Assessment of Pesticide Residues on Selected Vegetables of Pakistan

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Summary: The present study was conducted to determine the pesticide residues on selected summer vegetables. Five vegetables were grown with three replicates in a split plot randomized complete block design. Pesticides were sprayed on vegetables thrice at regular intervals each after 15 days. At maturity the pesticides residues were extracted from edible and leaf portions using anhydrous sodium sulfate and ethyl acetate while adsorption chromatography technique was used for cleanup. The extracts were subjected to high performance liquid chromatography (HPLC) for separation and analysis of the compounds. Significant differences ($p<0.05$) were found in the pesticides residues on edible portions whereas highly significant differences ($p<0.001$) were observed for the leafy portions. The residual level of cypermethrin was highest (16.2 $mgkg^{-1}$) in edible portion of bitter gourd, while Lambdacyhalothrin and Mancozeb residues were detected high (4.50 $mgkg^{-1}$, 6.26 $mgkg^{-1}$) in edible portion of bitter gourd and Cucumber respectively. Cypermethrin residues were high (1.86 $mgkg^{-1}$) in Okra leaves. Mancozeb and Lambdacyhalothrin residual level was high (1.23$mgkg^{-1}$, and 0.0002 $mgkg^{-1}$) in chili and tomato leaves. Cypermethrin residues were readily detected in edible and leaf portion of the selected vegetables.

Introduction

Pesticides are human-made and naturally occurring chemicals that control insects [1, 2], weeds, fungi and other pests that destroy crops. The prudent use of pesticides is considered to be indispensable for the control of insect-borne diseases in order to enhance food supply for an increasing world population. Pesticide application is still the most effective and accepted means for the protection of plants from pests, and has contributed significantly to enhance agricultural productivity and crop yield [3, 4]. According to the United Nations Food and Agricultural Organization, the world's potential human food losses are about 55 percent that include pre-harvest (35%) and post harvest (20%). In the developed countries, crop losses are estimated to be in the range of 10–30%, while in developing nations they are as high as 75% [5]. Thus, the use of pesticides is very common to increase the crop productivity in most of the developing countries. However, pesticides are toxic substances and persistent in character. Food exposure to pesticides accounts for more than 90% of total exposure [6]. Pesticide residues in food and crops are a result of direct application of pesticides to crops growing in the field, and to a lesser extent from pesticide residues remaining in the soil [7]. The problem of contamination of food sources, especially vegetables by pesticide residues constitutes one of the most serious challenges to public health. The hazards of toxic residues can be considerably reduced if pesticides are used in accordance with "good agricultural practice".

The total dietary intake of pesticide residues that remain on agricultural commodities are known as toxins and therefore, it is desirable to reduce these residues. The maximum residue limits (MRLs), is established by each country and sometimes causes conflicts because residue levels acceptable in one country could be unacceptable in another [8]. The required rates of application may vary, under different agricultural and climatic conditions, from country to country, and between regions of the same country [9]. With the growth of pesticide use in developing countries, the number of accidents, cases of poisoning and occurrence of environmental hazards are also likely to increase. In most countries, governments are endeavoring to regulate pesticide usage and increase farmer's awareness of hazards, while at the same time trying to promote the use of environmental friendly alternatives such as integrated pest management [10].

The environmental risk of a pesticide may be calculated as a function of its environmental hazard and the amount released into the environment. According to US department of Agriculture (USDA), no more than 2% (and less than 0.1%) of insecticides applied to crops by aerial or ground spraying reached the target pest, less than 5% of herbicides applied to crops reaches the target weed. Pesticides that miss

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their target pest end up in air, surface water, ground water, bottom sediment, food and non target organisms, including human and wildlife [11]. These side effects are well known worldwide [12]. Poisoning by pesticides in humans has been extensively studied [13], and frequently reported in forensic medicine [14, 15]. However, intoxications also appear to be of particular importance for affecting domestic animals and livestock [16-18]. Toxicological experiments carried out in 1998 proved that three hundred thousand farm workers in the US alone suffered from pesticide-related illnesses [19].

Toxic nature of different pesticides has been compared, it has been observed that metabolites of pyrethroid pesticides are relatively non-toxic or low toxicity compared with parent pyrethroid compounds [20], while pyrethroids were reported to have relatively low toxicity as compared to organophosphorus, organochlorine or carbamate pesticides [21].

Pesticide usage is not properly regulated due to ineffective legislation, lack of awareness and technical knowhow among the farming community in Pakistan. No comprehensive studies have been undertaken to determine the pesticide residues in vegetables and fruits in the country with the exception of few monitoring studies [22-24]. The present study aimed to investigate and quantify the residues of some commonly used pesticides on selected summer vegetables grown under local agro-climatic conditions.

Results and Discussion

Field Survey

Fifty farmers and pesticide dealers were interviewed regarding the use and application of pesticides on vegetables in Haripur district using a questionnaire. Results of the survey revealed that wide range of pesticides of different chemical classes were in routine use. The most frequently used insecticides were Cypermethrin (28.92%) and Lambdacyhalothrin (15.60%), followed by Mancozeb (10.32%), Methamidophos, (9.58%), Monocrotophos (8.56%), Chlorpyrifos (7.25%), Trichlorfon (7.00%), Dimethoate (5.59%), Fenvalerate (4.56%) and Diazinon (3.84%) Table-1. Cypermethrin was also observed as a major insecticide used (18.2%) in Botswana, followed by dimethoate (17%), chlopyriphos (12.5%), mancozeb (9.82%), methomyl (7.1%), dicofol (6.3%) and carbaryl (5.4%) [25].

Table-1: Most frequently used pesticides on vegetables in Haripur area of Hazara region.

Pesticide	Percent Use	Group
Cypermethrin	28.92	Pyrethroid
Lambda-cyhalothrin	15.60	Pyrethroid
Mancozeb	10.32	Dithiocarbamate
Methamidophos	9.58	Organophosphate
Monocrotophos	8.56	Organophosphate
Chlorpyrifos	7.25	Organophosphate
Trichlorfon	7.00	Organophosphate
Dimethoate	5.59	Organophosphate
Fenvalerate	4.56	Pyrethroid
Diazinon	3.87	Organophosphate

On the basis of survey and application to the vegetables, two insecticides and one fungicide was selected for the study, viz Cypermethrin, Lambdacyhalothrin and Mancozeb. The survey suggested that farmers of the area do not follow the proper crop rotational pattern and continuously grew exhaustive crops which leads to reduced soil fertility; mixed cropping was also observed during the study with improper irrigation practice which ultimately results in more humidity and fungal diseases. As a result of these improper practices crops were more prone to pest attack that leads to increased chemical use. Studies had indicated that pest attack can be reduced by good crop rotation practices which can decrease the need for fertilizers and pesticides [26].

The survey showed that farmers lack knowledge of pests, diseases and their management, and rely heavily on pesticides. Based on this information, researchers and extension workers in agriculture sector need to work with farmers in developing IPM strategies that will reduce their heavy reliance on pesticide usage.

Pesticides Residues on Edible Portions of Vegetables

Significant difference ($p<0.05$) was found for residual level of cypermethrin in bitter gourd. The mean residual level of cypermethrin in edible portion of bitter gourd was detected 16.2mg kg^{-1} while in edible portion of okra and chili the mean residual level were 4.89 $mgkg^{-1}$ and 4.85 $mgkg^{-1}$, respectively (Fig. 1). No significant differences ($p>0.05$) were found for Lambdacyhalothrine and Mancozeb for edible portion of five vegetables. Lambdacyhalothrin mean residual level in edible portion of bitter gourd was 4.50 mg kg^{-1}, while Mancozeb mean residual level in edible portion of Cucumber was 6.26 $mgkg^{-1}$, and in edible portion of Chili and Bitter gourd the mean residual levels were 3.70 $mgkg^{-1}$ and 1.99 $mgkg^{-1}$ respectively (Fig. 1).

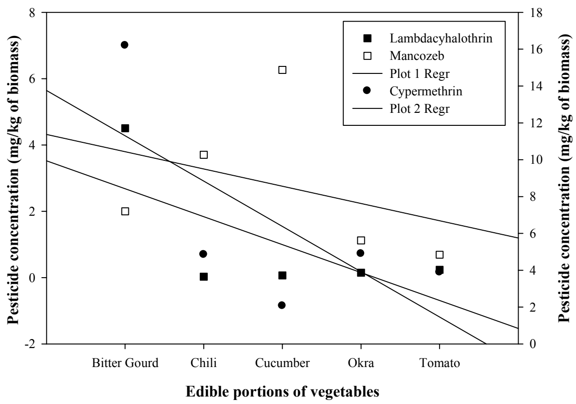

Fig. 1: Pesticide mean residual level on edible portion of vegetables.

Cypermethrin residues in edible portion of all the vegetables were above maximum residual limits (MRLs) prescribed by FAO/WHO Codex Alimentarius Commission, while Lambdacyhalothrin and Mancozeb residual level in edible portion of bitter gourd and cucumber samples were slightly higher. Zawiyah et al., [27] detected pesticide residue in tomato (30%), chilli (23.3%) and brinjal (20%). Our results were in agreement with European Coordinated Monitoring Program of Vegetal Products, which showed the presence of pesticides in samples of vegetables, cereals and fruits in 2002 [28]. The results obtained during this study showed that residues content varied depending on type of crop. The weather conditions were same for all the vegetables during the trial period sprayed with Cypermethrin, Lambdacyhalothrin, and Mancozeb, the differences may be due to type and response of vegetables to different insecticide and fungicide. Our results were also supported by the findings of Mayank and Ajay [29], who reported that in rainy season, the lowest contamination may be attributed to the washing out of the external surfaces of vegetables due to rain. Similar kinds of results were reported for organochlorine pesticide contamination in different season by Bhanti and Taneja [30]. Moreover agriculture practices may also be the reason of high residual level. Serpil [31] found that due to good agricultural practices and proper pesticide application in Turkey reduced the residual concentration of pesticide in vegetables.

Pesticides Residues on Leaf Samples of Vegetable

Leaf portion of all vegetables were analyzed for the three pesticides. Cypermethrin residues were found higher in almost all leaves samples as compare to other two pesticides. Cypermethrin mean residual level in okra leaves were 1.8 mgkg^{-1}, tomato leaves 0.842 mgkg^{-1} and bitter gourd leaves 0.765 mgkg^{-1}. Lambdacyhalothrin was detected in lowest concentration in chili and cucumber 0.000007 mgkg^{-1} each (Fig. 2). No significant differences ($p>0.05$) were found for leaf samples of vegetables in response to pesticides (Fig. 2).

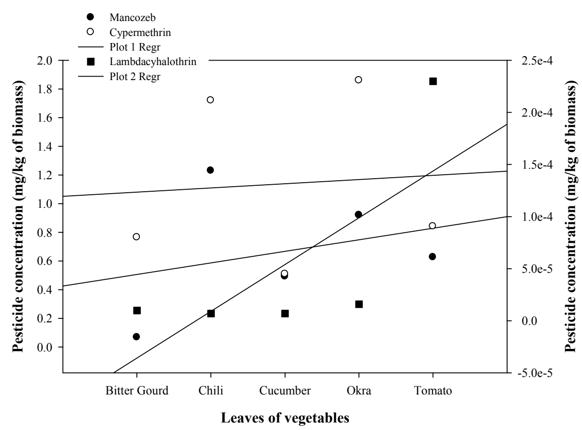

Fig. 2: Residues of pesticides on leaf portion of vegetables.

Our results are in agreement with Rosa et al., [32], who found that among the different insecticides in leafy vegetables, Cypermethrin was the insecticide with the highest number of positives above the MRL in leafy vegetables. Results are also comparable with the findings of Liu *et al.*, [33] who reported high pesticide residues in leaf and root vegetables than other kinds of fruits and vegetables. Our results showed that edible portion retained more residues while the leaf portion of vegetables was found less contaminated with pesticide. The fact may be attributed to the smooth surface of the leaves as compare to rough surface of the vegetables; moreover, runoff of pesticide from the leaves is high as compare to edible/fruits, which hang below the leaves.

Experimental

Plant Materials

Five vegetables viz, *Abelmoschus esculentus* **(**okra), *Capsicum annuum* (chilies), *Cucumis sativus* **(**cucumber), *Momordica charantia* (bitter gourd), and *Lycopersicon esculentum* (tomato) with three different pesticides were used in this study. The seeds were obtained from the National Agriculture Research Centre Islamabad, Pakistan. Field experiments were conducted to study dissipation rates of pesticides after application on to these vegetables. Results of the survey revealed that wide range of pesticides of different chemical classes were in routine use. The most frequently used insecticides were Cypermethrin (28.92%) and Lambdacyhalothrin (15.60%), followed by Mancozeb (10.32%), Methamidophos, (9.58%), Monocrotophos (8.56%), Chlorpyrifos (7.25%), Trichlorfon (7.00%), Dimethoate (5.59%), Fenvalerate (4.56%) and Diazinon (3.84%). On the basis of survey and application to the vegetables, two insecticides and one fungicide was selected for the study, viz Cypermethrin, Lambdacyhalothrin and Mancozeb.

Experimental Design

The initial step of the investigation was the conduct of a field survey to know the nature of pesticides used in the Hazara region. The vegetables were selected for the residual effects since pesticides were reported to be most frequently used on these crops in the Northwestern region of Pakistan, as indicated by the field survey at the initiation of the study (Table-1). For the experiment, the soil was collected from Research Nursery Farm, CIIT Abbottabad. The following parameters were determined prior to the experiments: pH solid: de-ironed water = 1:2.5 w/v); total organic matter (450 and 600 ºC, after heating for 6 h in a muffle furnace); total nitrogen content; total phosphorus (P) and water-soluble P; water-soluble N; water-soluble K. The selected physicochemical properties of the soil are presented in Table-2.

Table-2: Physico-chemical properties of soil.

Parameter	Value
pH	7.2
Organic matter (gkg^{-1})	20.3
Total Nitrogen (gkg^{-1})	0.95
Available Nitrogen (mgkg^{-1})	70.3
Total Phosphorus (gkg^{-1})	0.6
Available Phosphorus (mgkg^{-1})	8.2
Total Potassium (gkg^{-1})	16.5
Available Potassium (mgkg^{-1})	64.3

Three seedlings of each vegetable were transferred to the pots containing 1 kg soil. The soil moisture content was maintained at 60% water-holding capacity by weight adding deionized water after every 2 days. After 2 months of growth, plants were grown in a greenhouse at 30 and 24 ºC during the day and night, respectively.

For each vegetable three pesticides were applied following "Good Agricultural Practices" (GAP). One controlled block (unsprayed) was included in the experiment for blank analysis. Five vegetables were randomly assigned to the treatment units using split plot randomized complete block design (SPRCBD) with three replications. Each pesticide was applied with one recommended dose in practice among the farmers and agriculture practitioners. Pesticides were applied on vegetables three times, first spray was applied when half of the flowering completed, and keeping 15days difference the other two sprays were applied.

Sample Collection

Fresh samples of vegetables were randomly collected from each replicate to assess the residue level of pesticides applied. Edible portion and leaves from bottom, mid and top of the vegetable were sampled and immediately transported to laboratory in plastic bags after collection. The samples were stored at -20 °C using laboratory freezer, in order to avoid any degradation between sampling and analysis.

Sample Preparation and Extraction of Pesticide Residues

Pesticide residues from vegetable samples (Edible and leaf portion), were extracted with ethyl acetate. Vegetables were chopped with a sharp knife and mixed thoroughly. The chopped sample (200 g) was transferred to a high-speed blender. The sample was thoroughly blended to obtain a homogeneous representative sample for weighing. An aliquot of 25 g sample was taken in a 250 ml Erlenmeyer flask, 37 ml ethyl acetate, and 12 g anhydrous sodium sulfate were added to the flask and shaken for 2 hours at the speed of 220 cycles/min in a horizontal shaker (Orbital Shaking Incubator). The ethyl acetate extract was filtered through Whatmann (No. 4) filter paper.

After cleanup the samples were collected in a 100 ml round-bottom flask and were concentrated with rotary evaporator (Heidolph, Laborota 4000 efficient, Germany). The dried extract was dissolved in exactly 5 ml methanol. Extracts were filtered using 0.2µm filter paper before chromatographic analysis.

High Performance Liquid Chromatographic (HPLC) Analysis

Extracted samples of vegetables were analyzed by high performance liquid chromatography (HPLC) following the method of Ohlin and Hiemstra, et al. [34, 35]. HPLC analyses were performed in isocratic system using a PerkinElmer Chromatograph including Series 200 pump, Series 200 UV/VIS detector, and a Supelco C18 analytical column (25 cm x 4.6 mm (i.d)). Methanol/water was used as mobile phase. 20 µl sample was injected through auto sampler. The column temperature was kept 30 °C with a flow rate of 1ml min^{-1}.

Statistical Analysis

The combined experiment comprises 300 treatment units. The data on each treatment unit was obtained in chromatograms, the peaks of which showed the qualitative and quantitative measures. Each chromatogram was converted to quantitative measure (µV sec-1 to ppmsec-1). The new data obtained from the trials was subjected to statistical analysis. Statistical Analysis System (SAS) software (SAS, 1998, NC State, USA) was used for the analysis of split plot randomized block design. ANOVA and LSD were calculated on measures to separate the group of means of five vegetables.

Conclusion

Significant differences ($p<0.05$) were found for three pesticides applied to edible portion of vegetables while highly significant differences ($p<0.001$) were found for three pesticides on vegetables leaves. Among selected vegetables bitter gourd and okra exceeded the maximum residual limits (MRLs) in edible portions for all the three pesticides. It is concluded that cucumber, chili and okra when treated with recommended dosage of Lambdacyhalothrin, were safe for consumption. Tomatoes may be used for consumption if treated with recommended dosage of Mancozeb. Cypermethrin was readily detected in edible portion of most vegetables and exceeded the maximum residual limits (MRLs). High concentration of Cypermethrin and Mancozeb were detected in leaf portion of five selected vegetables. Chili and okra leaves were found highly contaminated with Cypermethrin and Mancozeb. It is recommended that there should be a regular check over the use of cypermethrin and mancozeb on vegetables. Cypermethrin should be immediately controlled by the concerned agencies so that the residues may not enter the food chain creating health and environmental hazards for the region. Vegetables should be subjected to a random inspection in order to monitor and regulate the use of sprays and products found, to curtain residue levels above the MRL. In context of this study it is recommended that vegetable leaves are more prone to pesticide residues and future research should focus on leafy vegetables.

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