Bull. Environ. Contam. Toxicol. (2006) 76:429–435

DOI: 10.1007/s00128-006-0939-5

Bulletin of Environmental Contamination and Toxicology

Estimation of Organochlorine Pesticide Residues in Two Popular Spices Extensively Used as Herbal Tea Ingredients in India

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Received: 14 June 2005/Accepted: 27 December 2005

Spices form an important ingredient of food as well as herbal teas in India. Indian spices such as Cloves (*Syzygium aromaticum* Linn.) and ginger (*Zingiber officinale* Rosc.) are famous all over the world for their natural aroma, flavor, taste and medicinal value. They have a wide range of therapeutic uses such as anti-microbial, anti-iral, anti-bacterial, anti-diabetic, anti-inflammatory, antioxidant activities, strengthening of immune system and for checking flatulence, dyspepsia, nausea and vomiting (Sambaiah and Srinivasan 1991; Srivastava and Mustafa 1992; Shobana and Naidu 2000; Lampe 2003). Due to the above mentioned therapeutic uses of these spices, they are commonly used as herbal tea ingredients in India (Naithani and Kakkar 2003;2004). Since these spices serve as raw materials in the preparation of herbal teas in India, they should be free from contaminants. There are some reports of presence of organochlorine pesticides in medicinal plants and herbal products such as Ayurvedic preparations (Srivastava et al. 2000), herbal products in Italy (Di Muccio et al. 1981), some major food crops of Northern Tanzania (Rusibamayila et al. 1998), Chinese herbal medicines and plants (Zhou et al. 1998; Zhang et al. 2000), fruits, vegetables and plants of France (Diop et al. 1999), medicinal plants sold in Portugal (Lino et al. 1999) and medicinal plants of Brazil (Zuin et al. 2003). There are reports of presence of metabolites of DDT i.e. DDE and DDD in soil even after banning its use for several years (Kiflom et al. 2001).

Our group has been making untiring efforts to check contaminant levels in therapeutically important medicinal plants and herbal products for heavy metals (Haider et al. 2004; Naithani and Kakkar 2005)) and persistent pesticides (Naithani and Kakkar 2004). The purpose of this study was to detect the presence of organochlorine pesticides in two spices (used as herbal tea ingredients in India), procured from different cities representing ecological zones from Central, North, South, East and West regions of India.

MATERIALS AND METHODS

Sygygium aromaticum (dried flower buds) and Zingiber officinale (rhizomes) were procured from different cities representing varied ecological zones of India. Identity of the samples was ensured with the help of experts. Market samples sold in medicinal plant stores were also procured from different regions. The samples

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Table 1. Medicinal plants commonly used as herbal tea ingredients.

Common and Botanical name	**Family**	**Part used with chemical constituents**	**Medicinal properties and uses***	**Place of Procurement**
Cloves *(Syzygium aromaticum* Merr & Perry*)*	Myrtaceae	Dried flower buds (eugenol)	Stimulant, aromatic and carminative; useful in flatulence and dyspepsia	Lucknow Shimla Rewa Srinagar Thiruvanantha puram Delhi Pune Kolkata
Ginger *(Zingiber officinale* Rosc.*)*	Zingiberaceae	Rhizome (Zinziberene, shogaol and camphene)	Stimulant, carminative, digestive and stomachic; useful in dyspepsia and flatulence	Lucknow Shimla Rewa Srinagar Kolkata Bangalore Pune

*(Chopra et al. 1956; Asolkar et al. 1992; Shobana and Naidu 2000; Gokhale et al, 2003).

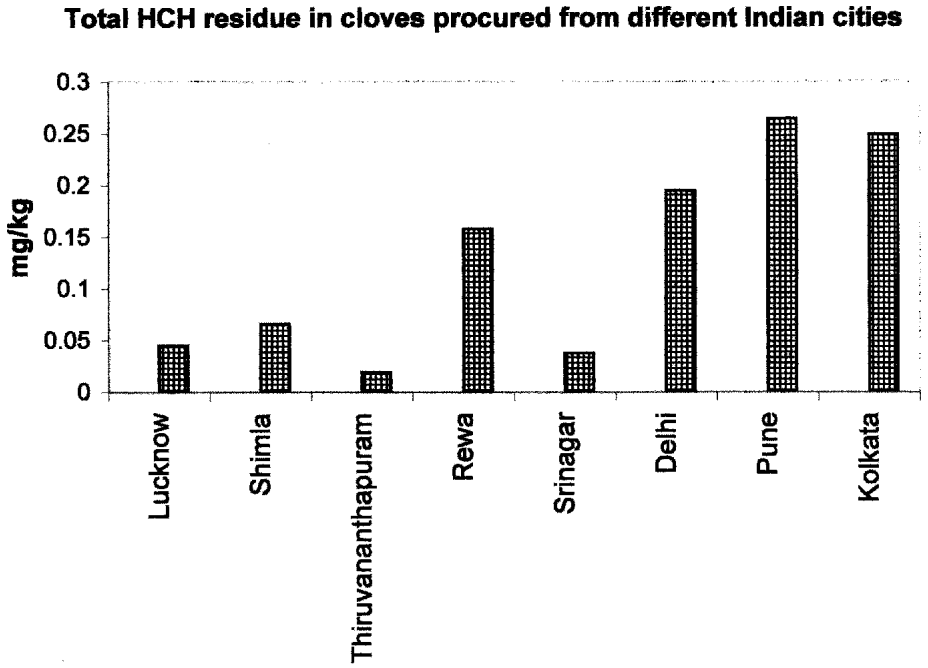
were dried in air and powdered prior to extraction. Table 1. shows place of procurement of samples with their therapeutic uses. All chemicals used in the study were of analytical grade (E.Merck). Quartz distilled water was used throughout the study, including rinsing of the glasswares. Solvents used such as n-hexane, acetonitrile were of HPLC grade and glassware used for making up the volume were all "A" grade Borosil, certified glassware. Estimation of organochlorine pesticides was carried out by taking two grams of the material and extracting it with 150 ml of n-hexane using a soxhlet apparatus (Naithani and Kakkar 2004). The extract was passed through anhydrous sodium sulphate filter column was prepared by putting about 10 g of anhydrous sodium sulfate in a glass column to remove traces of water. The hexane extract was then evaporated at 60 °C temperature of water bath under reduced pressure in a rotary flash evaporator (BUCHI-R114). The concentrated extract (1-2 ml) was transferred to the clean up column with small washing with hexane. The sample was further extracted with acetonitrile/hexane saturated solvent system for oil removal. Then clean up was done by hexane +benzene mixture elution method. Four gram of deactivated adsorbent (florisil) was packed in a Borosil glass column (150 x 5 mm), topped with anhydrous sodium sulfate and tapped firmly (Konstantinou et al. 2000). Concentrated extract (in hexane) was transferred to the top of the column and eluted with 5 ml of hexane. Elute was collected carefully and 5 ml of benzene-hexane mixture (80:20) was added to the column and collected. The elute was

again concentrated to 1-5 ml as required and analysed on GLC (NUCON 5765). The temperature of injection port (250 °C), oven (190 °C) and detector (250 °C) was adjusted as recommended for optimum efficiency and thermal stabilization ensured. Carrier (nitrogen) gas flow rate was adjusted to 60 ml per minute. Five μl of mixed standard solution of HCH and its isomers,α-endosulfan, DDT and its metabolites followed by 5 μl of the samples were injected in GLC and chromatograms were recorded. Detection limit of the equipment was 1μg/kg and recovery obtained under the experimental conditions was found to be 90-93%.

RESULTS AND DISCUSSION

In *Syzygium aromaticum* (Dried flower bud) (family: Myrtaceae), Lindane (γ-HCH) residue was 205 times higher in Pune sample as compared to Lucknow sample (Table 2A). It was below the Maximum Residual Limit (MRL) of 0.5 mg/kg and Acceptable Daily Intake (ADI) of 0.001 mg/kg-body weight (FAO/WHO, 1986, 1998) in all the samples. α-HCH ranged from 0.0014 mg/kg to 0.0954 mg/kg. β-HCH ranged from 0.0185 mg/kg to 0.0698 mg/kg whereas it was not detected in samples from Lucknow, Shimla, Thiruvananthapuram, Srinagar and Delhi. δ-HCH ranged from 0.0028 mg/kg to 0.0981 mg/kg whereas in samples from Shimla, Delhi and Kolkata, it was not detected. Total HCH (Figure 1) was highest in Pune sample which was 13.8 times more as compared to Thiruvananthapuram sample. It was below the ADI of 0.005 mg/kg-body weight in all the samples. DDE was detected in Rewa, Lucknow, Delhi and Pune samples but was far below the MRL of 0.1 mg/kg (FAO/WHO, 1986, 1998) (Table 2B).

Zingiber officinale (Rhizome) (family: Zingiberaceae), consumed regularly as a part of daily food in India, showed 100 times higher level of residual lindane in Bangalore sample as compared to Srinagar sample (Table 3). It was not detected in Shimla sample and was below the Maximum Residual Limit (MRL) of 0.5 mg/kg and Acceptable Daily Intake (ADI) of 0.001 mg/kg-body weight (FAO/WHO, 1986, 1998) in all the samples. α-HCH was detected in all the samples and ranged from 0.0258 mg/kg to 0.0593 mg/kg. β-HCH ranged from 0.0096 mg/kg to 0.0234 mg/kg and was not detected in samples from Lucknow, Shimla and Rewa. δ-HCH was detected only in Shimla sample. Total HCH residue (Figure 1) was highest in Bangalore sample i.e. 9.4 times more as compard to Lucknow sample. DDT, its metabolites and α-endosulphan were not detected in any sample of ginger. HCH and DDT in all the samples studied by us was below the maximum residue limit and acceptable daily intake for foods as recommended by Codex Alimentarius Commission. DDE, a persistent metabolite of DDT, was detected in few samples and it was also below the maximum residue limit. DDE has been detected in soil decades after banning its production and use (Simonich and Hites 1995). ADI for HCH is 0.005 mg/kg-body weight (0.30 mg/60 kg body wt. for humans) and for Lindane it is 0.001 mg/kg-bodyweight (0.06 mg/60 kg). However, the amount of spices consumed are not very high and the pesticide levels found in our samples are much below the prescribed ADI. Detection of OCP residues was more in samples from Bangalore, Kolkata, Delhi, Pune and Rewa and comparatively low contamination was found in samples from



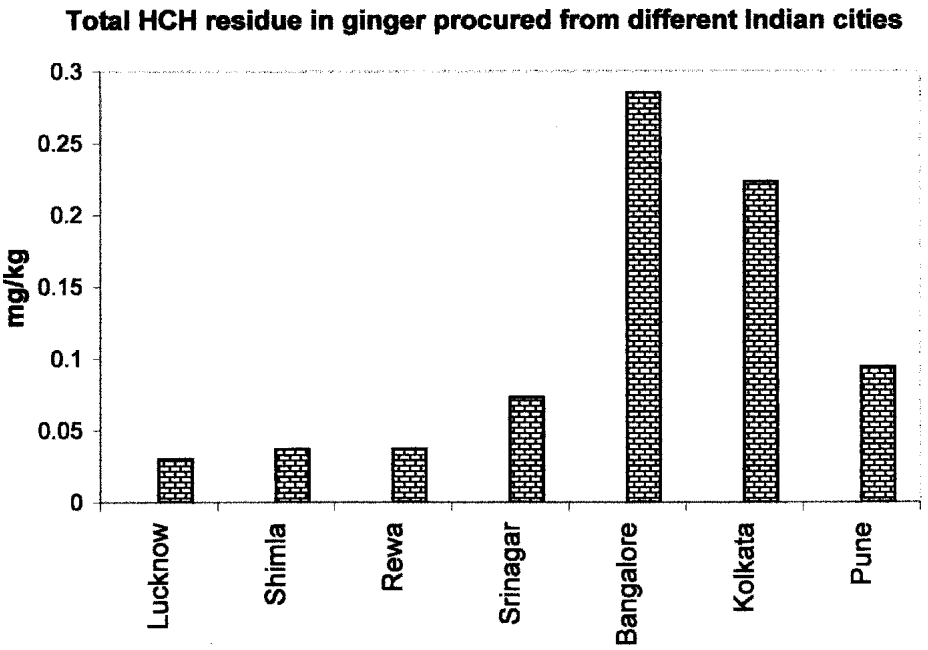


Figure 1. Total HCH residues in cloves and ginger samples.

Table 2 (A). Isomers of HCH (mg/kg) in *Syzygium aromaticum.*

Name	α-HCH	γ-HCH	β-HCH	δ-HCH
Lucknow	0.0042	0.0008	BDL	0.0401
Shimla	0.0640	0.0018	BDL	BDL
Thiruvananthapuram	0.0093	0.0015	BDL	0.0084
Rewa	0.0014	0.0390	0.0195	0.0981
Srinagar	0.0244	0.0036	BDL	0.0096
Delhi	0.0583	0.1363	BDL	BDL
Pune	0.0280	0.1640	0.0698	0.0028
Kolkata	0.0954	0.1350	0.0185	BDL

BDL: Below Detection Limit of instrument

Table 2(B). DDT and its metabolites and α-endosulphan (mg/kg) in *Syzygium aromaticum.*

Name	α-endosulphan	pp'DDE	op'DDT	pp'DDD	pp'DDT
Lucknow	BDL	0.0058	BDL	BDL	BDL
Shimla	BDL	BDL	BDL	BDL	BDL
Thiruvananthapuram	BDL	BDL	BDL	BDL	BDL
Rewa	BDL	0.0138	BDL	BDL	BDL
Srinagar	BDL	BDL	BDL	BDL	BDL
Delhi	BDL	0.0158	BDL	BDL	BDL
Pune	BDL	0.0112	BDL	BDL	BDL
Kolkata	BDL	BDL	BDL	BDL	BDL

BDL: Below Detection Limit of instrument

Table 3. Isomers of HCH (mg/kg) in *Zingiber officinale.*

Name	α-HCH	γ-HCH	β-HCH	δ-HCH
Lucknow	0.0258	0.0043	BDL	BDL
Shimla	0.0337	BDL	BDL	0.0033
Rewa	0.0343	0.0025	BDL	BDL
Srinagar	0.0472	0.0023	0.0234	BDL
Bangalore	0.0413	0.2312	0.0124	BDL
Kolkata	0.0287	0.1747	0.0196	BDL
Pune	0.0593	0.0254	0.0096	BDL

BDL: Below Detection Limit of instrument

Lucknow, Srinagar and Shimla. Lowest contamination was observed in samples from Thiruvananthapuram, the southern region of India which is also the belt for large scale cultivation of *Syzygium aromaticum.*

Acknowledgments. We thank Prof. Y.K. Gupta, Director, I.T.R.C., Lucknow, for his interest in this work. We are grateful for the support extended by Dr. Jairaj Bihari, Head, Analytical Chemistry Section, I.T.R.C. in carrying out this work. The authors are grateful to I.T.R.C. publication committee for critically reviewing this work and allocating manuscript number 2409.

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