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Analysis of Common Culinary Spices to Observe Multiclass Pesticides Residue Levels

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A B S T R A C T

Sixteen types of common culinary spices available in local market were procured and analyzed to observe the level of contamination of pesticide residues with special reference to organochlorines (OCs) and synthetic pyrethroids (SPs). A total twenty-four pesticides, i.e., fourteen types of OCs and ten OPs were analyzed using Quick, Easy, Cheap, Effective, Rugged and Safe: QuEChERS method. Method validation was performed satisfactorily prior to analysis. The percent (%) recovery, limit of detection (LOD) and retention time (RT) of 24 pesticides in 16 different spices sample matrix were also assessed. The level of pesticide residues in various spices were also compared with their maximum residues limit (MRL) as per food safety act/Codex guidelines. Maximum spice samples (90%) were found free from pesticide contamination. γ-HCH, endosulfan and dicofol were detectable amongst OCs in a limited number of samples of turmeric, coriander, red chilli, cumin and nutmeg. Isomers of cypermethrine as well as cyfluthrin were significant OPs observed in traces in a few samples of turmeric and aniseed. Contaminations of pesticide residues may occur during harvesting of post harvesting practices including prolong storage conditions. A periodic monitoring and assessment are needful for safe commercial and domestic uses of culinary spices.

Key words: Culinary spices, Contamination, Pesticide residue, Analysis, QuEChERS method, Food safety

Spices are high value and low volume crops which comprise specific part of a plant (root, rhizome, stem, bark, leaf, fruit, flower, stigma, seed etc.) enriched in essential oil and aroma become significant [1]. Spices have been basic agricultural commodity since the dawn of the human civilization. They are an inevitable part of our culinary art where different species of spices are utilized in a variety of ways specially to add flavour, nutrition, piquancy, condiment or seasoning the food articles and make them relish [2]. Spices are not only an appetizing agent but many of them also possesses specific medicinal as well as food persevering properties. Therefore, spices are high demand crops which are cultivated and mercantile to fulfil day to day requisites viz. cookery purposes, religious activities, medicinal usage, pharmaceuticals, cosmetics, perfumery, phytochemical industry etc. [3]. A majority of culinary spices are well known to exhibit antioxidant, antiseptic, anti-inflammatory, antipyretic, antimutagenic, anti-diabetic, antidiuretic and immune booster properties due to presence of specific biomolecules as well as active phytochemical components [4]. Their medicinal role as well as antiviral properties served as a boon to human health during COVID-19 pandemic [5-6]. Turmeric is one of the most demanded Indian culinary spices followed by cumin, ginger, black pepper (king of spices), cardamom (queen of spices), coriander, cinnamon etc. Specific medicinal properties and nutritive value of Garcinia species, star anise, mint, carom seeds, saffron, black cumin, oregano, lavender make them to classify under next generation crops.

India not only ranks no. 1 in spice production worldwide but also is a biggest consumer and exporter of about 75 spices of the 109 varieties listed by the International Organization of Standardization (ISO). Turmeric, ginger, pepper, cardamom, saffron as well as chilly are few values added Indian spices having high demand in international market. Chilly, cumin, turmeric, coriander and ginger are the most exported spice from India [2], [7]. Spice cultivation and processing have become a profitable agricultural sector in which there is a great potential in increasing the farmer's income substantially [8]. High per capita consumption of Indian culinary spices requires continuous supply of good quality products in adequate quantity

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also. Irregular rainfall, untenable climatic conditions, pest and disease problem are some major concerns of low productivity and poor quality of spice crops. Pesticide contamination in spices is also a major issue which results in its rejection for export or domestic use [9]. Food safety issues with special reference to contamination due pesticide residues in food commodities have been a global economic concern as well as unfavourably affecting Indian spice market also [10]. International standards have been laid out defining Maximum Residue Limit (MRL) of a pesticide residue in food commodities including culinary spices [11]. Food Safety and Standards Authority of India (FSSAI) as well as Indian Spice Board ensures food safety and quality related issues in Indian scenario in accordance with International agencies viz. Joint Food and Agriculture Organization (FAO), Codex Alimentarius Commission (CAC) and World Health Organization (WHO). All global spice producers and consumers must follow the standard guidelines during cultivation of raw material, processing, storage, transportation and trade of spices to maintain quality assurance and quality control. These environmental and food safety precautions are essential to minimize the exposure and impact of pesticide residues in human food chain [12].

In modern agrochemical based agricultural practices, application of pesticides is done to protect the crop against unwanted flora and fauna during cultivation period, storage or transport. Organochlorine, pyrethroids, organophosphates, carbamates, triazines as well as neonicotinoids are common chemical categories of pesticides which are either singly or in the form of a mixture applied to eradicate weeds, herds, fungus, insects, rodents as well as plant growth in agricultural fields during harvesting or in storage condition of food commodities [13]. Pesticide residues are persistent in nature and easily enter in food chain and surrounding environmental matrices. Due to which they are most frequent contaminants detected worldwide in agricultural food commodities including spices [14]. Screening and profiling of pesticide residues in culinary/medicinal spices and herbs has been significantly reported time to time due to well-known adverse health and environmental impacts of different agro-pesticide residues [15-16]. A research study on surveillance of pesticides, mycotoxins and heavy metals in commercial spices also emphasised the present status of contamination and adulteration [17]. Several International studies also reported the existence of pesticide residues in a variety of commercial culinary spices [18-19]. Outcomes of several research studies also indicating the omnipresence of pesticide residues in popular spices not only overseas but also in Indian scenario [20-21]. Organochlorine pesticides as well as synthetic pyrethroids are more prominent amongst the group of pesticides often detected in culinary spices [21-22].

Indiscriminate use of insecticides and herbicides during spice cultivation or post harvesting storage may enhance the productivity and shell life but act as major source of contamination. The present study aims to investigate the quality of popular culinary spices available in local market by emphasizing on pesticide residues assessment with special reference to organochlorine and synthetic pyrethroids. The study output will be helpful to provide contemporaneous status for pertinent researchers.

MATERIALS AND METHODS

An improvised Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method for multiresidue pesticide analysis was used in place of traditional methods. QuEChERS method has been considered as relatively modified, highly efficient, quick and applicable in processed matrices of spices, herbs, vegetables and crops [23-24].

Sample collection

Sporadic samples of sixteen types of common culinary spices (Table 1) namely turmeric, coriander, red chilli, cumin, black pepper, green cardamom, black cardamom, fenugreek, caraway, dry-ginger, mace, aniseed, nutmeg, cinnamon, cassia and cloves were collected as a whole from local markets. Five representative samples of each type of spices were analyzed to observe levels of selected pesticides.

Table 1 Trade name, botanical name and details of culinary spices under study criteria

S. No.	Trade name	Botanical name	Family	Edible part used as spice
1	Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome
2	Coriander	*Coriandrum stivum* L.	Apiaceae	Seed
3	Red chilli	*Capsiceum annuum*	Solanaceae	Fruit
4	Cumin seed	*Cuminum cyminum*	Apiaceae	Seed
5	Black pepper	*Piper nigrum*	Piperaceae	Seed
6	Green cardamom	*Elettaria cardamomum*	Zingiberaceae	Fruit
7	Black cardamom	*Amomum Subulatum*	Zingiberaceae	Fruit
8	Fenugreek	*Trigonella foenumgraecum*	Fabaceae	Seed
9	Caraway	*Carum carvi*	Apiaceae	Seed
10	Dry-ginger	*Zingiber officinall*	Zingiberaceae	Rhizome
11	Mace	*Myristica fragrans*	Myristicaceae	Flower
12	Aniseed	*Pimpinella anisum*	Apiaceae	Seed
13	Nutmeg	*Myristica fragrans*	Myristicaceae	Fruit
14	Cinnamon	*Cinnamomum zeylanicum*	Lauraceae	Bark
15	Bay leaf	*Cinnamomum tamala*	Lauraceae	Leaf
16	Cloves	*Syzygium aromaticun*	Myrtaceae	Bud

Pesticide residues under scope of study

A total of 24 different pesticides belonging to two major classes of pesticide residues viz. organochlorine (14 types) and synthetic pyrethroids (10 types) were assessed in popular culinary spices available in local market. Details of pesticide analyzed are as follows:

Organochlorines (OCs): α-HCH, β-HCH, γ-HCH, δ–HCH, Dicofol, Aldrin, α-endosulfan, β-endosulfan , p,p-DDE, o, p-DDE, o,p-DDD, p,p-DDD, o,p-DDT, p,p-DDT

Synthetic Pyrethroids (SPs): Bifenthrin, Fenpropathrin, λ-cyhalothrin, β-cyfluthrin-I, β-cyfluthrin-II, Cypermethrin-I, Cypermethrin-II, Fenvalerate-I, Fenvalerate-II, deltamethrin.

Chemicals and reagents

All solvents like n-hexane, acetone and ethyl acetate (HPLC grade) were purchased from Sigma-Aldrich. Co. USA, Spectrochem Pvt. Ltd. India and were glass distilled before use. Sodium chloride (NaCl), anhydrous Sodium sulphate (Na_2SO_4) and anhydrous Magnesium sulphate ($MgSO_4$) was procured from Himedia Pvt. Ltd., India. Pesticide standards were procured from Supelco Sigma-Aldrich USA, Fluka Sigma-Aldrich Schweis and Rankem Pvt. Ltd. New Delhi, India.

Sample extraction and cleanup

Spice samples (50 g each) were grinded in high-speed grinder for at least 5 minutes to obtain their homogenized powdery form. Powdered sample (2.0 g) of each spice was taken in triplicate for multi-pesticide residue analysis by a unique and improvised QuEChERS method. The homogenized powder sample was mixed with 8.0 ml mili-Q water, 10 ml ethyl acetate (EtOAc), 4.0 g of activated anhydrous $MgSO_4$, 1.0 g activated NaCl and shaken for 10 min. at 50 rpm on rotospin. The extract was centrifuged for 10 min at 8,000 rpm. 1.0 ml aliquot of extract was cleaned with the mixture of 100 mg primary secondary amine (PSA), 150 mg anhydrous $MgSO_4$ and 10 mg activated charcoal. The extract was again shaken for 10 min. at 50 rpm on rotospin and then centrifuged for 5 min at 8,000 rpm. The supernatant was collected in 2.0 ml vial mixed with 5.0 microliter acidified ethyl acetate (with 5% formic acid) to prevent microbial degradation.

Sample analysis

Clean extract (1.0 microliter) of sample was injected in Gas Chromatograph (GC) for the multi pesticides (OCs and SPs) residue analysis using ^{63}Ni electron-capture detector (ECD). Presence of pesticide residues were further confirmed on gas chromatograph - mass spectrometry (GC-MS) as per standard test method.

RESULTS AND DISCUSSION

The QuEChERS method proved to be a rapid and highly effective when applied to the determination and surveillance of a wide range of pesticides in spices. The validation results were highly satisfactory. The percent (%) recovery, limit of detection (LOD) and retention time (RT) of 24 pesticides in 16 different spices by QuEChERS method were also evaluated. (Fig 1) represents GC-ECD chromatogram of analyzed organochlorine and synthetic pyrethroids pesticide residues. The analyzed PESTICIDES (RT in min.) were α-HCH (6.46), β-HCH (6.89), γ-HCH (7.40), δ–HCH (7.62), Dicofol (11.76), Aldrin (11.88), o,p-DDE (14.88), α-endosulfan (15.19), p,p-DDE (16.41), o,p-DDD (16.65), β-endosulfan (17.50), p,p-DDD (18.23), o,p-DDT (18.67), p,p-DDT (20.36) belonging to organochlorine (OCs)class while Bifenthrin (23.85), Fenpropathrin (25.76), λ-cyhalothrin (26.41), β-cyfluthrin-I (27.05), β-cyfluthrin-II (31.51), Cypermethrin-I (31.61), Cypermethrin-II (32.40), Fenvalerate-I (34.45), Fenvalerate-II (35.16), deltamethrin (35.16) of Synthetic Pyrethroids (SPs) group. Limit of Detection (LOD) was also calculated for analysed pesticides and the range varied from 0.001-0.004 mg kg^{-1}. The percent recovery of OCs ranged from 78.50% (for o,p-DDD) - 99.36% (for γ-HCH) while in case of SPs it was varied from 71.24% (for Fenvalerate-II) - 80.25% (for Bifenthrin).The fortification level was 0.01 mg kg^{-1}. The pesticides residue recorded below the detection limit were considered as non-detectable (ND). The level of pesticide residues in various spices were compared with their maximum residues limit (MRL) fixed by Prevention of Food Adulteration Act. PFA, 1954 and Codex, 1986, 7-8.

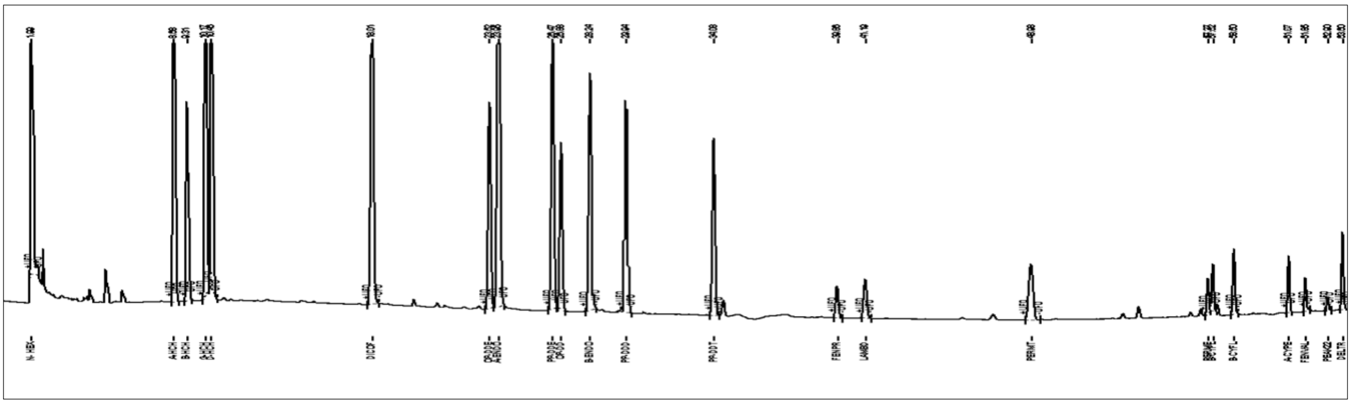

Fig 1 GC-ECD chromatogram of organochlorine and synthetic pyrethroids pesticides

Five samples of each sixteen types of spices were analyzed in replicates to observe the contamination level, if any. The observed results of analysis are represented in (Table 2). Dicofol (BDL - 0.019 mg kg^{-1}), endosulphan (ND - 0.257 mg kg^{-1}) amongst OCs and Cypermethrin (ND - 3.158 mg kg^{-1}), a synthetic pyrethroids, were observed in few turmeric samples. Amongst these, only one value of cypermethrin - 1 pesticide in one turmeric sample was found above MRL (MRL for cypermethrin in turmeric is 3.00 mg kg^{-1} as per PFA/CODEX guidelines). However, in coriander, one out of five samples were found to be contaminated with endosulfan (ND - 0.257 mg kg^{-1}) only. In red chilli samples, three out of five samples were found to be contaminated with γ-HCH (OC), observed levels ranging from 0.513 mg kg^{-1} - 1.593 mg kg^{-1} while two out of five samples have significant levels of dicofol (0.856 mg kg^{-1} - 2.310 mg kg^{-1}). Only level of dicofol (OC) in one sample (2.310 mg kg^{-1}) of red chilli was found above MRL (MRL for dicofol in red chilli is 1.0 mg kg^{-1} as per PFA/CODEX guidelines). In case of cumin seeds, γ-HCH (0.048 - 0.351 mg kg^{-1}) was present in two samples while endosulfan (ND-0.538 mg kg^{-1}) was only detectable in one sample. All the observed levels were found below MRL.

Trace amount of Cypermethrin-II (0.514 mg kg^{-1}), less than MRL, was observed in one sample of aniseed. In two samples of nutmeg, only γ-HCH was detected and the observed levels were 0.152 mg kg^{-1} and 0.586 mg kg^{-1} respectively. These pesticide levels are below to MRL.

The obtained results clearly revealed that several culinary spice samples like black pepper, cardamom (green and black), fenugreek, caraway, dry ginger, mace, cinnamon, bay leaf and clove have not shown the presence of pesticide residues in detectable range. Only γ-HCH, endosulfan and dicofol were frequently detected organochlorine pesticides in limited number of samples of turmeric, coriander, red chilli, cumin and nutmeg. γ-HCH, popularly known as Lindane, is used as insecticide to protect fruity portion of spice-oriented crops as well as in seed treatment. However, use of γ-HCH has been restricted due to its prolonged persistence and more adverse impacts on human health and surrounding environment, but, its indiscriminate use as well as illegal applications may be a source of contamination. Endosulfan (α- and β- isomers) has been an extensively used insecticide since several years. It is also a persistent organic pollutant having greater extent of bioaccumulation vis a vis well-known toxic and endocrine disruptive behaviour. Dicofol is a well-known miticide and used against spider mite during post harvesting process or storage of spices. Cypermethrine and Cyfluthrin-II belongs the class of synthetic pyrethroids which are found in detectable range in a few samples of turmeric and aniseed. Cypermethrine 1 & 2 as well as Cyfluthrin are effective insecticides having large scale commercial agricultural applications.

Pesticides enter and contaminate spices or other plant products via spillage and volatilization of residues from pesticide treated soil or storage. Though, the pesticides are known toxic chemicals their residues were found at very low level in few spices out of which only one observation has been reported above its MRL. These levels may even go much lower after home processing like washing, frying and cooking of food. It is known that certain physical and chemical conditions like washing, heat treatment, steaming treatment etc. are known to reduce the level of pesticides in food commodities. Traces of pesticide residues present in few spices samples may be due to their injudicious use and high exposure period during harvesting or storage. In view of above observations, it should be mandatory for the spices producers, consumers and stakeholders to periodically monitor pesticide residues in every batch so that the consumers are assured to food safety and quality.

Table 2 Observed levels (in mg kg^{-1}) of pesticide residues in common culinary spices

Pesticide	Class	Turmeric	Coriander	Red chili	Cumin seed	Black pepper	Green cardamom	Black cardamom	Fenugreek	Caraway	Dry-ginger	Mace	Aniseed	Nutmeg	Cinnamon	Bay leaf	Cloves
α-HCH	OC	BDL	BDL	ND - 0.573	ND - 0.048	ND	ND	ND	ND	ND	ND	ND	ND	ND - 0.152	BDL	ND	ND
β-HCH	OC	BDL	BDL	ND - 1.590	ND - 0.351	ND	ND	ND	ND	ND	ND	ND	BDL	ND - 0.586	BDL	ND	ND
γ-HCH	OC	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
δ-HCH	OC	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
Dicofol	OC	ND - 0.019	BDL	ND - 2.310	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
Aldrin	OC	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
o,p-DDE	OC	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
α-endosulfan	OC	ND - 0.174	ND - 0.257	BDL	ND- 0.538	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
p, p-DDE	OC	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
o, p-DDD	OC	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
β-endosulfan	OC	ND - 0.257	BDL	BDL	ND- 0.107	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
p,p-DDD	OC	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
o,p-DDT	OC	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
pp-DDT	OC	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
Bifenthrin	SP	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
Fenpropethrin	SP	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
λ -Cyhalothrin	SP	BDL	BDL	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
Cyfluthrin-I	SP	BDL	BDL	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	ND- 0.514	BDL	BDL	ND	ND
Cyfluthrin-II	SP	BDL	BDL	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
Cypermethrin-I	SP	ND – 0.528	BDL	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
Cypermethrin-II	SP	ND – 3.158	BDL	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
Fenvalerate-I	SP	BDL	BDL	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
Fenvalerate-II	SP	BDL	BDL	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND
Delta-methrin	SP	BDL	BDL	BDL	BDL	BDL	BDL	ND	ND	ND	ND	ND	BDL	BDL	BDL	ND	ND

OC- Organochlorine; SP-Synthetic Pyrethroids; BDL- Below Detection Limit; ND- Non-detectable

CONCLUSION

Tolerable limits of pesticide residues in spices are prime screening factors for their rejection or restrictions for export and other usage sectors. Besides adverse human health impacts, pesticide contamination severely affects the export potential of these high value commodity crops resulting in low income of farmers and reduced market value of their crop. Pesticide contamination in culinary spices may occur due to prolonged exposure during harvesting period or storage. In addition, substandard post harvesting operations viz. picking, drying, curing, initial packaging etc. may also cause contamination. Environmental and food safety issues in case of culinary spices encounter relatively weak attention during domestic use as well as market or export purpose, especially in low volume spice trade networks due to lack of proper storage conditions, processing system and packaging as well. Maximum local trade and marketing in rural and sub-urban area is still based on open/unpacked spice articles which may also be an indirect route of exposure of storage or domestic pesticides. Therefore, time to time science-based quality assessment of culinary spices is required as per standard food safety guidelines for quality assurance, quality control and to propose appropriate controlling measures.

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