Determination of Pesticides Residue by GC-ECD in Vegetables from Cameron Highlands, Malaysia and Their Human Health Risk Assessment

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Abstract

Gas chromatography-electron capture detection (GC-ECD) method has been developed to determine the residues of pyrethroid (PYRs), organophosphorus (OPPs) and organochlorine (OCPs) pesticide in 97 leafy vegetable samples collected from Cameron Highlands, Malaysia. The mean residual concentration of PYRs, OPPs and OCPs ranged from 0.04–17, 0.03–44.4 and 0.03–100 μg kg⁻¹ respectively. The sum of pesticides concentration increased in the order mustard < spinach < celery < cauliflower < lettuce < broccoli < cabbage. The results revealed that levels of maximum residue limit (MRL) for OCPs were violated by lettuce (7.7%), for OPPs, it exceeded mostly in cabbage (24.8%) and for PYRs only one cabbage sample exceeded the MRL. Health risk estimation revealed that hazard quotients (HQs) for OCPs, OPPs and PYRs were <1.0, showing less risk to consumers. However, the hazard indices (HIs) for OPPs were >1.0 for children (1.4) and for adults (0.4) which signify the potential health risk to consumers.

Introduction

Pesticides are used on crops to protect from diseases and pest infestation, before and after crop harvesting. It is used intensively in the European Union (EU) and in 2010; 208,000 tons of pesticides-active ingredients were consumed. The World Health Organization (WHO) reports (Organization, 2003) that vegetables and fruit constitutes 30% of consumer’s diet as they are good sources of vitamins, minerals, antioxidants and fiber. Its presence in human diet helps in preventing gastrointestinal and breast cancer (Lozowicka et al., 2012). Pesticides contamination can affect human health, animals and the environment in many ways, (Bhatti and Taneja, 2007; Park et al., 2016). About three million cases of pesticide poisoning have been estimated annually resulting in 220,000 deaths from both occupational as well as non-occupational exposure (Hossain et al., 2013). Consumers are exposed to pesticides dermally or through inhalation, dietary and non-dietary intake, however, vegetables and fruits have been recognized and characterized as one of the main exposure route (Quijano et al., 2016). Since 2005, the European Commission (EC) is considering the cumulative effect of pesticides for individuals since human beings can be simultaneously exposed to several chemicals (Jensen et al., 2013). Therefore, it is important to monitor these contaminants and assess the risk they pose to human health. A lot of attention has been given to it recently in monitoring programs for pesticides analysis in vegetables as most of them are eaten in the raw form.

Insecticides such as organophosphorus (OPPs), organochlorine (OCPs) and pyrethroid (PYRs) pesticides are commonly used in developing countries like Malaysia for controlling pests. Though OCPs were banned in Malaysia since 1990, however these are still prevalent illegally (Saadati et al., 2012). Due to lack of control programs, recourses and knowledge, farmers do not wait longer for the residues to be washed off by the sun or rain. They have less knowledge about the toxic effects of pesticides residue in vegetables and fruits (Chen et al., 2011). Since, pesticides poisoning and deaths are far greater in developing than in developed countries thus, risk assessment is necessary to study the effects on health.

Maximum Residue Limits (MRLs) encourages food safety programs by restricting the concentration level on specific commodities. Pesticide monitoring in food is usually carried out by referring to MRLs in tandem with the average daily intakes (ADI). The combination of MRL with ADI is considered to be a more precise criterion for elucidation of ‘safety’ of chemical constituents in food than MRL alone (Mansour et al., 2009). Estimated dietary exposure to pesticides is considered for consumers, if it exceeds the ADI, which is (mg kg⁻¹ body weight day⁻¹), over a life time daily ingestion does not pose a significant health risk to consumers (Akoto et al., 2013). In the United Kingdom, consumers are encouraged to eat at least five portions of fruits and vegetables daily (Chen et al., 2011). In cases of pesticides having a common mode of action by having same effects on the same organs and tissues through same sequence of biochemical processes like OPPs and PYRs, then the cumulative exposure can be assessed (Zhang et al., 2019).

Cameron Highlands is one of the main producers of vegetables for Malaysia therefore, assessing the risk of pesticides residues associated with these widely grown vegetables in this area for human consumption is necessary. We investigated the health risk assessment in the consumption of the vegetables of Cameron Highlands (Liu et al., 2020). The objective was to measure the residue of PYRs, OPPs and OCPs in vegetable samples e.g., cabbage, cauliflower, broccoli, spinach, lettuce, mustard and celery as well as in soil samples, to calculate the estimated dietary intake (EDI), hazard quotient (HQs) and hazard index (HIs) for adults and children via consumption of these vegetables.

Materials And Methods

Study Area and sampling

Cameron Highland is located in the Pahang state, with an estimated area. 712 km² its altitude is between 1280 – 1830 m above the sea level and its location is on the main range of Peninsular Malaysia between 4°20’ N-4°37’N and 101°20’-101° 36’E. Average annual rainfall and daily temperature is between 2800 mm and 14–21 °C respectively. Agriculture is the main occupation of people of the region. Continuous cultivation and use of pesticides in the highlands had contaminated the rivers with OCPs and OPPs (Chishti et al., 2013). About 67% of farmers grow two different species of vegetable at the same time and the most commonly grown vegetables are cabbage and lettuce (100 and 35%) followed by celery, spinach, mustard and cauliflower. OCPs, OPPs and PYRs pesticides are frequently used in the region (Ismaiel and Kalithasan, 2004; Mazlan and Mumford, 2005; Leong et al., 2007).

Various vegetables were purchased from grocery market were chopped, blended and stored at <16 °C. The vegetable and soil real samples were collected from seven conventional farms located in different regions of Cameron Highlands. Vegetable samples include spinach, mustard, lettuce, celery, cauliflower, cabbage and broccoli. For soil samples, the soil layer (0–10 cm) were taken and were divided for two kinds of analysis one for pesticides and other for soil geochemical properties. The edible parts of leafy portion of cabbage, celery, lettuce, mustard and spinach, and the heads of broccoli and cauliflower were collected in triplicate at each site. These samples were homogenized and stored at 4 °C prior to analysis.

Extraction
Vegetable samples were extracted (Farina et al., 2017) and the concentration of OPPs, OCPs and PYRs, were investigated based on a modified method (Fillon et al., 2000) in conjunction with GC-ECD method. A chopped vegetable sample of 10 g of all pesticides, 5.0 mL of spiking solution of 1.0 mg L⁻¹ were taken in a blender spiked and allowed to stand for 20 minutes for interaction and extracted with 65 mL acetonitrile for 1.0 min at high speed. A 12 g sodium chloride and 12.5 g anhydrous magnesium sulfate were added to remove moisture. The recovered 30 mL organic extract was evaporated in a rotary evaporated at 40°C to bring its final volume up to 1.0–2.0 mL. The extraction was followed by a cleanup step using a multilayer supelclean GCB/PSA SPE cartridge, pre-conditioned with a mixture of 5.0 mL solution of toluene-acetone (1:3 v/v). After loading the acetonitrile extract, the elution was brought about at a flow rate of 5.0 mL/min by the mixture of 5.0 mL acetone–toluene. The eluate was evaporated under nitrogen stream up to 0.5 mL followed by the addition of PCNB (1.0 µg mL⁻¹) solution in aceton as an IS and analysis by GC-ECD method (Farina et al., 2018).

### Pesticides Standards

Dr. Ehrenstorfer (Augsberg, Germany) provided the pesticides standards with purity ranging from 94–100%. Stock solutions (1000 µg mL⁻¹) of each standard were prepared by dissolving required quantity of the pesticide in 10 mL aceton and stored at < −16 °C in brown glass bottles. From these stock solutions working standards were prepared and stored at 4 °C. Internal standard (IS) in the form of pentachloronitrobenzene (PCNB), graphitized carbon (250 mg) and PSA (500 mg) packed solid phase extraction (SPE) tubes from Restek, (USA), solvents and reagents were obtained from Merck (J.T. Baker, USA and Darmstadt, Germany). The rotary evaporator (Model A 1000s) and glass blender (Model HGB 2QWTS3) were used during experimental work (Farina et al., 2017; Farina et al., 2018).

Experimental design was used for optimization and validation of the method by using the statistical analysis e.g., data processing was done by a mini-tab statistical package software version 17 (Minitab Inc., State College, USA).

### Apparatus and Chromatographic Conditions

A Varian chrome packed CP-3800 GC equipped with ⁶³Ni ECD was used. A HP-MS 5 (0.25 µm film thickness, 30 m-0.32 mm i.d.) fused silica capillary column was employed as the analytical column for vegetable samples containing pesticide residues. The carrier gas was high purity nitrogen with a flow rate of 1.5 mL min⁻¹. Detector and injector were operated at 300°C and 250°C respectively; the oven temperature was maintained at 90°C for 1.0 min and then programmed at 3.5°C/min to 170°C followed by a final ramp to 280°C at 5.0°C/min. A 1.0 µL of each sample was injected into GC using split-less mode. Retention time was used to identify the correct peak on the chromatogram (Farina et al., 2017).

The recovery and repeatability for three real samples analyzed by the method are shown in Table 3. The correlation coefficients (r) for all analyzed pesticides were achieved over the range of 0.990–0.995 with good linear dynamic ranges. Thus the efficiency of the method can be reflected from the lower limit of detection (LOD) and limit of quantification (LOQ), which were below than the maximum residue limits (MRL) set by the EU for the analyzed pesticides. The LOD ranged (0.00003–0.0045 µg g⁻¹) for all pesticides in these vegetables and recoveries were ranged in celery (60–114%), in lettuce (60–128%) and in cabbage (61.8–121%). These results indicate the method's good accuracy. Similarly, the method's precision is verified by the relative standard deviation (RSD) ranges, which were obtained as in celery from 3.0–19.8%, in lettuce from 0.5–18% and in cabbage from 0.2–15%.

### Human Health Risk Assessment of Pesticides through Vegetables Consumption

The EDI was obtained by calculating the mean residual concentration of pesticides in each vegetable, with the food consumption rate for vegetables in Malaysia which is 0.078 kg person⁻¹ day⁻¹, and dividing the product with the standard body weight (kg) (Bempah et al., 2011; Akoto et al., 2015). The per capita vegetable consumption rate for vegetables in Malaysia according to the Federation of Agricultural Malaysia (FAMA) was 36 kg per capita for the year 1991 (Izzah et al., 2012). The average body weight of Malaysian adults and children are considered to be 70 and 10 kg respectively. The reference doses (ADI) are considered to be the dose without risk effects (Bempah et al., 2011; Yu et al., 2016). The target hazard quotients (THQ) were calculated as ratios between the estimated pesticide exposure dose, and the reference dose for adults and children. The THQ is calculated according to the following equation;

\[
\text{THQ} = \frac{\text{EDI}}{\text{ADI}} \quad (1)
\]

If value for THQ is <1.0, then the people would unlikely experience the toxic effects. However, if it exceeds 1.0, then toxic effects are possible. The Hazard Index (HI) was calculated as a measurement of health risk associated with mixture of chemical constituents in vegetables (Yu et al., 2016). The HI is calculated as

\[
\text{HI} = \sum_{i=1}^{n} \text{THQ}_i \quad (2)
\]

If the HI value is less than 1.0 then the consumers unlikely to experience toxic effects via exposure (Bempah et al., 2012; Yu et al., 2016). In case if HI exceeds 1.0, it would indicate that the consumption of vegetables probably causes toxic effects. The HI was calculated by summation of HI for all pesticides of specific group.

### Result And Discussion

#### Pesticides in Vegetables

Table 1 shows the mean residual pesticides concentration of OCPs. The total concentration of DDTs (sum of P-P' DDT, P-P' DDE, P-P'DDD) ranges from ND–66.6, ND–66, ND–20, ND–53.3, ND–50 ND–16.6 and ND–16.7 µg kg⁻¹, HCHs (α- HCH, β-HCH, γ-HCH) from ND–133.3, ND–53.3, ND–16.6, ND–103, ND–26.6, ND–6.6 and ND–23.3 µg kg⁻¹ and in the case of endosulphan, from ND–36.63, ND–16.2, ND–73, ND–0.3, ND–70, ND–33.3 and ND–33.3 µg kg⁻¹ in cabbage, cauliflower, spinach, celery, lettuce, broccoli and mustard respectively. The sum of OCPs in these crops decrease in the following order cabbage >
broccoli > lettuce > cauliflower > celery > spinach > mustard. The detection frequency for HCHs and DDTs was 81% and for endosulphan it was 71% respectively. The dense, waxy, and hairy foliage of brassica in combination with the lipophilic character of pesticides offers more deposition of pesticides in these vegetables. The uptake of pesticides from roots can be influenced by many factors e.g., plant species, its growth, loss from the surface of the leaf due to transpiration, pesticides metabolism and physico-chemical characteristics of pollutants (Liu et al., 2020) (Singh et al., 1990). The application rate of pesticides also varies at different sampling points that can also influence the uptake of pesticides. Cabbage is the widely grown crop; therefore more pesticides are used by the farmers for maintaining its quality under tropical conditions (Mazlan and Mumford, 2005). The ratio of DDT/DD and y-HCH/α-HCH indicated that the environment in which these vegetables are grown is polluted by both the new sources of HCHs and the old sources DDT. The new sources could be dangerous for human health as well as for environment. The order of percentages of OCPs above MRL set by EU was as follows: lettuce (7.7%) > mustard (6.0%) > spinach (4.7%) > cabbage (5.7%) cauliflower (4.3%) > broccoli (3.0%). The cabbage farms are the mostly contaminated, occupying 1170 ha out of 7050 ha of total vegetables cultivated area (92%) (Mazlan and Mumford, 2005).

OPPs are effective in controlling a variety of insects on vegetable crops. The concentration of OPPs decreases in the order; cabbage > cauliflower > lettuce > broccoli > spinach > celery > mustard. It is evident from the results that all vegetables were contaminated by OPPs and cabbage seemed to be contaminated the most. The concentration of OPPs were in the range of ND–233.3, ND–200, ND–10, ND–200, ND–200, ND–166.7 and ND–27.7 μg kg⁻¹ in cabbage, cauliflower, celery, spinach, broccoli, lettuce and mustard respectively. Among OPPs, dimethoate was found abundantly and the sum of its average mean concentration in all vegetable samples was 149.6 μg kg⁻¹. Cabbage was found to be the most contaminated followed by cauliflower as its head and leaves were badly damaged by pests that might cause both qualitative and quantitative loss to crops. The least contaminant was malathion with an average mean concentration of 40.8 μg kg⁻¹. The observed trend in concentration of studied OPPs were dimethoate (36.8%) > diazinon (14.3%) > parathion methyl (12.3 %) > parathion ethyl (20.3%) > chlorpyriphos (14.0%) > malathion (9.9 %). The EU order of percentages of OPPs above MRLs is as; cabbage (24.8%) > cauliflower (13.6%) > broccoli (11%) > lettuce (13.6%) > cauliflower (4%) > spinach (3.7%) > celery (1.8%) > mustard (1.8 %). Various factors are responsible for OPPs residue greater than MRLs. Some of these factors are lack of safety education and awareness about harvesting, pesticides treatment procedures, application dose and appropriate time (Sapbarnr and Hongisbsong, 2014). As most of the farmers are illiterate, they usually do not follow the labeled instructions. PYRs have less photosensitivity, greater insecticidal activity, and relatively low toxicity as compared to OPPs. Its concentration in crops decreases in the order; cabbage > cauliflower > lettuce > celery > spinach while in broccoli and mustard it was not detected. It is clear that not all vegetables were contaminated by PYRs and cabbage seemed to be the most affected one. The concentration of all these PYRs were in the range of ND–1.6, ND–16.7, ND–6.6, ND–10.0 and ND–100 μg kg⁻¹ in mustard, lettuce, celery, cauliflower and cabbage respectively. Among PYRs, fenvelerate was abundantly found, the sum of its average mean concentration in all vegetable samples was 20.2 μg kg⁻¹ and cabbage was found to be the most contaminated. In spinach and broccoli, it was not detected while mustard was the least contaminated. Only one sample of cabbage has crossed the MRL value. The observed trend in concentration of OPPs in this study was fenvelerate (29.8%) > permethrin (7.7%). The difference in its concentration might be due to its different application rates at different sites on different vegetables or fast degradation of permethrin on these vegetables.

**Risk Assessment**

Health risk estimates associated with the total of 80 samples (cabbage, cauliflower, broccoli, spinach, lettuce, celery and mustard) was analyzed for OCPs, OPPs and PYRs (Table 2) for each vegetable by considering the mean concentration for each pesticide in the vegetables. Table 2 comprises estimated daily intake (EDI), reference daily dose (RDD) and average maximum daily intake and corresponding hazard quotients (HQs) during the study period for both adults and children.

The EDI of OCPs in vegetables ranges as 3.3x10⁻⁸–1.0x10⁻⁴ for adults and 2.3x10⁻⁷–7.8x10⁻⁴ for children. For adults the HQs for OCPs in vegetables decrease as broccoli > lettuce > cabbage > cauliflower > celery > mustard > spinach. For children, the maximum THQ decreases as DDT > β-HCH > α-HCH > γ-HCH > endosulphan > DDE >DDD.

The combined risk due to OCPs for adults in vegetables decreases in the order cabbage (0.2) > cauliflower (0.09) > broccoli (0.05) > celery (0.03) > lettuce (0.02) > mustard (0.005) > spinach (0.003) for children. The combined risk were in the order broccoli (3.0) > cabbage (1.7) > cauliflower (0.6) > lettuce (0.2) > mustard (0.04) > and celery (0.02) respectively. This indicated that children are the most vulnerable group as they eat more of single unit of one food commodity in one day. This might cause a systematic toxicity due to exposure to OCPs [2]. For children the HQs of OCPs in vegetables decreases in the order; broccoli > lettuce > cabbage > cauliflower > mustard > celery > spinach.

For OPPs, the HQs for each of the pesticides in vegetables does not exceed 1.0 though some had residue levels above MRLs. The EDI of OPPs in vegetable sample ranges as 3.3x10⁻⁸–3.7x10⁻⁵ for adults and 2.3x10⁻⁷–3.0x10⁻⁴ in children. For adults, the HQs in vegetables decreases in the order; cauliflower > broccoli > spinach > lettuce > celery > mustard and for children, cabbage > broccoli > cauliflower > spinach > lettuce > celery. This is dangerous as cabbage is sometimes eaten in raw form by consumers as a salad. For adults the maximum THQs for OPPs (dimethoate, diazinon, parathion ethyl, parathion methyl, chlorpyriphos and malathion) were 2.5x10⁻², 5.3x10⁻², 6.8x10⁻², 8.1x10⁻³, 2.3x10⁻⁴ respectively. The maximum THQ increased in the order malathion > chlorpyriphos > parathion methyl > parathion ethyl > diazinon > dimethoate. For children the maximum THQ decreases in the order diazinon > dimethoate > parathion methyl > parathion ethyl > malathion > chlorpyriphos. The average THQ decrease in the same order as for adults.

The combined risk due to OPPs for adults in vegetables decreased in the following order cabbage (0.6) > cauliflower (0.4) > celery (0.1) > broccoli (0.06) > lettuce (0.01) > spinach (0.01) > celery > mustard (0.004) and for children cabbage (4.3) > cauliflower (2.0) > broccoli (1.7) > lettuce (1.4) > spinach (0.1) > celery (0.09) > mustard (0.02). The highest HQ was that of diazinon and dimethoate of 0.47 and 0.2 respectively. Diazinon is an acetylcholinesterase inhibitor and neurotoxicant and had the lowest ADI among all other studied pesticides. It is believed that lower level of enzymes might increase the susceptibility of children to neurotoxicity via OPPs exposure (Yu et al., 2016). Its higher HI might cause an adverse health effect on inhabitants in the study area specially
farmers who are directly exposed to it. They might suffer from blurred vision, respiratory problems, muscle weakness and pulmonary edema, and its severity varies with the exposure dose and duration (Jaipieam et al., 2009). The presence of higher concentration of dimethoate might cause malignant brain tumors in farmers as reported previously (Sinha et al., 2012). In Kashmir, where farmers suffered from brain tumor due to exposure of multiple neurotoxic and carcinogenic mixture of OPPs, which might also result in Parkinson disease. However, the hazard indices (HIs) of OPPs (cumulative risk) was > 1.0 in children (1.4) than adults (0.4) indicating that children are exposed to health risk via consumption of these vegetables since many vegetable samples contained more than one OPPs. These results are in contrast to studies in Ghana and China (Akoto et al., 2015; Yu et al., 2016), where combined health risk for OPPs was < 1.0. Vegetables are the main route for exposure to OPPs of local people. It can bioaccumulate in the human body in cases of continuous exposure causing long lasting neurobehavioral impairment due to accumulation of neurotransmitters, acetylcholine, at nerve terminals (Chen et al., 2011) therefore, a continuous environmental monitoring and health survey should be conducted to prevent the possible future risks with OPPs. This study is also helpful in facilitating a risk assessment for children's health due to higher OPPs in vegetables.

In the case of PYRs, HQs were very low indicating its less contamination of vegetables. For adults the HQ values for PYRs decrease in the order of cauliflower > cabbage > celery > mustard and was absent in broccoli, spinach and lettuce. The maximum THQ in adults was 8.3x10^{-3} for fenvelerate and 6.6x10^{-3} in children. The combined health due to PYRs via consumption of vegetables for adults decreases in the order cabbage (8.3x10^{-3}) > cauliflower (1.9x10^{-3}) > celery (4.1x10^{-4}) > lettuce (1.4x10^{-4}) > mustard (1.2x10^{-4}) and for children cabbage (5.8x10^{-5}) > cauliflower (7.1x10^{-5}) > lettuce (1.0x10^{-5}) > celery (2.9x10^{-6}) > mustard (8.7x10^{-5}). For children, the HQs decreases as cauliflower > cabbage > celery and mustard. The highest quotient (HQ) index was that of fenvelerate (0.005). The HIs (cumulative risk) for adults was 0.01 and for children it was 0.01. The highest HQs for OCPs were 32.7 and 30% in adults and children, in OPPs were 65 and 69.5% for adults and children, and for PYRs as 1.6 and 0.5%. OPPs like diazinon are carcinogenic and mutagen an endocrine disruptor effecting the reproductive development (Vijgen et al., 2011). OCPs like β-HCH could pose toxicity by effecting the endocrine system, causing cancer, and bioaccumulation due to its toxicity by persistence (Vijgen et al., 2011). The results provided important information regarding the contamination level and toxicity of OPPs in one of the important agricultural area of Malaysia, and needs an urgent action for appropriate use of pesticides on vegetable.

PCA analysis was used to identify the sources of pollutants. Principal component 1 (PC1) accounted for 38.1 % of data variability and is related to DDTs, chlorpyriphos and fenvelerate indicating that they are coming from the same sources. PC2 contains 21.7% of data variability and its main contributors were β-HCH, endosulphan and parathion ethyl. PC3 (17.9% variance) highlights the combination of α-HCH and parathion ethyl. PC4 (11.8% variance) includes α-HCH, β-HCH and permethrin and PC5 (8.6% variance) α-HCH, parathion methyl and malathion (Table 4). These observations indicated that; (i) farmers are using mixtures of pesticides over the same crop; (ii) vegetables in the field are laying side-by-side and (iii) high rotation frequency. The biplot indicates that vegetables like cabbage, spinach, cauliflower and celery lie close together and contaminated by the majority of pesticides as compared to broccoli, lettuce and mustard. These observations again signify the importance of pesticides application on crop type (Figure 1).

Conclusions

The present study shows the residual concentration and risk assessment of pesticides in vegetables. The MRLs for OCPs given by EU were exceeded mostly in lettuce, and least in broccoli. For OPPs, the MRLs were exceeded most in cabbage which is the most widely grown vegetable in area, and the least in mustard, while for PYRs only one cabbage sample has crossed the MRLs of EU. The health risk estimation revealed that despite having few samples exceeding the MRLs for average pesticides concentration, the HI value is less than one for all pesticides for both children and adults. However, the hazard quotient (HQ) for cumulative risk for OPPs is higher than 1.0 for children, the group which is most vulnerable to significant health risk in cases of ingesting different kinds of OPPs. Cabbage is also the most affected by pesticides indicating the intense use of pesticides by farmers due to increasing demand. Good agricultural practices must be followed by the farmers and they must use the appropriate pesticides doses, and to restrict them using pesticides before harvesting. Pesticide residue can bioaccumulate across a food chain over time, therefore a continuous and strict monitoring program should be enforced to check the residual levels in food items.

This research provided information regarding the contamination and toxicity of OPPs to children, and suggests that spraying pesticides at high doses should be discouraged because of the diseases associated with it via exposure in developing countries like Malaysia.

Declarations

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Table 1. Pesticides detected in vegetables from Conventional farms.

| Compounds  | Cabbage | Cauliflower | Spinach | Celery | Lettuce | Broccoli | Mustard |
|------------|---------|-------------|---------|--------|---------|----------|---------|
| α-HCH      | 3.2±0.44| 5±0.8       | 1.2±0.44| 2.2±2  | 16.2    | 0.9±0.5  | 5.7±2.18|
| β-HCH      | 3.1±5.2 | 8.3±7.37    | 1.4±1.2 | 1.4±3  | 12.6    | 16.7±5.7 | 3.7±1.98|
| γ-HCH      | 15±1.8  | 5.6±1.51    | 5±1.86  | 0.7±7.5| 13±2.4  | 100±1.8  | 1.3±6.42|
| DDE        | 32±5.5  | 14.7±1.9    | 0.2±2.94| 8.4±2.37| 5.1±1.4 | 2.1±2.4  | 7.6±4.5 |
| DDD        | 28.5±0.75| 4.5±1.59   | 2.6±0.12| 4.4±4.14| 5.5±1.22| 0.2±2.8  | 0.1±1.22|
| DDT        | 10.3±4.9| 10.8±5.1    | 0.4±10.9| 4.4±3.4 | 4.3±8.7 | ND       | ND      |
| Endosulphan| 11±0.75 | 3±1.03      | 0.8±0.8 | 0.03±2.5| 15.6±4.8| 33.3±2.7 | 4.6     |
| Dimethoate | 35.8±1.8| 44.4±3.55   | 28.9±0.81| 18.5±3.2| 4.8±3.49| 16.7±1.7 | 0.5±5.7 |
| Diazinon   | 15.8±1.1| 8±2.2       | 0.3±1.8 | 0.03±5.6| 0.3±3.8 | 33.3     | 0.7±0.9 |
| Parathion methyl| 11.8±1.6| 4.6±1.78   | 0.9±3.67| 18.5±2.4| 12.6±2.4| ND       | 3.8±1.1 |
| Parathion ethyl| 36.8±1.27| 3.1±1.54 | 3.3±5.6 | 0.7±3.5 | 37.4±2.7| 0.4±1.4  | 2.3±3.55|
| Chlorpyriphos| 36.8±0.3 | 1.5±0.49  | 0.8±1.51| 15.1±4.8| 2.1±0.5 | ND       | 0.7±1.24|
| Malathion  | 10.8±1.13| 7.3±0.78   | 12.5±0.3| 0.7±0.3 | 2.1±3.6 | 2.1±6.2  | 5.3±6.5 |
| Fenvelerate| 15±5.8  | 17±7.5      | ND      | 15.1±2.67| ND     | ND       | ND      |
| Permethrin | ND      | 0.3±7.6     | ND      | ND      | ND      | ND       | 0.04±8.9|
| Σ pesticides| 267.4   | 122.6       | 58.4    | 60      | 143.4   | 205      | 36.7    |

ND = not detected

Table 2. The health risk estimation for mean pesticides in vegetables.
| Pesticide | RD (lg kg⁻¹day⁻¹) | Cabbage | Cauliflower | Spinach | Celery | Lettuce | Broccoli |
|-----------|------------------|---------|-------------|---------|--------|---------|----------|
| Endosulphan |                  |         |             |         |        |         |          |
| 4.0      | 3.5·10⁻³         | 5.7·10⁻³ | 1.8·10⁻³ | 4.3·10⁻³ | 2.5·10⁻³ | 8.1·10⁻⁴ | 1.8·10⁻⁴ |
|          | 1.1·10⁻¹         | 2.6·10⁻¹ | 7.1·10⁻¹ | 1.7·10⁻¹ | 5.8·10⁻¹ | 1.3·10⁻¹ | 4.2·10⁻¹ |
| 4.0      | 2.5·10⁻⁵         | 3.9·10⁻⁵ | 1.3·10⁻⁵ | 3.3·10⁻⁵ | 1.7·10⁻⁵ | 5.8·10⁻⁶ | 1.3·10⁻⁶ |
|          | 8.2·10⁻⁵         | 9.1·10⁻⁵ | 3.3·10⁻⁵ | 1.7·10⁻⁵ | 5.8·10⁻⁶ | 1.3·10⁻⁶ | 4.2·10⁻⁶ |
| 2.8      | 9.2·10⁻⁶         | 1.6·10⁻⁶ | 5.3·10⁻⁶ | 1.5·10⁻⁶ | 5.1·10⁻⁶ | 1.4·10⁻⁶ | 4.7·10⁻⁶ |
|          | 3.0·10⁻⁶         | 1.1·10⁻⁶ | 3.6·10⁻⁶ | 1.1·10⁻⁶ | 3.6·10⁻⁶ | 9.8·10⁻⁷ | 3.3·10⁻⁷ |
| 2.8      | 6.4·10⁻⁶         | 2.1·10⁻⁶ | 8.2·10⁻⁶ | 7.8·10⁻⁶ | 6.6·10⁻⁷ | 1.7·10⁻⁷ | 4.0·10⁻⁷ |
|          | 2.1·10⁻⁶         | 1.1·10⁻⁶ | 5.8·10⁻⁷ | 7.8·10⁻⁷ | 6.6·10⁻⁷ | 1.7·10⁻⁷ | 4.0·10⁻⁷ |
| 2.8      | 3.0·10⁻⁷         | 1.1·10⁻⁷ | 5.8·10⁻⁷ | 7.8·10⁻⁷ | 6.6·10⁻⁷ | 1.7·10⁻⁷ | 4.0·10⁻⁷ |
|          | 1.0·10⁻⁷         | 5.8·10⁻⁸ | 7.8·10⁻⁸ | 6.6·10⁻⁸ | 1.7·10⁻⁸ | 4.0·10⁻⁸ | 1.6·10⁻⁸ |
|          | 5.8·10⁻⁸         | 7.8·10⁻⁸ | 6.6·10⁻⁸ | 1.7·10⁻⁸ | 4.0·10⁻⁸ | 1.6·10⁻⁸ |          |
|          | 7.8·10⁻⁸         | 6.6·10⁻⁸ | 1.7·10⁻⁸ | 4.0·10⁻⁸ | 1.6·10⁻⁸ |          |          |

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| Compound      | Recovery | Repeatability |
|--------------|----------|---------------|
| Chlorpyriphos| 3.0⋅10⁻⁵  | 4.1⋅10⁻⁴      |
|              | 1.2⋅10⁻³  | 1.7⋅10⁻¹      |
|              | 8.1⋅10⁻⁸  | 9.9⋅10⁻¹      |
|              | 6.2⋅10⁻¹  | 2.0⋅10⁻¹      |
| Malathion    | 1.0⋅10⁻²  | 1.2⋅10⁻³      |
|              | 1.0⋅10⁻⁵  | 8.1⋅10⁻⁴      |
|              | 8.0⋅10⁻⁰  | 5.7⋅10⁻³      |
|              | 9.7⋅10⁻⁰  | 1.1⋅10⁻⁰      |
| Fenvelerate  | 2.0⋅10⁻²  | 1.7⋅10⁻³      |
|              | 8.3⋅10⁻⁴  | 1.9⋅10⁻¹      |
|              | 6.6⋅10⁻³  | 1.3⋅10⁻⁴      |
| Permethrin   | 5.0⋅10⁻²  | 0.0          |
|              | 0.0       | 0.0          |
|              | 3.7⋅10⁻⁸  | 6.7⋅10⁻⁸      |
|              | 2.3⋅10⁻⁵  | 4.7⋅10⁻⁵      |

RD = Reference Dose, ED = Estimated Dose, HQ = Hazard Quotient, HR = Health Risk, A = Adults, C = Children

**Table 3.** Recovery and repeatability of the method for three sample matrix.
| Pesticide    | Conc. (mg kg$^{-1}$) | Recovery (%) | Repeatability (%) |
|--------------|----------------------|--------------|-------------------|
|              |                      | Cabbage | Lettuce | Celery | Cabbage | Lettuce | Celery |
| α-HCH        | 1.0                  | 78.7    | 110     | 86.5   | 15      | 0.6     | 19.1   |
|              | 0.5                  | 108     | 60      | 66.6   | 10      | 1.5     | 4.0    |
|              | 0.01                 | 61.8    | 72      | 70     | 5.0     | 8.9     | 5.5    |
| β-HCH        | 1.0                  | 105     | 126     | 114    | 5.0     | 2.5     | 4.0    |
|              | 0.5                  | 101     | 74      | 109    | 12      | 6.4     | 19     |
|              | 0.01                 | 105     | 80      | 67     | 5.0     | 3.2     | 4.5    |
| δ-HCH        | 1.0                  | 84      | 111     | 128    | 6.0     | 15.8    | 5.6    |
|              | 0.5                  | 109.9   | 101     | 70     | 4.0     | 10      | 6.5    |
|              | 0.01                 | 121     | 79      | 72     | 2.0     | 12      | 8.9    |
| DDE          | 1.0                  | 91      | 72      | 102    | 5.0     | 13.9    | 10.5   |
|              | 0.5                  | 105     | 65      | 84     | 1.6     | 4.5     | 11.8   |
|              | 0.01                 | 103     | 90      | 85     | 3.4     | 9.0     | 7.9    |
| DDD          | 1.0                  | 82      | 116     | 64     | 6.4     | 2.6     | 6.9    |
|              | 0.5                  | 77      | 89      | 65     | 4.6     | 3.4     | 9.5    |
|              | 0.01                 | 103     | 75      | 76     | 9.0     | 7.8     | 3.6    |
| DDT          | 1.0                  | 100     | 119     | 101    | 0.6     | 8.5     | 1.5    |
|              | 0.5                  | 107     | 81      | 88     | 3.0     | 1.4     | 8.8    |
|              | 0.01                 | 84      | 78      | 60     | 2.4     | 6.9     | 9.0    |
| Endosulphan  | 1.0                  | 72      | 116     | 89     | 8.3     | 18      | 5.9    |
|              | 0.5                  | 101     | 102     | 60     | 5.5     | 5.7     | 9.0    |
|              | 0.01                 | 83      | 76.5    | 64     | 0.2     | 3.4     | 6.0    |
| Dimethoate   | 1.0                  | 88      | 85      | 70     | 7.8     | 7.9     | 11     |
|              | 0.5                  | 105     | 63      | 119    | 12      | 10.2    | 19.8   |
|              | 0.01                 | 81.8    | 77      | 78     | 10.5    | 0.5     | 5.0    |
| Diazinon     | 1.0                  | 83      | 70      | 103    | 1.5     | 12      | 1.3    |
|              | 0.5                  | 88      | 65      | 120    | 2.6     | 0.6     | 4.4    |
|              | 0.01                 | 107     | 93      | 3.9    | 3.9     | 1.5     | 6.9    |
| Parathion methyl | 1.0          | 104     | 60      | 60     | 6.0     | 9.2     | 6.4    |
|              | 0.5                  | 108     | 64      | 72     | 1.5     | 3.9     | 3.2    |
|              | 0.01                 | 78      | 78      | 81     | 3.5     | 7.4     | 16     |
| Parathion ethyl | 1.0              | 70      | 65      | 66     | 2.8     | 1.0     | 7.0    |
|              | 0.5                  | 80      | 72      | 67     | 3.0     | 7.0     | 1.8    |
|              | 0.01                 | 62      | 101     | 78     | 4.0     | 9.6     | 10     |
| Chloropyrifos| 1.0                  | 103     | 128     | 75     | 1.7     | 0.5     | 5.6    |
|              | 0.5                  | 70      | 65.5    | 80     | 12.3    | 14      | 10.5   |
|              | 0.01                 | 87.8    | 72.5    | 82     | 7.0     | 12.4    | 8.4    |
| Malathion    | 1.0                  | 85      | 72      | 60     | 6.0     | 1.6     | 13.4   |
|              | 0.5                  | 106     | 80      | 75.6   | 16      | 2.5     | 1.0    |
|              | 0.01                 | 81      | 71      | 80     | 12      | 2.3     | 6.6    |
| Fenvelerate  | 1.0                  | 106     | 62      | 70     | 4.5     | 3.8     | 12     |
|              | 0.5                  | 97      | 62      | 77     | 9.0     | 2.9     | 9.5    |
|              | 0.01                 | 101     | 70      | 90     | 1.5     | 2.7     | 8.4    |
| Permethrin   | 1.0                  | 65      | 62      | 70     | 11      | 18      | 3.0    |
Table 4: Principal Loading of Main Factors (in bold) of Principal Component Analysis

| Variable   | PC1  | PC2  | PC3  | PC4  | PC5  |
|------------|------|------|------|------|------|
| â-HCH      | -0.02| -0.11| -0.44| -0.38| 0.34 |
| ß-HCH      | -0.25| 0.32 | -0.10| -0.37| 0.08 |
| ß-HCH      | -0.25| 0.42 | -0.05| 0.02 | -0.13|
| DDE        | 0.36 | 0.22 | -0.04| 0.01 | 0.02 |
| DDD        | 0.33 | 0.25 | -0.15| 0.18 | 0.11 |
| DDT        | 0.35 | 0.14 | 0.02 | -0.34| 0.04 |
| Endosulphan| -0.24| 0.43 | -0.11| 0.01 | 0.05 |
| Dimethoate | 0.25 | 0.16 | 0.38 | -0.10| 0.09 |
| Diazinon   | -0.11| 0.51 | 0.12 | 0.03 | -0.11|
| P-methyl   | 0.22 | -0.10| -0.37| -0.09| 0.43 |
| P-ethyl    | 0.10 | 0.16 | -0.44| -0.02| 0.36 |
| Chlorpyriphos| 0.33| 0.20 | -0.15| 0.26 | -0.17|
| Malathion  | 0.19 | 0.005| 0.27 | 0.32 | 0.5  |
| Fenvelerate| 0.34 | 0.06 | 0.14 | -0.22| -0.35|
| Permethrin | 0.1  | -0.03| 0.35 | -0.55| 0.129|

Figures

Figure 1

Biplot Indicating the Concentration of Pesticides in Vegetable Samples