ent Res 9:57–59

Gebreegzi YT, Foster GD, Khan SU (2000) Simultanous determination of carbaryl, malathion, fenitrothion and diazinon residues in sesamum seeds (*Sesamum indicum* L.). J Agric Food Chem 48:5165–5168

Iwata Y, DiLsch ME, Carman GE, Gunther FA (1979) Worker environment research: residues from carbaryl, chlorobenzilate, dimethoate and trichlorfon applied to citrus trees. J Agric Food Chem 27:1141–1145

Mathew L, Reddy MLP, Rao TP, Iyer GSP, Damodaran AC (1995) Simple spectrophotometric method for the determination of carbaryl in soil and insecticide formulations. Analyst 120:1799–1801

O'Malley M (1997) Clinical evaluation of pesticide exposure and poisonings. Lancet 349:1161–1166

Rao NB, Ramasubbaiah K (1988) Dissipation of monocrotophos and carbaryl in tomato *Lycopersicon lycopersicum* L. Indian J Ent 50:209–214

Sachan AP, Singh G (1994) Dissipation of carbaryl and monocrotophos residues from mango fruits during storage. Indian J Ent 56:408–417

Singh KP, Pandey SY, Galhotra R, Srivastava OP, Singh S, Paralikar AB (1980) Dissipation of carbaryl residues in/on cabbage. J Ent Res 4:25–27

Sun J, Dong T, Zhang Y, Wang S (2010) Development of enzyme linked immunoassay for the simultaneous detection of carbaryl and metolcarb in different agricultural products. Anal Chim Acta 666:76–82

Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, Shah VP, Skelly JP, Swann PG, Weiner R (2007) Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res 24:1962–1973

Yucel U, Ilim M, Aslan N (2006) ^{14}C-carbaryl residues in hazalnut. J Environ Sci Health B 41:585–593