PESTICIDE CONTAMINATION IN CAULIFLOWER AND RELATED HEALTH RISK ASSESSMENT IN GURUGRAM, INDIA

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ABSTRACT

Imprudent use of pesticides in agriculture has resulted in increased concentrations of pesticides in food. Consumption of pesticide-contaminated vegetables can cause many chronic and acute health problems for consumers. Vegetables are an important part of the human diet as they are a source of vitamins and minerals. Cauliflower (*Brassica oleracea var. botrytis)* is a frequently consumed vegetable in India and has high nutritional value. In the present study, cauliflower was scanned for organochlorines, organophosphates, synthetic pyrethroids and some other classes of pesticides. The pesticides were extracted from the vegetable using quick, easy, cheap, effective, rugged, and safe extraction techniques, and the extracts were analysed on Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS). All samples were contaminated with pesticide residues. The pesticides detected in the samples were aldrin (0.018 mg/kg), chlorpyrifos (0.02 - 0.04 mg/kg), cypermethrin (0.03 mg/kg), deltamethrin (0.018 - 0.04 mg/kg), malathion (0.02 mg/kg), o,p-dichloro-diphenyl-trichloroethane (0.02mg/kg), and phorate (0.02 - 0.03 mg/kg). Cauliflower samples collected from Manesar showed the presence of chlorpyrifos and cypermethrin. Concentrations of chlorpyrifos were above the maximum residue limits recommended by the Prevention of Food Adulteration Act, 2004. The health risk index was highest for aldrin (0.08) and lowest for malathion ($2.67 \cdot 10^{-5}$).

Keywords: *cauliflower, GC-MS/MS, health risk index, pesticide, maximum residue limit*

INTRODUCTION

Vegetables are a good source of vitamins, fibres, folic acid, and minerals, and hence they are important integrant of the human diet. To meet the growing need for vegetables of a large population, the Indian agriculture sector is under the burden. The situation is further aggravated due to the attack of the pests on the crops. The loss of crop production can be reduced to a great extent by the use of pesticides in agriculture. The pesticides are chemicals or mixtures of chemicals that repel, reduce, and destroy the population of pests. Eighty-five to ninety percent of sprayed pesticide does not reach the target organisms, and may get stockpiled in the environmental compartments like air, water, and soil [1]. The

unregulated use of pesticides can harm the farmers, local populace, and the environment. The effects of pesticides on human beings depend upon the dose, route (inhalation, ingestion, and dermal), duration, and frequency of exposure [2]. The sensitivity of human beings also varies toward the toxicity of the same pesticide. Children, pregnant women, and senior population are more sensitive as compared to adults. India is the second-largest producer of pesticides amongst Asian countries and has the twelfth position globally in the use of pesticides with 3.75 % of the total pesticide consumption of the world [3]. Thirteen to fourteen percent of the pesticide consumption of the country goes to vegetable production [4]. Cauliflower is a member of the cruciferous family and has high nutritional value. It contains vitamin C, fibre, folic acid, and minerals. The vegetable also holds sugars, water, starch, protein, fat, energy, phytochemicals, riboflavin, ascorbic acid, and carotene [5, 6]. Most of the pesticides used on cauliflower in India are listed as extremely, highly, and moderately hazardous (classes Ia, Ib, and II) by the World Health Organization [7]. Central Insecticides Board and Registration Committee (CIBRC) have registered 234 pesticides for agricultural use. Cypermethrin, chlorpyrifos, carbofuran, dimethoate and phorate come under the commonly used pesticides in India [8].

MATERIALS AND METHODS

Study area

Gurugram is the district of the national capital region (NCR) India, situated at a latitude and longitude of 28.4595° N, 77.0266° E. A total of 4203 industrial units are registered in the industrial area of Gurugram, which are employing 329340 people [9]. The large population of the district results in huge consumption of vegetables, resulting in pressure on agriculture to increase production. The farmers may use an exorbitant amount of pesticides to fulfil the gap between demand and supply. Keeping in view the above facts, the assessment of different pesticides has been carried out in cauliflower in Gurugram, national capital region, India.

Sampling

Twelve different sites were identified by using the grid selection method for the sampling of cauliflower (*Brassica oleracea* var. *botrytis*.) (Figure 1). Around 2 kg of cauliflower samples were collected in polyethylene bags from selected sites during November 2019. The samples were labelled and placed in an icebox and brought to the laboratory within 24 hours of collection. Samples were kept in a deep freezer until analysis, which was accomplished within three days of collection [10].

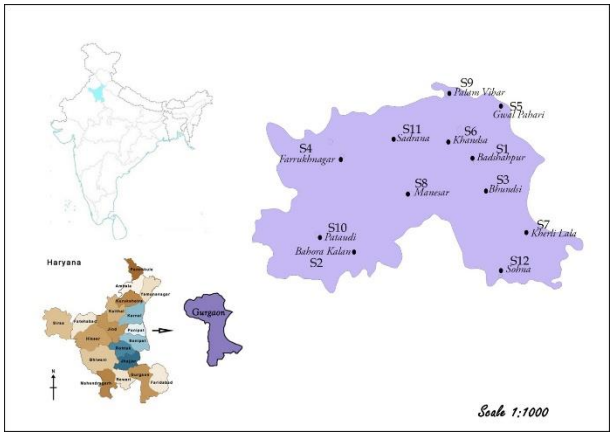

Figure 1. Map of Gurugram and the sampling locations [11, 12]

Extraction and clean-up of pesticide residues

Certified reference grade chemicals and pesticide standards (Sigma Aldrich grade) with purity from 98.5 to 99.5 % were used in the experiments. Quick, easy, inexpensive, effective, rugged, and safe (QuEChERS) extraction technique was used for the extraction of pesticides residue, as shown in Figure 2 [13]. The average recoveries of the pesticides were found to be in the range of 80 to 120 % and linearity lying between 0.991 - 0.999.

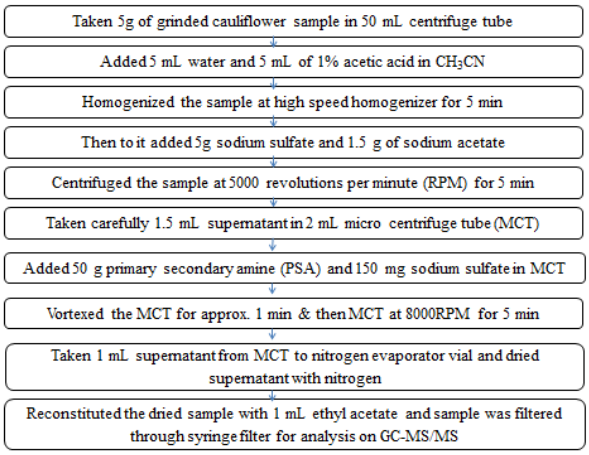

Figure 2. Extraction and clean up procedure of pesticides from cauliflower [13]

Instrument and instrumental condition

GC-MS/MS Triple Quadrupole 7000 D (Agilent) was used for pesticide residue analysis. Separation of compounds was done using a capillary column (ResTek 5Sil MS, 20 M, 0.18 mm ID, 0.18 µm). The injector temperature was 280 °C, and injector volume was 2µL (split injection). The column oven temperature program is shown in Table 1. Argon gas (purity 99.99 %) and helium gas (purity 99.99 %) were used as collision induced gas and carrier gas in the MS detector.

Table 1. Column oven temperature program

Temp. (°C)	Rate (°C/min)	Hold time (min)	Total time (min)
70		1	1
180	20	0	6.5
240	6	0	16.5
300	8	8	32

Health risk assessment

According to the United States Environmental Protection Agency (USEPA) "human health risk assessment is the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future" [14]. The health risk index (HRI) was calculated by using equations 1 and 2. HRI > 1 shows that the vegetable is not safe for human consumption [15].

$$HRI = EDI/ADI \quad (1)$$

$$EDI = \frac{(\text{Mean residue level} \cdot \text{food consumption})}{(\text{Body weight})} \quad (2)$$

where is: EDI - estimated daily intake (mg/kg bw/day), and ADI - an acceptable daily intake (mg/kg bw/day). In the present study, the average daily intake of cauliflower and the average body weight of adult were taken as 24 g/day and 60 kg, respectively [16].

RESULTS AND DISCUSSION

A total of twelve samples of cauliflower were analysed on GC-MS/MS in triplicates for organochlorines (OCs), organophosphate (OPs), pyrethroid, and synthetic pyrethroid (SP) classes of pesticides (Table 2). The concentrations of pesticides detected in the vegetable and their maximum residue limits are presented in Table 3. All samples were found to be contaminated with various types of pesticides. The identified pesticides include aldrin, chlorpyrifos, cypermethrin, deltamethrin, malathion, o,p-dichlorodiphenyltrichloroethane (DDT), and phorate. chlorpyrifos, cypermethrin, deltamethrin, malathion, and o,p-DDT belong to WHO hazard class II, meaning that they are moderately dangerous, and phorate belongs to WHO class Ia which means it is highly dangerous.

Organophosphates (OPs), synthetic pyrethroids, and organochlorines (OCs) pesticide residues were present in 50 %, 41.67 %, and 16.67 % of the total samples collected from Gurugram, respectively. Amongst OPs, chlorpyrifos was present in 3 samples of cauliflower, phorate in 2 samples and malathion in 1 sample. Amongst OC's, aldrin (0.018 mg/kg), and DDT (0.02 mg/kg) were detected in the cauliflower samples collected from Bhondsi and the New PalamVihar. Deltamethrin (a pyrethroid), was detected in four samples collected from Badshapur (0.018 mg/kg), Gwal Pahari (0.028 mg/kg), Khandsa (0.027 mg/kg), and Sohna (0.037 mg/kg).

Table 2. Pesticide residues scanned in the cauliflower samples collected from Gurugram

Pesticides class	Name of the compound(s)
Organochlorines (OCs)	aldrin, endosulfan, hexachlorocyclohexane (HCH), chlorbenzilate, chlordane, chlorfenapyr, cis chlordane, dicofol, dieldrin, endrin, endosulfan sulfate, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxide, methoxychlor, mirex, hexachlorobenzene (HCB), o,p-dichlorodiphenyldichloroethane (DDD), o,p–dichlorodiphenyldichloroethylene (DDE), o,p dichlorodiphenyltrichloroethane (DDT)
Organophosphates (OPs)	chlorpyrifos, chlorfenvinphos, coumaphos, diazinon, dichlorvos, edifenphos, ethion, ethyl paraoxan, etrimfos, fenitrothion, formothion, malathion, methyl paraoxon, parathion ethyl, parathion methyl, fonophos, phorate, phorate sulfone, phorate sulfoxide, prothiofos.
Pyrethrins and synthetic pyrethroid	allethrin, bifenthrin, cypermethrin, cyfluthrin, cyhalothrin, deltamethrin, esfenvalerate, etofenprox, fenpropathrin, fenvalerate, flucynthrinate, fluvalinate, permethrin, lambda permethrin, tefluthrin, transfluthrin
Others	alachlor, butachlor, captan, chiorothalonil, chlorpropham, etoxazole, fluchloralin, metribuzin, molinate, oxyflourfen, pendimethalin, propanil, 4,4 dichlobenzophenone, 4-Bromo-2 chlorophenol, trifluralin, vinclozolin

Table 3. Concentration of pesticides residues in cauliflower

Sites	Pesticides residue (mg/kg)[a]						
	A	CH	CP	DM	MH	D	PH
S1	BDL	BDL	BDL	0.018±0.025	BDL	BDL	BDL
S2	BDL	BDL	BDL	BDL	BDL	BDL	0.03 ± 0.173
S3	0.018 ± 0.023	BDL	BDL	BDL	BDL	BDL	BDL
S4	BDL	0.03 ± 0.02	BDL	BDL	BDL	BDL	BDL
S5	BDL	BDL	BDL	0.028 ± 0.02	BDL	BDL	BDL
S6	BDL	BDL	BDL	0.027 ± 0.011	BDL	BDL	BDL
S7	BDL	0.02 ± 0.021	BDL	BDL	BDL	BDL	BDL
S8	BDL	0.04 ± 0.12	0.032 ± 0.0	BDL	BDL	BDL	BDL
S9	BDL	BDL	BDL	BDL	BDL	0.02 ± 0.002	BDL
S10	BDL	BDL	BDL	BDL	0.02 ± 0.033	BDL	BDL
S11	BDL	BDL	BDL	BDL	BDL	BDL	0.023 ± 0.001
S12	BDL	BDL	BDL	0.037 ± 0.01	BDL	BDL	BDL
MRLs* [17, 26]	0.1	0.01	1 (2009)	0.1 (2004)	3.0	3.5	0.05

[a] Mean value of triplicates, BDL- Below detectable limit, Limit of detection (LOD): 0.005mg/kg, *MRLs refer to maximum residue limits, A - Aldrin, CH - Chlorpyrifos, CP - Cypermethrin, DM - Deltamethrin, MH - Malathion, D - o,p-DDT, PH - Phorate, S1 - Badshapur, S2 - Bahora kalan, S3 - Bhondsi, S4 - Farrukhnagar, S5 - Gwal Pahari, S6 - Khandsa, S7 - Kherli lala, S8 - Manesar, S9 - New PalamVihar, S10 - Pataudi, S11 - Sadrana, S12 – Sohna

Cypermethrin, a synthetic pyrethroid, was found in one sample collected from Manesar along with chlorpyrifos. The concentration of chlorpyrifos in all the samples was higher than the recommended values of the food safety and standards authority of India (FSSAI) [17]. Chlorpyrifos was 2, 3, and 4 times higher than the maximum residue limit in samples collected from the S7, S4, and S8 sites, respectively. The concentrations of other pesticides were below the maximum residue limits of FSSAI (2011). The presence of detected pesticide residue in the cauliflower was due to the use of these pesticides by the farmers in their fields. According to a study conducted by Yadav et al. (2016), chlorpyrifos, aldrin, DDT, phorate, and malathion are used by 7.2 %, 24.5 %, 40 %, 87.2 %, and 90 % of the farmers in the Gurugram, respectively [1]. The chlorpyrifos concentrations found in the present study are consistent with the concentration reported in cauliflower of the Andaman Islands, India, and Faisalabad, Pakistan [18, 19]. The pesticide

residues reported in this study are higher than the reported pesticide residues in Hisar, Agra, and Andhra Pradesh [16, 20, 21]. Chlorpyrifos residues reported in cauliflower from Hisar are in line with the concentration found in the present study [22]. Malathion was detected in one of the cauliflower sample, and its concentration was higher than the reported concentration in Agra and Hisar [16, 20]. One sample of cauliflower was contaminated with DDT. The concentration of DDT found in the present study is consistent with the concentration reported in cauliflower in Hisar [22]. DDT concentrations in the present study were higher than the concentrations reported in cauliflower in Agra and West Bengal [10, 23]. Even after ceasing the use of aldrin and DDT in agriculture, they were found in cauliflower samples. The presence of DDT may be attributed to its long half-life period in the environment. Unregulated use of DDT and aldrin may also be one of the causes of the presence of these pesticides in the samples. Cypermethrin was reported in one sample, and its concentration was in good agreement with the findings of Mukherjee [24]. Deltamethrin residues were found in four samples. Deltamethrin, chlorpyrifos, and cypermethrin were also found in cauliflower samples collected from the Faisalabad district of Pakistan. Some of our samples had higher concentrations of pesticides than reported concentrations in Pakistan, while some had lower concentrations of all three pesticides [5]. In Greece field trials, the average concentration of cypermethrin (0.198 mg/kg) and deltamethrin (0.047 mg/kg) was found to be higher than the concentrations in this study, but the case was reversed in the case of market samples [6]. In the present study, two samples of cauliflower were reported to have phorate contamination. The presence of chlorpyrifos, cypermethrin, malathion, and phorate has also been found in cauliflower samples collected during 2014 - 2015 from different parts of India under the monitoring of pesticide residues at the national level. The maximum number of cauliflower samples collected during this mission had higher chlorpyrifos concentrations than the maximum residue limit (MRL) determined by the Food Safety and Standard Authority of India (FSSAI), which are consistent with the results of the present study [25].

Estimated daily intake and health risk assessment

Estimated daily intake (EDI) and health risk index (HRI) for the various pesticides calculated in the samples were shown in Table 4. The health risk index was the highest for aldrin ($8 \cdot 10^{-2}$) and the lowest for malathion ($2.67 \cdot 10^{-5}$). These findings are consistent with the results of other researchers [16, 27]. There is no potential health risk to the consumers by the lifetime consumption of cauliflower as the indices for all the pesticides are less than 1 (Table 4).

Table 4. Health risk assessment in consumers due to consumption of cauliflower

Pesticide	EDI (mg/kg/day)	ADI (mg/kg/day) [28]	HRI	Health risk
A	$8.0 \cdot 10^{-6}$	0.0001	0.08	No
CH	$1.2 \cdot 10^{-5}$	0.01	$1.2 \cdot 10^{-3}$	No
CP	$1.2 \cdot 10^{-5}$	0.02	$6 \cdot 10^{-4}$	No
DM	$8.78 \cdot 10^{-6}$	0.01	$8.78 \cdot 10^{-4}$	No
MH	$8.0 \cdot 10^{-6}$	0.3	$2.67 \cdot 10^{-5}$	No
D	$8.0 \cdot 10^{-6}$	0.01	$8.0 \cdot 10^{-4}$	No
PH	$1.06 \cdot 10^{-5}$	0.0007	$1.5 \cdot 10^{-2}$	No

CONCLUSION

All the samples were contaminated with various types of pesticide residues indicating variable use of pesticides. Two organochlorines (aldrin, and o,p-DDT), three organophosphates (chlorpyrifos, malathion, and phorate), and two pyrethroids (cypermethrin and deltamethrin) were detected among the analysed pesticides. Cauliflower sample collected from Manesar showed the presence of two pesticides that are chlorpyrifos (0.04 mg/kg), and cypermethrin (0.03 mg/kg), while the remaining samples showed a single pesticide residue. The presence of two different types of pesticide residue in the

cauliflower sample collected from Manesar pointing towards the use of various pesticides in the Manesar. The concentration of chlorpyrifos in all samples was higher than the recommended values of PFA 2004. Chlorpyrifos concentration was four times higher than the FSSAI MRL in one sample. Aldrin (0.018 mg/kg) and DDT (0.02 mg/kg) have been detected in cauliflower samples even after their use was banned in agriculture in India. The health risk index (HRI) was highest for aldrin (0.08) and lowest for malathion ($2.67 \cdot 10^{-5}$). There is no significant health effect due to the consumption of cauliflower in the study area as the HRI values were below 1. Although there is no significant health risk due to consumption of the cauliflower in the study area, it does not ensure that the consumers are free from health risks. It is recommended from the current study that the government authorities should conduct a larger level study to assess the health risk in the study area.

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