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Magnitude and Frequency of Pesticide Residues in Farmgate Samples of Cauliflower in Punjab, India

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Abstract Fifty samples of cauliflowers were collected from intensive vegetable growing areas in Punjab during 2007–08 to determine the magnitude and frequency of pesticidal contamination. The estimation of pesticide residues representing three major chemical groups i.e. organochlorine, organophosphorus and pyrethroids was done by using a multiresidue analytical methodology employing GC-ECD and GC-FTD systems with capillary columns and confirmed by GC–MS. Forty-two per cent samples were found to be contaminated with low but measurable amounts of residues. Among the three chemical groups, the organophosphates were dominant followed by organochlorines and pyrethroids. However, none of the samples were found to contain the residues of these insecticides above their respective maximum residue limits (MRL). On the basis of these limited observations, it is suggested that more extensive surveillance studies covering major cauliflower growing areas in the state be carried out to know the status of contamination, which may serve as a base for the national regulatory authorities to decide future policy of chemical use on vegetables.

Keywords Maximum residue limit (MRL) · Surveillance · Organochlorine · Organophosphorus · Pyrethroids · Cauliflower

Cauliflower (*Brassica oleracea var. botrytis*. L.) is one of the important cruciferous vegetable crops of India. It is widely cultivated throughout the sub-tropical parts of north India. In Punjab, the area under cauliflower was 5.59 thousand hectares with a production of 131.40 thousand tones (Anonymous 2007). The crop is attacked by a number of insect pests such as stem borer [*Hellula undalis* (Fabricius)], diamond-back moth [*Plutella xylostella* (Linnaeus)], tobacco caterpillar [*Spodoptera litura* (Fabricius)] and aphid [*Lipaphis erysimi* (Kaltenbach)]. These insect pests are attacked at various stages of crop growth, which require the use of pesticides for quality produce. Repeated application of pesticides may lead to undesirable residues on consumable parts of cauliflower. It is well recognised, that there are risks associated with the consumption of pesticide-treated crops because of the presence of residues on them. Therefore, the rational recommendation of a pesticide requires that it must not only provide an effective control of pests, but at the same time its residues on the commodity must also be toxicologically acceptable. These residues, if present in excess, may prove hazardous to the health of the consumers.

Several studies on the contamination of different vegetables over the years have been conducted in the state and residues of different insecticides, including those which are not recommended for use on vegetables, have been invariably detected (Chahal et al. 1997 and Chahal et al. 1999). On the other hand, analysis of farm gate vegetables carried out by various workers in India, revealed contamination mostly with organophosphorus and pyrethroids insecticides, indicating clearly the changes in usage pattern from organochlorine to other groups of pesticides (Mukherjee and Gopal 1996; Shah et al. 2000; Kole et al. 2002; Kumari et al. 2003; Deka et al. 2005; Battu and Joia 2006). Keeping in view this fact, present studies were undertaken to know the extent and magnitude of contamination of cauliflower with residues of pesticides belonging to different groups.

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Materials and Methods

Fifty samples of marketable size cauliflower (1 kg each) were collected from intensive vegetable growing areas viz Jalandhar, Amritsar and Hoshiarpur districts of Punjab state in India at monthly intervals, in the crop season during 2007–08. The relevant information regarding pesticide usage on cauliflower crop was collected from the farmers at the time of sampling. Composite sample consisted of 1 kg was cut into small pieces and macerated in a grinder.

A representative 50 g sample of chopped and macerated cauliflower curds was dipped for overnight into 100 mL acetone in an erlenmayer flask. The extract was filtered through glass wool plugged in filtering funnel into 1 L separatory funnel. The residual material was rinsed with acetone and transferred to the same separatory funnel. The contents of separatory funnel were diluted with 600 mL brine solution and partitioned twice into 100 and 75 mL dichloromethane and twice into 100 and 75 mL hexane. The combined organic layers containing the insecticide residues were drained into 500 mL beaker through 1.5 inch layer of anhydrous sodium sulphate supported on a pre washed glasswool in a funnel. The sodium sulphate was washed with an additional 25 mL of dichloromethane. The combined extracts thus obtained were concentrated to 2–3 mL in *vacuo* in a rotary evaporator at a temperature below 35°C.

The extracts thus obtained were cleaned up by column chromatography using silica gel (60–120 mesh) as an adsorbent. Before use, the silica gel was activated at 110°C for 2 h. The extract was mixed with a mixture of silica gel (10–12 g), anhydrous sodium sulphate (10 g) and 2 g activated charcoal. A glass column (60 cm × 1.5 cm i.d.) containing 40 mL dichloromethane supported on a cotton plug was used for cleanup. Sample slurry was prepared using dichloromethane and transferred to the column. The glass beaker containing extract was rinsed with acetone and was transferred to the column. The column was allowed to stand for 90 min. Then the dichloromethane present in the column was eluted drop wise. When about 5 mL dichloromethane remained on the surface of the adsorbent, the extract was eluted with 150 mL of freshly prepared solvent mixture of dichloromethane: hexane (1:1, v/v). The eluate was concentrated to near dryness in a rotary evaporator under *vacuum* and transferred to 5 mL of acetone for further analysis.

The quantification of residues was carried out with a gas liquid chromatography (GLC) (Shimadzu Model GC-2010) equipped with an electron-capture detector (ECD) and flame thermionic detector (FTD). Organochlorines (OC) and pyrethroids (SP) insecticides were analysed with the help of ECD (^{63}Ni) and a capillary column DB-5 (30 m × 0.32 mm i.d. × 0.25 μm film thickness) with split ratio 1:10. The GLC working conditions were as follows: nitrogen flow rate, 30 mL min^{-1}; injection port, 290°C; and detector, 310°C. The column temperature was initially maintained at 170°C for 13 min, then increased at the rate of 3°C min^{-1}, for 12 min, to the final temperature of 270°C. Organophosphates (OP) insecticides were analysed with the help of FTD and a capillary column DB-1 (10 m × 0.53 mm i.d. × 2.65 μm film thickness) splitless. The GLC working conditions were as follows: nitrogen flow rate, 60 mL min^{-1}; hydrogen flow rate, 3 mL min^{-1}; air flow rate, 145 mL min^{-1}; injection port, 280°C; and detector, 310°C;. The column temperature was initially maintained at 170°C for 5 min, then increased gradually to 240°C.

Table 1 Recovery of different insecticides from fortified samples of cauliflower

Insecticide(s)	Level of fortification (mg kg^{-1})	Mean per cent recovery ± S.D.
p,p-DDE	0.06	83.33 ± 0.00
p,p′-DDD	0.11	84.85 ± 5.25
p,p′-DDT	0.11	84.85 ± 5.25
Lindane	0.02	91.67 ± 10.41
α- Endosulfan	0.20	90.00 ± 5.00
β- Endosulfan	0.20	83.33 ± 2.89
Quinalphos	0.20	86.67 ± 5.77
Methyl parathion	0.24	88.89 ± 4.82
Chlorpyriphos	0.20	83.33 ± 5.77
Monocrotophos	0.20	88.33 ± 2.89
Cypermethrin	0.40	84.17 ± 7.64
Deltamethrin	0.20	83.33 ± 7.64

Residues were estimated by comparison of peak heights/peak areas of the standards with that of the unknown or spiked samples run under identical conditions. Cauliflower samples were spiked with organochlorines, pyrethroids and organophosphorus insecticides at different levels ranging from 0.02–0.40 mg kg^{-1} and analyzed separately as per the methodology described above. Per cent recovery of these insecticides were found to be consistent and more than 80 per cent (Table 1). Therefore, the results are reported as such without applying any correction factor. The limit of quantification (LOQ) was found to be 0.01 mg kg^{-1} for organochlorines, 0.02 mg kg^{-1} for pyrethroids and 0.05 mg kg^{-1} for organophosphorus (except chlorpyriphos LOQ = 0.01 mg kg^{-1}) insecticides.

Results and Discussion

These studies were conducted by collecting samples of cauliflower from various farms located in extensively

Table 2 Residues of various insecticides (mg kg^{-1}) detected in farmgate samples of cauliflower

Insecticide(s) detected	Number of samples contaminated	Range (mg kg^{-1})	Mean value (mg kg^{-1})	MRL (mg kg^{-1})	No. of samples > MRL
α-Endosulfan	8	0.01–0.04	0.02	2.0	NIL
β-Endosulfan	2	0.01–0.03	0.02	2.0	NIL
Endosulfan sulfate	1	0.01	0.01	2.0	NIL
Acephate	3	0.05–0.37	0.19	2.0	NIL
Chlorpyriphos	3	0.01–0.02	0.02	0.05	NIL
Fenamiphos	5	0.05–0.17	0.08	Not available	–
Profenophos	1	0.10	0.10	0.5	NIL
Quinalphos	3	0.05–0.07	0.06	0.1	NIL
λ-cyhalothrin	1	0.14	0.14	0.2	NIL
β-cyfluthrin	3	0.11–0.18	0.15	Not available	–
Fenpropathrin	2	0.10–0.11	0.11	Not available	–

vegetable growing areas of Punjab, viz; Jalandhar, Hoshiarpur and Amritsar districts. A total of 50 samples (16 from Jalandhar, 17 from Hoshiarpur and 17 from Amritsar) of cauliflower were collected from these locations. All the contaminated samples were confirmed with the help of the gas chromatograph-mass spectrometry (GC–MS).

Samples were analysed to detect organochlorine pesticides like α-HCH, β-HCH, γ-HCH, δ-HCH, heptachlor, aldrin, dicofol, p,p-DDE, p,p′-DDD, pp′-DDT, α-endosulfan, β-endosulfan, and endosulfan sulfate. In some of the samples of cauliflower (11), residues of endosulfan (α-endosulfan, β-endosulfan and endosulfan sulfate) were found to be present. However the levels of residues of these insecticides were found to be below the MRL of 2.0 mg kg^{-1} (Table 2).

The analysis of cauliflower samples revealed the presence of some commmonly used organophosphate insecticides like acephate, chlorpyriphos, fenamiphos, profenophos and quinalphos. However, none of the samples were found to contain residues of any of these insecticides above their respective MRL's (Table 2). Out of these insecticides, only quinalphos is recommended and the occurance of residues of other insecticides reflect the misuse of insecticides on cauliflower.

Samples were analysed to detect pyrethroid insecticides like λ-cyhalothrin, β-cyfluthrin, cypermethrin, deltamethrin, fenpropathrin and fenvalerate. Among the pyrethroids, residues of λ-cyhalothrin, β-cyfluthrin and fenpropathrin were detected in 1, 3 and 2 samples, respectively. The frequency and magnitude of pyrethroid residues in cauliflower was found to be low as compared to organophosphates as only 12 per cent samples were found to contain residue of pyrethroids at low levels. However, the level of residues of these pyrethroids were less than their respective MRL's (Table 2).

In the present studies, though 42 per cent samples of cauliflower were found to be contaminated, but none of the samples revealed the presence of residues of these insecticides above their respective maximum residue limits (MRL's). These results are contrary to the findings of Chahal et al. (1999) who reported 78 per cent samples of cauliflower contaminated with 32 per cent samples showing residues of chlorpyriphos, cypermethrin, deltamethrin, endosulfan, methyl parathion, monocrotophos and quinalphos above the MRL. The analysis of fruits and vegetables samples for pesticide residues under AICRP on Pesticide Residues revealed that majority of the samples were found to be contaminated though the levels were generally below the prescribed MRL's (Agnihotri 1999). In some countries of EEC it was verified that concentration of pesticides in fruits and vegetables in many cases exceeded the permissible levels (Fytianos et al. 1985).

The decline in frequency and magnitude of pesticide residues in cauliflower could be attributed to the fact that farmers are becoming more aware as far as the use of pesticides on vegetables is concerned. This awareness is the result of training and education being regularly imparted by the university to the state farmers regarding safe and judicious use of pesticides. On the basis of these limited observations, it is suggested that more extensive studies need to be carried out to know the frequency and magnitude of pesticide residues in cauliflower, which may serve as a base for the state and national regulatory authorities to take appropriate measures so that pesticide residues on cauliflower are within permissible limit.

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