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Use Patterns and Residue Levels of Pesticides on Mukunuwenna, a Leafy Vegetable Grown in Sri Lanka

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Intensive use of chemical inputs such as pesticides and fertilizers as a means of expanding domestic food production in Sri Lanka began in the 1960's (Abeysekera 1988). Pesticides also showed spectacular success in control of vector borne diseases such as malaria (Fernando 1989). Because of these beneficial uses of pesticides, they were used indiscriminately both in agriculture and in public health programs. However, recently, there has been growing awareness and concern over such extensive use of pesticides and agricultural malpractices (i.e. Farmers not adhering to the prescribed pre-harvest interval, usage of higher than recommended concentrations of pesticides etc.) which could result in harmful levels of pesticides in crops (Seneviratne & Thambipillai 1974; Jeyaratnam *et al* 1982; Gunawardena *et al* 1987; Abeysekera 1988; Fernando 1988). Residue information which is vital for effective control through regulations is however rather limited in Sri Lanka. Thus, there is an urgent need for pesticide monitoring programs in the country both for the direct protection of the consumer and in relation to the acceptabilities of commodities in trade. In this context it is important to screen a variety of vegetables for pesticide residues. Such a study has the potential to detect the magnitude of this problem and would allow remedial action to be taken. In this study we restricted ourselves to determining residue levels of selected pesticides in Mukunuwenna (*Alternanthera sissilis*), the most widely produced and consumed leafy vegetable in Sri Lanka. To aid this study it was thought necessary to obtain information regarding the cultivation of Mukunewenna. Hence this was another objective of this study.

MATERIALS AND METHODS

Alachlor, captan, chlorpyrifos, diazinon, oxyfluorfen, permethrin (*cis and trans*), phenthoate, profenofos, quinalphos (purity >98%) were purchased from Accu.Standard Inc. (New Haven, USA). HPLC grade water, acetone and hexane were purchased from Merck (Poole, UK). Pesticide grade florisil (60–100 mesh, Merck, Poole, UK) was activated by heating at 650°C for 3 h in a muffle furnace (KL-280, Advantec, Japan) and stored at 130°C in a foil covered glass bottle. Glass wool was rinsed with acetone and placed in a muffle furnace for 1 hr at 400°C. Anhydrous sodium sulphate (Merck, Poole, UK) was heated for 4 hrs at 600°C in a muffle furnace to remove phthalate esters. A Buchi rotary evaporator (Type: R-114A29, Buchi Labortechnik AG, Switzerland) fitted with a water bath

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(B-481, Buchi Labortechnik AG, Switzerland) was used to evaporate solvents. A Mettler Toledo AB104 digital balance (Mettler-Toledo GmbH, Switzerland) was used for weighing measurements.

Stock solutions of pesticide standards (1000 ppm) and individual working standard solutions (0.01 to 1 ppm) were prepared in hexane. Mixed pesticide working standard solutions (0.01 to 1 ppm) were prepared by mixing stock standard solutions as appropriate and diluting to volume with hexane.

The Colombo district was selected for this investigation as it is the most densely populated of the 22 districts in Sri Lanka. The Colombo district consists of eleven "Assistant Government Agent" divisions (AGA divisions): Colombo, Kotikawatta-Mulleriyawa, Nugegoda, Dehiwala-Mt. Lavinia, Moratuwa, Kesbawa, Maharagama, Kaduwela, Homagama, Hanwella and Padukka. These "AGA" divisions are sub-divided into 557 "Grama Niladhari" divisions (GND divisions). Farmers in each of the "GND" divisions are represented by 1-2 farmer representatives. Randomly, 94 farmers were selected and information regarding the cultivation of Mukunewenna was obtained through a questionnaire and personal interviews. Information on the pesticides used, regions where Mukunuwenna and other leafy vegetables were cultivated, sale of crops, diseases and other crops cultivated was sought. Farmers were also interviewed on their practices regarding use and application of pesticides and the subsequent harvest and sale of crops. Information regarding the cultivation of Mukunuwenna, although not extensive, was also obtained from the Department of Census and Statistics and the Ministry of Agriculture, Sri Lanka.

The sampling sites were selected according to a statistical plan to represent the Colombo district. Of the eleven "AGA" divisions in the district, four were selected randomly for sampling: Kaduwela, Kotikawaththa-Mulleriyawa, Kesbawa, Maharagama. From each of these divisions six "GND" divisions were randomly identified (i.e. a total of 24 GND divisions) as sampling sites. A cluster sampling technique was employed to collect six samples from each "GND" division (i.e. a total of 144 samples).

The Mukunuwenna samples (1 kg) were collected (Oct. 2001 to Aug. 2002), placed in polythene bags and brought to the laboratory in coolers maintained at 4°C. The samples were kept in the freezer until analyzed.

Codex Alimentarius protocols were closely followed in obtaining the sub-sample (Clifton 1996). Mukunuwenna (100 g) was chopped and blended using a Waring blender (Cole-Parmer International, USA), acetone was added (200 ml) and mixed well. The mixture was filtered under suction. The filtrate (80 ml) was extracted with a 1:1 mixture of petroleum ether and methylene chloride (200 ml). The aqueous and the organic layers were separated and the organic layer was dried by passing through an anhydrous sodium sulfate column. Sodium chloride (7 g) was added to the aqueous phase and shaken till most of the sodium chloride was dissolved. The aqueous phase was extracted twice more with methylene chloride (2 x 100 ml) and the organic layer was dried by passing through an anhydrous sodium sulphate column. The extracts were combined and concentrated to 2 ml. Petroleum ether (100 ml) was then added to the extract and re-concentrated to about 2 ml. This process was repeated with a second portion of petroleum ether (50 ml) and finally with acetone (20 ml). The final volume

of the extract was adjusted to 5 ml with acetone.

The extract obtained for each sample was diluted with hexane to 20 ml and passed through an activated florisil (1g)/anhydrous sodium sulphate column. The column was eluted with a mixture of 50% methylene chloride : 1.5% acetonitrile : 48.5% hexane (50 ml). The solution obtained was concentrated to 2 ml and the final volume of the extract was adjusted to 5.00 ml with hexane. The extract was analyzed using gas chromatography (GC) coupled with electron capture, nitrogen phosphorus or mass spectrometric detection system.

Analysis of the final extracts was conducted using a Hewlett Packard 6890 gas chromatograph equipped with the following chromatographic systems: a) a splitless injector with a HP 5 trace analysis 5% Phenyl methyl siloxane capillary column (30 m x 0.25 mm x 0.25 μm) and an electron capture detector (ECD). Helium was used as the carrier gas at flow rate of 2ml/min. Operating temperatures were 150°C for the injector and 300°C for the detector. b) a cold-on column injector, with a trace analysis cross linked phenyl methyl silicone capillary column (30 m x 0.53 mm x 0.88 μm) connected to a nitrogen phosphorus detector (NPD). The carrier gas helium flow was set at 3.9 ml/min. Operating temperatures were 200°C for the injector and 250°C for the detector. The temperature program was the same for both chromatographic systems: programmed from 150°C to 175°C at a rate of 3.3 °C/min and held at 175°C for 10 min; from 175°C to 290°C at a rate of 20°C/min and held at 290°C for 6 min. The injection volume was 1 μl.

The GC/MS system consisted of a Hewlett-Packard 5890 series II gas chromatograph coupled with a 5989A mass selective detector in electron impact ionization mode (70eV). The GC was equipped with a BP-1 cross linked dimethyl siloxane fused silica capillary column (15 m x 0.22 mm x 0.25 μm). Operating conditions were: helium carrier gas flow was set at 0.9 ml/min. Operating temperatures were 270°C for the injector and 250°C for the detector. Temperature programme: The temperature was maintained at 110°C for 1 minute and then programmed at 30°C/ min to 220°C and held at 220°C for 0.5 min.

The unknown peaks in the chromatograms were identified by comparing the retention times (RT) of the unknown peaks to the RTs of the reference standards. In order to confirm the identity of the pesticides found, the retention times obtained from the other capillary column was used, while further confirmation, wherever necessary was achieved by the use of GC-MS. Quantification was carried out with a computer integrator by comparing the detector response for the sample to that measured for the calibration standard within a linear range. The Limit of Quantification (LOQ) was calculated as the quantity of the chemical (in ng) causing 10% full scale detector response/ mg sample equivalent in determinative step.

For statistically validating the efficiencies of the method a recovery study was performed by spiking in the laboratory the acetone extract of untreated Mukunuwenna samples (100 g) with the target pesticides at two concentration levels (0.05 ppm, 0.1 ppm), extracting according to the method and determining how much is recovered.

Analyses were performed in sets consisting of a blank (double distilled water run through the method), two spikes (0.05 ppm and 0.1 ppm) and 5 samples. The

repeatability of the method was checked by measuring the relative standard deviation from the recovery experiments. Duplicate analysis was performed for the samples in which pesticide residues were detected.

RESULTS AND DISCUSSION

Local farming practices concerning the application of pesticides and subsequent harvest of treated crops have raised concerns over the possibility of excessive residues on crops sold in the market. Agricultural malpractices such as application frequencies and doses exceeding manufacturer recommendations, disregard of recommended pre-harvest intervals are common. Therefore, studies that monitor residue levels in fruit and vegetables are essential in order to ensure the "safety" of consumers.

Mukunuwenna is the most popular of nine leafy vegetables cultivated and sold on a commercial scale in Sri Lanka (MAL 1999 & MAL 2000). It is easy and inexpensive to cultivate, damage during transport is minimal and once cultivated it can be harvested on a monthly basis for about two years. The following information was obtained from the Field Study/ survey conducted among farmers in the Colombo district.

The crop protection methods used by farmers were hand weeding, hoeing, application of weedicides for weed control, removing insects by hand or by use of insecticides. Disease control strategies involved destroying infected plants, crop rotation, seed treatment, use of soap water with kerosene and use of other traditional methods and application of fungicides. The most commonly encountered weeds were *Isachene kunthania* (Bamboo grass), *Fimbristylis miliacea* (Buffalo grass), *Cyprus iria, Isachneglobasa* and *Oxalis corniculata.* Leaf eating caterpillar, pod borer, leaf hopper, white fly and beetle were the most frequently encountered insect pests. Blight, mildew and rust were the three most common diseases affecting the growth of leafy vegetable. The average pre-harvest interval was 10 days. Farmers sold their vegetables at farmers markets, fairs, roadside stands, retail and wholesale stores. The crop was harvested in many cases after 5 p.m., stored in the open and brought to the market in open meshed cord or rope bags around 6-8 a.m. the following day. A few vendors harvested the crop early in the morning and brought it to the market on the same day. All the farmers interviewed did not wash the crop. However, some of the retailers sprayed water on the crops throughout the day to prevent wilting. Others believed that contact with water would increase spoilage and therefore did not spray water. In most vegetable stalls, the crops were displayed in traditional open wooden structures which allow for good drainage of water.

Through the survey, it was found that in addition to other pest control methods a wide range of pesticides of different chemical categories were widely used in the cultivation of Mukunuwenna (Table 1). Of the common pesticides used on Mukunuwenna, ten were selected for this study. Use of GC as the only analytical tool limited us in determining the carbamates commonly used by farmers on Mukunuwenna (aldicarb, carbaryl, carbofuran and carbosulfan) while dimethoate and propanil were not recoverable by this procedure. The retention times and percentage recoveries of the selected pesticides are given in Tables 2 and 3 respectively.

The recoveries of the pesticides (Table 3) studied ranged from 81% - 110% (fortification level, 0.05 ppm) and 85% - 106% (fortification level, 0.1 ppm). Standard

deviations associated with each determination were < 10%. The LOQ values obtained for the pesticides were in the range 0.01-0.05 ppm.

Table 1. Pesticides used by farmers in the Colombo district in the cultivation of Mukunuwenna.

Pesticide	**Type/ Chemical class**
Alachlor	pyrethroid
Aldicarb	carbamate
Captan	phthalimide
Carbaryl	carbamate
Carbofuran	carbamate
Carbosulfan	carbamate
Chlorpyrifos	organophosphate
Diazinon	organophosphate
Dimethoate	organophosphate
Oxyfluorfen	diphenyl ether
Permethrin	pyrethroid
Phenthoate	organophosphate
Profenofos	organophosphate
Propanil	pyrethroid
Quinalphos	organophosphate

Table 2. Retention times of pesticides.

Pesticide	**Retention time (min)**	
	With ECD	**With NPD**
Alachlor	11.92	7.85
Captan	16.03	8.20
Chlorpyrifos	13.56	9.70
Diazinon	9.96	6.08
Oxyfluorfen	17.85	--
Permethrin (*cis*)	25.74	--
Permethrin (*trans*)	26.04	--
Phenthoate	15.49	11.44
Profenofos	17.26	13.01
Quinalphos	15.48	11.36

Pesticides were detected in 45 samples of the 144 Mukunuwenna samples analyzed (Table 4). The concentrations of the pesticides ranged from 0.011 ppm to 1.942 ppm. The most widely detected pesticide was chlorpyrifos followed by captan > profenofos > diazinon > permethrin (*cis*) and permethrin (*trans*) > oxyfluorfen. Alachlor was not detected in any of the samples. Quinalphos and phenthoate could not be quantified as they co-eluted under the GC conditions used. Of the 45 samples captan , chlorpyrifos, diazinon , oxyflurofen, permethrin (*cis*), permethrin (*trans*) and profenofos were detected above the LOQ in 7(4.9%), 22 (15.3%), 4 (2.8%), 1 (0.7%), 2 (2.0%), 3 (2.0%) and 5 (3.5%) respectively.

Table 3. Percentage recoveries of pesticides.*

Pesticide	Fortification at 0.05 ppm *(n=3)*		Fortification at 0.1 ppm *(n=3)*	
	Mean	€*SD*	**Mean**	€*SD*
Alachlor	88	8.4	94	1.5
Captan	103	4.2	106	4.7
Chlorpyrifos	100	4.3	102	2.0
Diazinon	98	3.4	98	2.3
Oxyfluorfen	110	5.7	88	1.5
Permethrin (*cis*)	102	5.0	99	1.6
Permethrin (*trans*)	81	9.1	85	6.7
Phenthoate	#nq	--	nq	--
Profenofos	88	5.2	86	7.1
Quinalphos	#nq	--	nq	--

* Quantification by ECD

\# nq - Since phenthoate and quinalphos co-eluted under these conditions, quantification was not possible

€ Standard Deviation

Table 4. Pesticide residue levels (mg/kg) and their frequency of appearance (given in parenthesis) in Mukunuwenna samples.

Pesticide	No. of samples positive	Residue level (mg/kg)
Alachlor	none	--
Captan	07	0.016, 0.018, 0.125, 0.147, 0.249, 0.487, 0.889
Chlorpyrifos	22	0.014, 0.019, 0.040(2), 0.050, 0.051(2), 0.054, 0.070, 0.075, 0.090(2), 0.149, 0.151, 0.163(2), 0.200, 0.207, 0.875, 1.225, 1.309, 1.942
Diazinon	04	0.012, 0.023, 0.070, 0.090
Oxyfluorfen	01	0.011
Permethrin (*cis*)	03	0.017, 0.060, 0.149
Permethrin (*trans*)	03	0.036, 0.121, 0.345
Profenofos	05	0.011, 0.012, 0.027, 0.050(2)

Of the 22 samples in which chlorpyrifos was detected 4 samples exceeded the MRL value of 0.5 ppm specified by the Sri Lanka Standards Institution. MRL values for other pesticides are not available for comparison.

In Sri Lanka, stringent regulations are in existence in order to address all aspects of the proper management of pesticides (Control of Pesticides Act No. 33 of 1980, Amendment Act No 6 of 1994). However, in spite of this, many farmers tend to use pesticides indiscriminately, for example not adhering to the specified pre-harvest interval, use of higher than the recommended concentrations etc. Such practices have serious implications since they may result in high pesticide residue levels in crops. Therefore, regular monitoring programs whereby pesticide residue levels in crops/water are determined are necessary and important. Such programs have the potential to act as deterrents in the misuse of pesticides. It would also enable the

relevant authorities to enforce the law by the provision of the requisite data in this regard. In the given circumstances, the need to safeguard the interests of the consumer and thereby the health of the nation cannot be over emphasized.

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