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Multi-residue determination of pesticides in vegetables and assessment of human health risks in Western Himalayan region of India

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Abstract This study was aimed to determine pesticides concentrations in fresh vegetables and assess human health risks in North-Western Himalayan region of India. Vegetable samples ($n = 300$) collected randomly from different agro-climatic zones were analyzed for 19 pesticides using gas chromatography. Pesticide residues were detected in 116 samples, of which 49 samples exceeded maximum permissible limits established by European Commission. Hexaconazole was most frequently detected in 9.3% samples followed by aldrin (8.3%), alachlor (5.3%), bifenthrin (4.3%), chlorpyrifos (3.7%), metribuzin (2.7%), β-endosulfan, ethion, β-HCH (2%, each), γ-HCH (1.3%), α-HCH, δ-HCH, malathion, heptachlor (1%, each), and α-endosulfan, pendimethalin in 0.7% samples. Human health risk assessment revealed that the percent contribution to acceptable daily intakes of pesticides via dietary intake of vegetables ranged from 0.014 to 39.4% in children and 0.003 to 9.85% in adults. Although hazard index values were < 1 but considering the concentrations of detected pesticide in samples, children were found to be at more risk. Since pragmatic investigations on occurrence of pesticides in vegetables and human health risk assessment from study area have not yet been worked out, so, this study highlights the importance of adopting good agricultural practices, awareness on food safety, monitoring of harmful chemicals in food commodities, and execution of food safety regulations to safeguard environmental and human health.

Keywords Pesticide residues · Vegetables · Gas chromatography · Risk assessment · India

Introduction

Agriculture sector contributes approximately 20% to gross domestic product (GDP) and provides employment to

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over 50% of working population in India (Anonymous, 2021). Agriculture, once considered a rural livelihood sector, is now emerging as a business enterprise. There has been a substantial increase in production of agricultural commodities, especially staples like rice, wheat, pulses, and vegetables. Among various agricultural produce, fruits and vegetable cultivation provide good opportunities for the farmers to achieve rapid economic growth. Therefore, adopting intensive vegetable cultivation practices are now being considered highly profitable and remunerative business among the mountainous farming community of India. Owing to India's diverse agro-climatic conditions, it is the second largest producer of vegetables in the world. According to the National Horticulture Board of India, the agrarian country produced 185.88 million metric tonnes of vegetables during 2018–2019 under cultivation area of 10.1 million hectares (NHB, 2019). Vegetables are good source of vitamins, minerals, dietary fibers, and other micronutrients. Therefore, they are one of the preferred dietary sources and find an important place in food basket of people (Maity & Tripathy, 2004). The average Indian diet constitutes of about 160–250 g of vegetables in the total meal per day.

Presently, high agricultural output is essential to fulfil food and nutritional requirements of over 135 billion Indian population. However, vegetable production is subjected to various pest damages and pesticide usage becomes indispensable to deal with pest-related problems (Sharma et al., 2014). Therefore, to ensure food security, pesticides are used in modern agricultural practices, but absolute dependency on pesticides is not considered sustainable because of their detrimental effects on human, environment, and animal health. The unplanned and unapproved use of pesticides in agricultural practices is leading to contamination of vegetables. Subsequent exposure of these toxic residues through food chain pose significant health risks to consumers (Kumar et al., 2018a). Hence, evaluation of pesticides in commonly grown and consumed fresh vegetables is a vital concern for safeguarding consumer's health.

Since food contamination with pesticides is a major cause of concern for various global food safety regulators, national and international food safety organizations like Food Safety and Standards Authority of India (FSSAI), European Commission, and Codex Alimentarius Commission (CAC) have established maximum residual limits (MRLs) and acceptable daily intake (ADI) values for pesticide residues in vegetables (EC, 2005; FSSAI, 2011).

The comprehensive information on contamination status of fresh vegetables and human health risks associated with their dietary intake from North-Western Himalayan region of India is meager. Therefore, keeping in view the aforementioned facts, the present study was aimed to evaluate pesticide usage by the farmers in prominent vegetable growing hilly areas of Himachal Pradesh (HP) in India, to gain insight into the awareness level of farmers regarding food safety and estimate the levels of pesticides in collected samples. Furthermore, health risks associated with dietary intake of pesticides were also evaluated. Presumably, this is the first extensive study conducted on estimating multiple pesticide residues in freshly collected diverse varieties of vegetables from HP, a hill state in North-Western Himalayan region of India.

Materials and methods

Chemicals and reagents

The pesticides targeted for analysis in this study were organochlorines (α-, β, γ-, δ-HCH, α, β-endosulfan, heptachlor, aldrin, dieldrin, endrin), organophosphates (chlorpyrifos, malathion, ethion), synthetic pyrethroids (bifenthrin), herbicides (metribuzin, pendimethalin, oxyfluorfen, alachlor), and fungicide (hexaconazole). All the individual certified pesticide standards (> 98% purity) were purchased from Sigma-Aldrich. The storage and handling of pesticides was carried out by following the standard operating procedures (Sharma, 2016). The multicomponent standard working solution was formulated by dissolving individual pesticide solutions in n-hexane. All other analytical reagents and solvents used were procured from Merck, India.

Study area and survey on pesticide usage

Himachal Pradesh is a hill state situated in the North-Western Himalayan region of India. The state has wide range of climatic conditions due to which plenty of agri-horticultural products including vegetables are successfully grown in this region. In order to acquire information on type of vegetables grown in the study area, practices associated with pesticide usage, etc., the cross-sectional surveys were conducted in the major vegetable producing blocks of study areas using participatory rural appraisal technique. The information was collected on a pre-validated questionnaire from randomly selected respondents (Online Resource Table S1).

Sample collection

Sampling was performed in accordance with the Codex Alimentarius Commission and Food Safety and Standards Authority of India Guidelines (CAC, 2011; FSSAI, 2015). Three hundred fresh vegetable samples, covering 21 species, were collected by simple random sampling directly from agricultural farms located in three districts of Himachal Pradesh, India (Fig. 1). Sampling points' locations for analyses are presented in Online Resource Table S1. The vegetable samples included tomato, cucumber, beans, okra, brinjal, green chilies, cabbage, onion, cauliflower, potato, bitter guard, radish, garlic, capsicum, green peas, mustard, spinach, cucurbits, coriander, fenugreek, and rye. The sample size was at least 1 kg of edible portion of vegetables. Samples were wrapped in aluminum foil and sealed in sterile polyethylene bags with distinctive identity labels. The sealed packets were then placed in an insulated ice box and transported to residue analysis laboratory. Samples were kept at 4 °C for maximum of 24 h before being analyzed. The edible parts of the vegetables were chopped, macerated, and analyzed to estimate the concentrations of pesticide residues.

Sample preparation

Sample extraction and clean-up for the analysis of pesticides were carried out as per the methods of Selim et al. (2011) with slight modifications. Briefly, 20 g of vegetable sample was mixed with 50 mL of acetonitrile in a stoppered conical flask. The solution was then placed on shaker (Remi India Pvt. Ltd.)

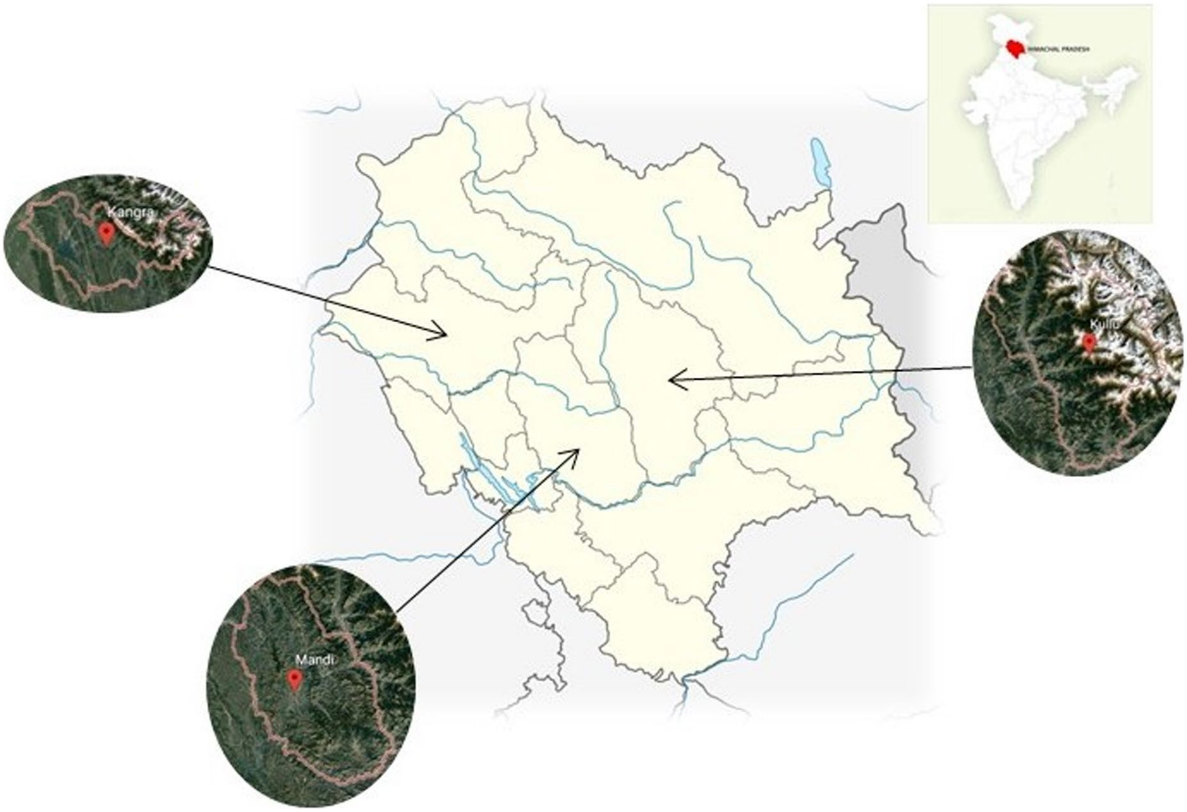

Fig. 1 Map showing different districts of Himachal Pradesh in North-Western Himalayan region of India from where samples were collected

for 3 h. The mixture was filtered and this extraction process was repeated twice. Then, the collected filtrate was transferred to a separating funnel followed by addition of saturated solution of sodium chloride (25 mL) and deionized water (125 mL). The above mixture solution was partitioned with petroleum ether (50 mL) thrice after discarding of aqueous layer every time. The collected organic layer was washed thrice with 50 mL of deionized water. Thereafter, the organic layer was passed over Na_2SO_4 bed and then volatilized to dryness in a vacuum concentrator at 40 °C. The pesticide residues were then re-dissolved in 2 mL of hexane. Finally, 2 µl of reconstituted sample was injected into the gas chromatographic system.

Chromatographic analyses

Chromatographic analysis was carried out by using Perkin Elmer gas chromatograph (Clarus 500 model) equipped with a Ni^{63} electron capture detector and chromatographic column Rtx-5 (30 m × 0.25 mm i.d.). The temperature programmed was as follows: initial temperature of 120 °C (held for 1 min), a 20 °C/min ramp to 180 °C (held for 0 min), a 5 °C/min ramp to 280 °C (held for 10 min) (120/1–20-180/0–5-280/10). The injector and detector temperature were set to 240 °C and 350 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 9.0 mL/min, maintaining 1.0 mL/min through column at split ratio of 1:5. Total Chrom Workstation v 6.3 chromatography software was used for instrument control and data processing.

Method validation and quality assurance

Method validation and quality assurance were done as per European Commission guidelines (EC, 2016). Recovery experiments were performed by fortifying 20 g of the ground vegetable sample with pesticide standards at two fortification levels of 0.05 mg/kg and 0.1 mg/kg. The precision of the procedure (relative standard deviation, % RSD) was evaluated by spiking three identical samples with working pesticide standard solutions at similar and different concentrations. Multi-level calibrations were executed in the concentration range of 0.001–0.01 mg/kg to evaluate method linearity. The peak signal-to-noise ratio of 3/1and 10/1 was considered for determining limit of detection (LOD) and limit of quantification (LOQ), respectively. The method's selectivity was assessed by performing blank analyses to check for any significant interference at the retention time of targeted compounds in the sample.

Health risk assessment

The human health risks due to consumption of vegetables containing pesticide residues were evaluated. The risk assessments were carried out by comparing estimated daily intakes of pesticides (EDI) with their acceptable daily intakes (ADIs). To estimate pesticide exposure, samples that had no detectable levels of pesticide were computed as 0 (zero) for the estimation of dietary exposure (YI et al., 2020). The estimated daily intakes (EDIs) of the pesticides found in vegetables were calculated using the equation $EDI = \frac{Ci \times F}{W}$

Where C_i is average pesticide residue concentration of i^{th} pesticide in analyzed vegetable samples (mg/kg), F is the vegetable intake per person in study area according to National Sample Survey Office, India (NSSO, 2014). W is average human body weight (60 kg for adult and 15 kg for children) (Kumar et al., 2022).

Results and discussions

Survey results

The survey results revealed that the average land holding under vegetable cultivation is 50.42% of the total average land holding of farmers in selected blocks of Mandi, Kangra and Kullu district of HP, India. The major vegetable crops grown in the study area were tomato, cucumber, beans, chilies, onion, potato, okra, radish, brinjal, capsicum, spinach, and cucurbits. Farmers were using chloropyriphos, methyl parathion, malathion, acephate, dimethoate, fenvalerate, α–cyhalothrin, fungicides: bavistin, indofil-45, ridomil, mancozeb and herbicides: pendimethalin, alachlor, metribuzin, etc. to protect their crops (Online Resource Table S1). Furthermore, it was observed that farmers were aware of the purpose for which pesticides are being used. However, their awareness level regarding MRLs, food safety, and human health risks was found to be inadequate.

Verification of the analytical method

The results for LOD, LOQ, recovery percentage, and RSD are shown in Table 1. LOD values were found to be in the range of 0.0002 to 0.0034 (mg/kg) and the LOQ ranged from 0.0005 to 0.01 (mg/kg), which were well below the permitted MRLs, fulfilling the validation requirement. The overall recovery percentage range of spiked samples was 75.25–102.35% for organochlorines, 81.45–98.26% for organophosphates, 89.12% for synthetic pyrethroids, 81.25–101.14% for herbicides, and 79.15% for fungicides, which are within the acceptable recovery range of 70–120%. Similarly the obtained RSD of < 10% also fulfilled the validation requirements. The method was found to be linear with R^2 value between 0.9989 and 0.9999 for all the investigated compounds. The optimized chromatographic conditions led to functional separation of pesticide residue showing symmetrical peaks with good resolutions (Fig. 2a).

These results confirm that the analytical method used in this study was acceptable for determining pesticide residues in real vegetable samples.

Pesticide residue in vegetables

India's North-Western Himalayan region is one of the emerging agricultural areas owing to its fertile land, appropriate climate, heavy rainfall, and agricultural diversification through vegetable cultivation. From the major vegetable growing regions of HP, vegetable samples were analyzed for presence of 19 pesticide residues. The results of analyses are presented in Tables 2 and 3. Out of the 300 samples analyzed, residues of 17 pesticides were detected in 116 samples (38.7%). Cauliflower (61.6%), cucumber (51.5%), beans (50%), tomato (45.2%), okra (43.3%), and green chilies (42.1%) had the highest percentage of detected residues. None of the spinach, cucurbits, coriander, fenugreek, and rye samples contained pesticide residues. Tomato and beans were found to be contaminated with most pesticides followed by cucumber and okra. Since it is a common practice among some people to eat raw, unprocessed tomatoes and cucumber especially as salads, so the occurrence of harmful pesticides can be a serious threat to the consumer's health (Szpyrka et al., 2015). Our results are also in accordance with several other studies

Table 1 Validation parameters for determination of pesticides in vegetables

Peak no	Pesticides	Retention time (min)	LOD (mg/kg)	LOQ (mg/kg)	Recovery (%)	R^2
1	α-HCH	11.795	0.0032	0.011	88.46 ± 0.0017	0.9999
2	β-HCH	13.031	0.0030	0.011	82.14 ± 0.0013	0.9997
3	γ-HCH	13.835	0.0010	0.004	102.35 ± 0.0008	0.9999
4	δ-HCH	14.807	0.0030	0.009	92.34 ± 0.0007	0.9998
5	Chlorpyrifos	15.256	0.0023	0.005	81.45 ± 0.0015	0.9989
6	Metribuzin	16.081	0.0020	0.005	81.25 ± 0.0013	0.9996
7	Pendimethalin	16.350	0.0020	0.005	88.20 ± 0.0004	0.9998
8	Malathion	16.358	0.0021	0.005	89.014 ± 0.0019	0.9989
9	Alachlor	17.334	0.0020	0.005	101.14 ± 0.004	0.9998
10	Aldrin	17.810	0.0011	0.004	92.10 ± 0.0012	0.9999
11	Heptachlor	18.748	0.0002	0.0005	98.12 ± 0.0017	0.9996
12	Oxyflourfen	19.395	0.0003	0.001	90.62 ± 0.0009	0.9999
13	α-endosulfan	19.970	0.0034	0.010	86.08 ± 0.0003	0.9988
14	Dieldrin	21.244	0.0032	0.010	94.15 ± 0.0016	0.9999
15	Hexaconazole	21.550	0.0030	0.010	89.12 ± 0.0015	0.9999
16	Endrin	22.052	0.0030	0.014	102.35 ± 0.0021	0.9991
17	β-endosulfan	22.190	0.0003	0.001	75.25 ± 0.0012	0.9997
18	Ethion	23.483	0.0002	0.0005	98.26 ± 0.0016	0.9994
19	Bifenthrin	26.924	0.0006	0.002	79.15 ± 0.0012	0.9999

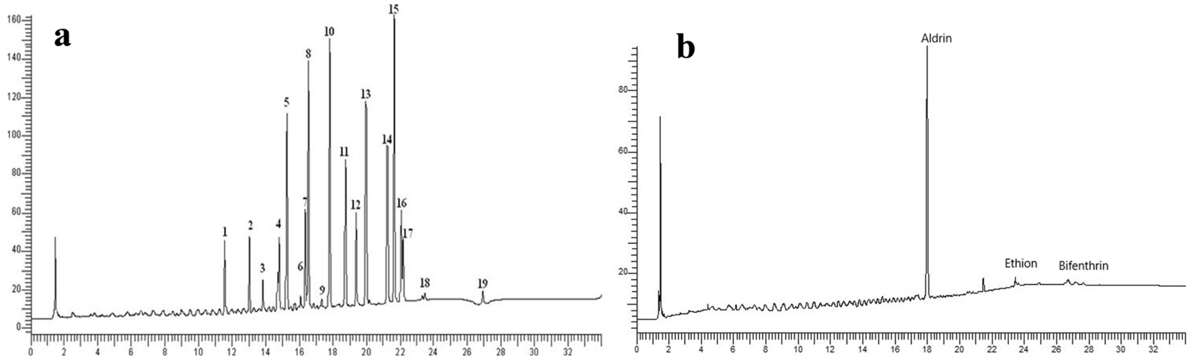

Fig. 2 **a** GC-ECD representative chromatogram of targeted pesticides (1, α-HCH; 2, β-HCH; 3, γ-HCH; 4, δ-HCH; 5, chlorpyrifos; 6, metribuzin; 7, pendimethalin; 8, malathion; 9, alachlor; 10, aldrin; 11, heptachlor; 12, oxyflourfen; 13, α-endosulfan; 14, dieldrin; 15, hexaconazole; 16, endrin; 17, β-endosulfan; 18, ethion; 19, bifenthrin). **b** GC-ECD chromatogram showing presence of aldrin, ethion, and bifenthrin in cucumber sample

conducted globally. The monitoring of pesticide residues in 22 types of fruits and vegetables from Brazil exhibited positive results in 48.3% of the 13,556 samples analyzed (Jardim & Caldas, 2012). Furthermore, it was observed that 59.8% of tomato, 55.1% of beans, and 53.4% of cucumber samples contained one or more pesticide, which is in agreement with the current findings. Studies from South-eastern Poland also revealed the presence of pesticide residues in 36.6% of fruit and vegetable samples (Szpyrka et al., 2015). In an analysis of 506 vegetables from Chinese market during 2010–2013, 30.2% samples were found to be positive for at least 1 pesticide residue (Qin et al., 2015). Similarly, pesticide residues above MRLs were detected in 12.5% of the fruits and vegetable samples collected from Algeria by Mebdoua et al. (2017). From Saudi Arabia, studies on pesticide residues in vegetables revealed that 68.7% of the samples had pesticide residues with concentrations ≤ MRLs, whereas 20.9% exhibited detectable pesticide residues above MRLs (Ramadan et al., 2020).

Among targeted pesticides, hexaconazole was most frequently detected with concentration ranging from 0.01 to 0.2 mg/kg in 9.3% of the vegetable samples followed by aldrin (8.3%), alachlor (5.3%), bifenthrin (4.3%), chlorpyrifos (3.7%), metribuzin (2.7%) in the concentrations range of 0.0054–0.24, 0.010–0.061, 0.0063–0.2, 0.01–0.2, 0.007–0.3 mg/kg, respectively. Isomers of endosulfan, ethion, HCH, malathion, heptachlor, pendimethalin were found scarcely. The most commonly detected hexaconazole, has also been reported to be extensively used as a systemic fungicide on wide variety of vegetables (Liang et al., 2012). The existence of hexaconazole has been attributed to its reduced dissipation rate due to lower temperature and humidity in the region (Maria & Lennart, 2007). However, Codex and European Commission databases reveal that the tolerance limits for hexaconazole in many varieties of vegetables have not yet been established. Samples of tomato (7), cabbage (4), cauliflower (4), okra (4), cucumber (2), beans (2), potato (2), capsicum (2), bittergourd (1) were found to contain hexaconazole residues and out of these 28 positive samples, in 23 samples, the tolerance limits also exceeded. Therefore, its occurrence in high levels is very alarming. Hence, current evidences highlight the need for national and international regulatory attentions to safeguard human health.

The occurrence of organochlorine pesticides such as endosulfan in vegetables which has been banned for its agricultural use in India indicated its unapproved use. Its presence could be attributed to its persistence in the environment or its availability in the local market from previous stocks. Similar findings regarding detection of banned pesticides have also been reported in various food commodities such as fruits and vegetables, honey, and milk from India (Bedi et al., 2016; Kumar et al., 2018b; Ranu, 2015).

There is lack of homogeneity with regards to the established values of MRLs for pesticides in vegetables as various food safety regulators have set their own limits. Population size, density, demographic characteristics, stringency of law enforcement agencies, the awareness among producers and consumers

Table 2 Frequency of samples with pesticide residues in the North-Western Himalayan Region, India

Commodity (*scientific name*)	**Samples analyzed**	**Samples without quantifiable residues**	**Samples with quantifiable residues**		**Samples with residues > MRL**	**Detected pesticides** (concentrations range in mg/kg)
			Single residue	**Multiple residue ($n \geq 2$)**		
Tomato	62	34 (54.8%)	22	6	7 (11.3%)	β-HCH (BDL–0.02) Chlorpyrifos (0.01–0.2) Metribuzin (0.007–0.021) Malathion (0.011–0.1) Alachlor (0.01–0.021) Aldrin (0.015–0.083) Heptachlor (BDL–0.001) α,β-endosulfan (0.020–0.022) Hexaconazole (0.013–0.14) Bifenthrin (BDL–0.059)
Cucumber	33	16 (48.5%)	14	03	4 (12.1%)	α, β, γ, and δ-HCH (0.02–0.5) Alachlor (0.01–0.04) Aldrin (0.031–0.079) Hexaconazole (0.01–0.03) Ethion (0.076–0.12) Bifenthrin (0.0148–0.015)
Beans	32	16 (50%)	10	6	9 (28.2%)	β-HCH (BDL–0.014) Chlorpyrifos (0.021–0.086) Metribuzin (0.007–0.014) Malathion (BDL–0.1) Alachlor (0.02–0.061) Aldrin(0.008–0.042) Heptachlor (BDL–0.001) Hexaconazole (0.04–0.082) β-endosulfan (0.02–0.14) Ethion(BDL–0.078) Bifenthrin (0.011–0.035)
Okra	30	17 (56.7%)	11	2	7 (23.3%)	α-HCH (BDL–0.02) γ-HCH (BDL–0.005) Chlorpyrifos (BDL–0.1) Pendimethalin (BDl–0.01) Alachlor (BDl–0.01) Aldrin (BDL–0.06) Hexaconazole (0.01–0.068) Ethion (0.001–0.02) Bifenthrin (0.03–0.1)
Brinjal (*Solanum melongena*)	25	19 (76%)	6	0	0	α, β, γ-HCH (0.01–0.1) Chlorpyrifos (BDL–0.13) Aldrin (BDl–0.0054)

Table 2 (continued)

Commodity (*scientific name*)	**Samples analyzed**	**Samples without quantifiable residues**	**Samples with quantifiable residues**		**Samples with residues > MRL**	**Detected pesticides** (concentrations range in mg/kg)
			Single residue	**Multiple residue ($n \geq 2$)**		
Chilies (Green)	19	11 (57.9%)	7	1	3 (15.8%)	β-HCH (BDL–0.014) Chlorpyrifos (BDL–0.2) Metribuzin (BDL–0.01) Aldrin (0.012–0.028) Bifenthrin (0.0063–0.015)
Cabbage	17	12 (70.5%)	4	1	4 (23.5%)	γ-HCH (BDL–0.2) Chlorpyrifos (BDL–0.2) Hexaconazole (0.02–0.04)
Onion	15	12 (80%)	3	0	0	Chlorpyrifos (BDL–0.03) Alachlor (BDL–0.013) β-endosulfan (BDl–0.0031)
Cauliflower	11	4 (36.4%)	6	1	5 (45.5%)	γ-HCH (BDL–0.04) Chlorpyrifos (BDL–0.04) Pendimethalin (BDl–0.03) Aldrin (BDl–0.13) Hexaconazole (0.01–0.2)
Potato	9	6 (66.7%)	3	0	2 (22.2%)	Hexaconazole (0.069–0.1) Bifenthrin (BDL–0.03)
Bitter guard	8	6 (75%)	1	1	2 (25%)	Metribuzin (BDL–0.3) Hexaconazole (BDL–0.06) Heptachlor (BDl–0.15)
Radish	7	6 (85.7%)	1	0	1 (14.3%)	Bifenthrin (BDL–0.2)
Garlic	7	5 (71.4%)	2	0	0	Aldrin (BDL–0.04) β-endosulfan (BDL–0.05)
Capsicum	6	4 (66.7%)	2	0	2 (33.3%)	Hexaconazole (0.1–0.2)
Peas (Green)	5	3 (60%)	2	0	2 (40%)	Metribuzin (BDL–0.02) α-endosulfan (BDL-0.03)
Mustard	5	4 (80%)	0	1	1 (20%)	Aldrin (BDL–0.14)
Spinach	2	2 (100%)	0	0	0	None detected
Cucurbits	2	2 (100%)	0	0	0	None detected
Coriander	2	2 (100%)	0	0	0	None detected
Fenugreek	2	2 (100%)	0	0	0	None detected
Rye (***Secale cereale***)	1	1 (100%)	0	0	0	None detected
Total	**300**	**184 (61.3%)**	**94 (31.3%)**	**22 (7.3%)**	**49 (16.3%)**	

are the major factors accounting for this perplex situation. FSSAI (India) and European Union has established MRLs for most of the pesticides but some of the guidelines are still lacking w.r.t. the pesticides in some varieties of vegetables. Hence, for non-registered MRLs, the default MRL of 0.01 mg/kg

Table 3 Summary of pesticide residues detected in GC-ECD analyzed vegetable samples

Pesticide	MRLs established by FSSAI, CAC, and EU (mg/kg)	No. of samples	No. of samples with detectable residues	No. of samples > MRL (%)	Range of residue detected (mg/kg)	Positive vegetables (no. detected)
α-HCH	1.0	300	3 (1%)	0 (0%)	0.02–0.06	Cucumber (1), okra (1), brinjal (1)
β-HCH	1.0	300	6 (2%)	0 (0%)	0.014–0.5	Cucumber (2), tomato (1), beans (1), brinjal (1), chillies (1)
γ-HCH	1.0	300	4 (1.3%)	0 (0%)	0.005–0.2	Brinjal (2), cucumber (1), okra (1), cabbage (1)
δ-HCH	1.0	300	3 (1%)	0 (0%)	0.020–0.133	Cucumber (2), cauliflower (1)
Chlorpyrifos	0.01–0.2	300	11 (3.7%)	1 (0.3%)	0.01–0.2	Tomato (3), beans (2), okra (1), brinjal (1) Chillies (1), cabbage (1), onion (1), cauliflower (1)
Metribuzin	0.01*–0.05	300	8 (2.7%)	2 (0.7%)	0.007–0.3	Tomato (3), beans (2), chillies (1), bitterguard (1), peas (1)
Pendimethalin	0.05	300	2 (0.7%)	1 (0.3%)	0.01–0.03	Cauliflower (1), okra (1)
Malathion	0.5–1.0	300	3 (1%)	0 (0%)	0.011–0.1	Tomato (2), beans (1)
Alachlor	0.01*	300	16 (5.3%)	8 (2.7%)	0.010–0.061	Tomato (6), beans (4), cucumber (4), okra (1) Onion (1)
Aldrin	0.01*–0.1	300	25 (8.3%)	6 (2%)	0.0054–0.24	Tomato (8), beans (4), brinjal (1), cauliflower (1), chillies (3), cucumber (3), garlic (2), mustard (1), okra (1), onion (1)
Heptachlor	0.010*	300	3 (1%)	1 (0.3%)	0.001–0.15	Tomato (1), beans (1), bitterguard (1)
Oxyflourfen	-	300	0 (0%)	0 (0%)	-	None
α-endosulfan	0.01*–0.5	300	2 (0.7%)	1 (0.3%)	0.022–0.030	Peas (1), tomato (1)
Dieldrin	-	300	0 (0%)	0 (0%)	-	None
Hexaconazole	0.01*–0.02	300	28 (9.3%)	23 (7.7%)	0.01–0.2	Tomato (7), cabbage (4), cauliflower (4), okra (4), cucumber (2), beans (2), potato (2), capsicum (2), bitterguard (1)
Endrin	-	300	0 (0%)	0	-	None
β-endosulfan	0.01*–0.5	300	6 (2%)	4 (1.3%)	0.0031–0.14	Beans (3), onion (1), tomato (1), mustard (1)
Ethion	0.5–1.0	300	6 (2%)	0	0.001–0.12	Cucumber (3), okra (2), beans (1)
Bifenthrin	0.010*–0.3	300	13 (4.3%)	6 (2%)	0.0063–0.2	Chillies (3), beans (2), cucumber (2), okra (3) Potato (1), tomato (1), radish (1)

*Default tolerance limit of 0.01 mg/kg in cases of pesticides for which MRL have not been fixed for targeted commodity (EU, 2002; FSSAI, 2018; CAC) established by European Commission were employed (EC, 2005). In present study, out of 116 vegetable samples showing pesticide residues, 49 samples exceeded the MRLs for the following ten pesticides: chlorpyrifos ($n=1$), metribuzin ($n=2$), pendimethalin ($n=1$), alachlor ($n=8$), aldrin ($n=6$), heptachlor ($n=1$), α-endosulfan ($n=1$), hexaconazole ($n=23$), β-endosulfan ($n=4$), bifenthrin ($n=6$) (Table 3). Most often, MRL values were surpassed in cauliflower (45.5%) and green peas (40%) followed by capsicum (33.3%), beans (28.2%), bitter guard (25%), cabbage (23.5%), okra (23.3%), potato (22.2%), mustard (20%), radish (14.3%), green chilies (15.8%), cucumber (12.1%), and tomato (11.3%) (Table 2). Frequent occurrence of pesticide residue above MRL has also been reported earlier in chili pepper and cucumber from Saudi Arabia (Ramadan et al., 2020).

Since most vegetables grown in the study areas are supplied to the nearby metro cities, so farmer usually has to face pressure for meeting the demands. Probably, to maintain this equilibrium between demand and supply, the extensive use of pesticides is being practiced especially for the production of high-priced off-season vegetables. Hence, this could be the reason for occurrence of pesticide residues in vegetables such as tomato, cauliflower, cucumber, beans, okra, and green chilies which are supplied to serve major cities of India throughout the year.

Critical perusal of the results revealed that, of the 300 tested samples, 31.3% (94 samples) contained a single residue, and 7.3% (22 samples) contained multiple pesticide residues (Fig. 2b). The simultaneous occurrence of different pesticides was observed predominantly in beans, tomato, cucumber, okra, and mustards. Exorbitant levels of contaminants in vegetables can probably be due to non-judicious and excessive use of pesticides, inadequate supervision by relevant agencies, insufficient understanding among farmers with regard to doses of pesticides, their applications, mechanisms, and standard pre-harvest intervals (Kumari & John, 2019).

Human health risk assessments

The occurrence of pesticides in food products such as vegetables and their exposure to humans via oral intake can produce harmful effects on consumer's health. Moreover, it has also been realized that the human exposure to pesticides is relatively more frequent through food commodities in comparison to their exposure through air or water (Juraske et al., 2007). Therefore, to protect human health from avoidable pesticide exposures, routine monitoring of pesticide levels in commonly consumed food items and their dietary intake assessment is of utmost importance. The human health risk evaluations were carried out based on mean levels of pesticides detected in the collected samples and consumption data of vegetables in the local population. The results revealed that the EDIs of quantifiable pesticide residues were lower than the established ADIs. This implies that the hazard index is less than one and there is minimum hazard risk to consumers (Table 4). Hence, the consumers appear to be reasonably protected from harmful effects of pesticides present in the vegetables at current contamination levels. However, the contribution of vegetables to acceptable daily intakes of pesticides (i.e. % ADI) was found to be ranging from 0.014 to 39.4% in children and 0.003 to 9.85% in adults. This indicates that children are relatively at more risk. Moreover, considering the high concentration levels of pesticides found in some of the samples and the consumption pattern of vegetables in hilly state, preventive steps are warranted and should always be taken in light of snowballing and enduring effects of these chemicals in impending time. The Indian dietary patterns are predominantly vegetarian type and slightly skewed towards cereals, fruits and vegetables (FVs), pulses, etc. However, FVs contribute only less than 9% of the total calorie intake (Green et al., 2016). Therefore, theoretically, if other key items of daily food ration like rice, pulses, edible oil, and animal sourced foods together with drinking water containing pesticide residues are consumed by the people, then the long-term chronic outcomes could be very fatal and alarming. Assessing the toxicological effects of factual, simultaneous, and long-term exposure to variety of pesticides with probable synergetic effects has been a strenuous task, which requires an exhaustive research. It has been pointed out in some of the earlier conducted studies that the concurrent occurrence of different pesticides may cause more alarming human health issues (Kortenkamp, 2007; Singh et al., 2017). Thus, risk assessment studies encompassing wide range of food commodities along with environmental and occupational exposures to humans especially the farming community should be

Table 4 Estimated daily intakes (EDIs) and ADIs of pesticide residues found in raw honey samples

Compound	**ADI** (mg/kg b.wt.)	**Age group** (body weight)	**EDI*** (mg/kg body weight/day)	**Hazard Index (EDI/ADI)**	**% ADI**	**Hazard risk** yes/no
α-HCH	0.005	Adult	4.77E-06	9.55E-04	9.55E-02	No
		Children	1.91E-05	3.82E-03	3.82E-01	No
β-HCH	0.005	Adult	1.51E-05	3.03E-03	3.03E-01	No
		Children	6.06E-05	1.21E-02	1.21E+00	No
γ-HCH	0.005	Adult	1.47E-05	2.95E-03	2.95E-01	No
		Children	5.90E-05	1.18E-02	1.18E+00	No
δ-HCH	0.005	Adult	1.04E-05	2.08E-03	2.08E-01	No
		Children	4.16E-05	8.32E-03	8.32E-01	No
Chlorpyrifos	0.01	Adult	2.29E-05	2.29E-03	2.29E-01	No
		Children	9.16E-05	9.16E-03	9.16E-01	No
Metribuzin	0.013	Adult	8.69E-06	6.68E-04	6.68E-02	No
		Children	3.47E-05	2.67E-03	2.67E-01	No
Pendimethalin	0.125	Adult	4.32E-06	3.46E-05	3.46E-03	No
		Children	1.73E-05	1.38E-04	1.38E-02	No
Malathion	0.03	Adult	1.70E-05	5.68E-04	5.68E-02	No
		Children	6.81E-05	2.27E-03	2.27E-01	No
Alachlor	0.0025	Adult	5.05E-06	2.02E-03	2.02E-01	No
		Children	2.02E-05	8.09E-03	8.09E-01	No
Aldrin	0.0001	Adult	9.85E-06	9.85E-02	9.85E+00	No
		Children	3.94E-05	3.94E-01	3.94E+01	No
Heptachlor	0.0001	Adult	9.30E-06	9.30E-02	9.30E+00	No
		Children	3.72E-05	3.72E-01	3.72E+01	No
α-endosulfan	0.006	Adult	6.62E-06	1.10E-03	1.10E-01	No
		Children	2.65E-05	4.41E-03	4.41E-01	No
Hexaconazole	0.005	Adult	1.50E-05	3.00E-03	3.00E-01	No
		Children	6.01E-05	1.20E-02	1.20E+00	No
β-endosulfan	0.006	Adult	8.39E-06	1.40E-03	1.40E-01	No
		Children	3.36E-05	5.59E-03	5.59E-01	No
Ethion	0.002	Adult	9.04E-06	4.52E-03	4.52E-01	No
		Children	3.62E-05	1.81E-02	1.81E+00	No
Bifenthrin	0.01	Adult	7.53E-06	7.53E-04	7.53E-02	No
		Children	3.01E-05	3.01E-03	3.01E-01	No

*$EDI = C \times F \times W^{-1}$, C is the mean of residue concentration in vegetables (mg/kg), F is mean intake of total dietary vegetables per person, and W is mean human body weight (which was considered 60 kg for adults and 15 kg for children)

performed to protect their health and to ensure overall food quality.

Conclusions

The application of pesticide in modern agricultural practices has significantly contributed to enhanced agricultural production but the presence of pesticide residues in foods has also posed serious threats to human health and the environment. The present study revealed that 16.3% of fresh farm vegetables contained pesticides above the established maximum residual limits. This could be a serious human health issue especially for vulnerable populations (children, old and ill people). Therefore, strict compliance of food safety laws, usage of pesticide in accordance with approved label directions and restricting their spray prior to crop harvesting can minimize the level of residues on agricultural commodities. Furthermore, increasing the education level of farmers on their use and awareness towards food safety will also reap the benefit for generations to come.

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Declarations

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References

Anonymous. (2021). *Economic Survey 2020–21*. Ministry of Finance, Government of India. Retrieved July 25, 2021, from https://pib.gov.in/PressReleasePage.aspx?PRID=1693205

Bedi, J. S., Gill, J. P. S., Aulakh, R. S., & Kaur, P. (2016). Occurrence and spatial distribution of pesticide residues in butter and ghee (clarified butter fat) in Punjab (India). *Environmental Monitoring and Assessment, 188*(2), 100. https://doi.org/10.1007/s10661-016-5115-8

CAC. (2011). *Procedural manual (20th ed.)*. Rome: WHO/FAO. Retrieved March 29, 2020, from http://www.fao.org/3/i5079e/i5079e.pdf

EC. (2005). Regulation (EC) No 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending council directive 91/414/EEC. *Official J. Eur. Commun*, 1–16. Retrieved March 15, 2020, from http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R0396

EC. (2016). Document number SANTE/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Retrieved August 13, 2020, from https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_11945.pdf.

FSSAI. (2011). *Food safety and standards (contaminants, toxins and residues) regulations, 2011*. Retrieved January 10, 2021, from https://www.fssai.gov.in/upload/uploadfiles/files/Compendium_Contaminants_Regulations_20_08_2020.pdf

FSSAI. (2015). *Food safety and standards authority of India - general guidelines for sampling*. Retrieved January 10, 2021, from https://fssai.gov.in/upload/uploadfiles/files/GENERAL_GUIDELINES_ON_SAMPLING.pdf

Green, R., Milner, J., Joy, E. J., Agrawal, S., & Dangour, A. D. (2016). Dietary patterns in India: A systematic review. *British Journal of Nutrition, 116*(1), 142–148.

Jardim, A. N. O., & Caldas, E. D. (2012). Brazilian monitoring programs for pesticide residues in food- Results from 2001 to 2010. *Food Control, 25*, 607–616.

Juraske, R., Antón, A., Castells, F., & Huijbregts, M. A. J. (2007). Human intake fractions of pesticides via greenhouse tomato consumption: Comparing model estimates with measurements for Captan. *Chemosphere, 67*, 1102–1107.

Kortenkamp, A. (2007). Ten years of mixing cocktails: A review of combination effects of endocrine-disrupting chemicals. *Environmental Health Perspectives., 115*, 98–105. https://doi.org/10.1289/ehp.9357

Kumar, A., Gill, J. P. S., & Bedi, J. S. (2018a). Multiresidue determination of pesticides in market honey from northern India using QuEChERS approach and assessment of potential risks to consumers. *Current Science, 115*(2), 283–291.

Kumar, A., Gill, J. P. S., Bedi, J. S., & Kumar, A. (2018b). Pesticide residues in Indian raw honeys, an indicator of environmental pollution”. *Environmental Science and Pollution Research, 25*, 34005–34016. https://doi.org/10.1007/s11356-018-3312-4

Kumar, A., Panda, A. K., & Sharma, N. (2022). Determination of antibiotic residues in bovine milk by HPLC-DAD and assessment of human health risks in Northwestern Himalayan region, India. *Journal of Food Science and Technology., 59*(1), 95–104. https://doi.org/10.1007/s13197-021-04988-8

Kumari, D., & John, S. (2019). Health risk assessment of pesticide residues in fruits and vegetables from farms and markets of Western Indian Himalayan region. *Chemosphere, 224*, 162–167.

Liang, H., Li, L., Li, W., Wu, Y., & Liu, F. (2012). The decline and residues of hexaconazole in tomato and soil. *Environmental Monitoring and Assessment, 184*, 1573–1579.

Maity, T. K., & Tripathy, P. (2004). Organic farming of vegetables in India: Problems and prospects. Retrieved April 16, 2021, from www.share4dev.info/kb/documents/2997.pdf

Maria, P. C., & Lennart, T. (2007). Effect of biobed composition, moisture and temperature on the degradation of pesticides”. *Journal of Agricultural and Food Chemistry, 55*, 5725–5733.

Mebdoua, S., Lazali, M., Ounane, S. M., Tellah, S., Nabi, F., & Ounane, G. (2017). Evaluation of pesticide residues in fruits and vegetables from Algeria". *Food Additives and Contaminants: Part B Surveillance, 10*(2), 91–98. https://doi.org/10.1080/19393210.2016.1278047

NHB. (2019). National Horticulture Board, Government of India - Annual Report 2018–19. Retrieved January 11, 2021, from http://nhb.gov.in/PDFViwer.aspx?enc=3ZOO8K5CzcdC/Yq6HcdIxMvJ0RY/w0FTznMeREnr5ok (accessed 11 Jan 2021)

NSSO. (2014). National Sample Survey Office, household consumption of various goods and services in India 2011–12. Report No. 558 (68/1.0/2)". Retrieved June 19, 2020, from http://www.mospi.nic.in/sites/default/files/publication_reports/Report_no558_rou68_30june14.pdf

Qin, G., Li, Y., Chen, Y., Sun, Q., Zuo, B., He, F., Shen, N., Jia, G., & Ding, G. (2015). Pesticide residues determination in China vegetables in 2010–2013 applying gas chromatography with mass spectrometry. *Food Research International, 72*, 161–167.

Ramadan, M. F. A., Abdel-Hamid, M. M. A., Altorgoman, M. M. F., AlGaramah, H. A., Alawi, M. A., Shati, A. A., Shweeta, H. A., & Awwad, N. S. (2020). Evaluation of pesticide residues in vegetables from the Asir region, Saudi Arabia. *Molecules, 25*, 205.

Ranu, B. (2015). Pesticide residues in vegetable and fruits", *International Journal of Advanced Research in Chemical. Science, 2*(1), 11–17.

Selim, M. T., & EL-Saeid, M.H., & Al-Dosasari, I.M. (2011). Multi-residues analysis of pesticides using gas chromatography mass spectrometry: I-leafy vegetables. *Research Journal of Environment Science, 5*(3), 248–258.

Sharma, K. K. (2016). Storage and Handling of Reference Standards. In: International workshop and training program on good food laboratory practices. Retrieved March 10, 2022, from http://www.ilsi-india.org/International_Workshop_and_Training_Program_on_Good_Food_Laboratory_Practices/Storage%20and%20Handling%20of%20Reference%20Standards,%20by%20Dr.%20K.K.%20Sharma,%20Network%20Coordinator.pdf

Sharma, N., Kumar, S., Chopra, P., Joshi, R., & Kumari, S. (2014). Herbicide usage and residues in vegetable crops of Kangra district of Himachal Pradesh. *Journal of Environment and Bio-Sciences, 28*(2), 261–264.

Singh, N., Gupta, V. K., Kumar, A., & Sharma, B. (2017). Synergistic effects of heavy metals and pesticides in living systems. *Frontiers in Chemistry, 5*, 70. https://doi.org/10.3389/fchem.2017.00070

Szpyrka, E., Kurdziel, A., Matyaszek, A., Podbielska, M., Rupar, J., & Słowik-Borowiec, M. (2015). Evaluation of pesticide residues in fruits and vegetables from the region of south-eastern Poland. *Food Control, 48*, 137–142.

Yi, Y. J., Joung, H. J., Kum, J. Y., Hwang, I. S., & Kim, M. S. (2020). Pesticide residues in vegetables and risk assessment for consumers in Korea during 2010–2014. *Food Additives & Contaminants: Part a. Advance Online Publication.* https://doi.org/10.1080/19440049.2020.1769198