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RESEARCH ARTICLE

Assessment of Neurotoxic Pesticides Residues in Fruits and Vegetables by Bioassay Technique

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Abstract

A study was preformed to investigate the status of organophosphate and carbamate pesticide residues present in the fruits and vegetables. For this purpose, samples were collected from the local markets of Godawari municipalityand Dhangadhi sub-metropolitian city of Sudurpaschim province in Kailali district of Nepal. Samples were analyzed for the pesticide residues by the rapid bioassay for pesticide residues (RBPR) technology. The obtained results showed that in fruits the highest inhibition (%) of carbamates (CMs) was present in the banana 29.488%and the highest inhibition (%) of organophosphates (OPs) was present in orange 17.433%. The highest acetylcholinesterase (AChE) inhibition (%) of CMs and OPs pesticides was found to be associated with sting gourd i.e. 20.574% and 25.357% respectively. The findings point out the urgent need to address the potential risk of exposure to multiple pesticide residues via the stringent monitoring programs on daily basis from the pesticides present in the diet.

Key words: Pesticide residue; Fruits; Vegetables; Bioassay; Food safety

Introduction

Chemical contamination of agricultural products has become a global issue. Pesticides are indispensable to modern farming. They are used to control or eliminate pests. They are either synthetic or natural in origin. Pesticides are compound or a mixture of a several chemical compounds that may be employed to eliminate or reduce insect population, rodents, fungi, weeds or every kinds of aquatic plants, animals and viruses, bacteria and microorganisms that are considered pests.In this way, the application of these compounds intends to ensure the quantity and quality of agricultural productsrequired for consumers. However, this leads to the bioaccumulation of pesticide residues in them. For this reason and considering the negative effects of the pesticides on human health; such as genotoxicity, inhibition of acetyl cholinesterase activity, hepatic, and renal toxicity pesticides monitoring is important to ensure a minimal exposure to them [1–4].

Residues of multiple pesticides are exposed to human via agro-ecosystem. So far as vegetables and fruits are concerned, pesticides are employed to control diseases and pests. Theinappropriate and indiscriminate use of pesticide results in adverse environmental and healtheffects [5]. Some of these pesticides are often persistent, highly mobile in nature and get bio-accumulated [6-7]. Owing to this, the maximum residue limits (MRLs) for various pesticides in food producehas been set by the United Nations and the European Union [8-9].The maximum residue level is the uppermost statutorylevel of concentration for a pesticide residue in or on food when chemical pesticides are employed appropriately in accordance with good agricultural practice [10]. In context of chemical pesticides in food in Nepal, no specific residue limits are established yet [11].

Although use of pesticides led to massive increase in yield of agricultural products, certain chemical pesticides have been abandoned due to health and environmental issues. Highly persistent and lipophilic organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) and its metabolites such as dichlorodiphenyldichloroethylene (DDE) have been banned in many countries because of their carcinogenic, mutagenic and endocrine disrupting properties [12-13]. This promoted organophosphates [14] and carbamates [15] as more ecological alternatives to organochlorine chemical pesticides with lower persistence [16-17]. Despite of being commonly employed chemical pesticides, both the organophophates and carbamates has the ability to cause various health effects such as cancer, diabetes, strokes, kidney failure, Parkinson's disease, dementia, neurobehavioral effects, reproductive issues, non-Hodgkin,s lymphoma and Alzheimer's disease [18-19]. Organophosphates, such as dichlorovos, glyphosate, chloropyriphos, chloropyriphos-methyl, malathion, parathion, diazinon and dimethoate are the commonly employed ones' in agriculture [20]. These form one of the versatile organic pesticides in agriculture to protest variety of crops. They fetch their effect via inhibiting acetylcholinesterase (AChE) the enzyme that ceases the action of neurotransmitter acetylcholine (Ach) within neuromuscular confluence and nerve tissue, leading overstimulation of the post-synaptic membrane. As a consequence, accumulation of acetylcholine takes place which causes neurobehavioral malfunction in the pest [21-22].

Another class of chemical pesticides include esters of carbamic acid that have been associated with the effects on the function of acetylcholinesterase enzyme, are carbamates. This class includes variety of pesticides such as aldicarb, carbaryl, carbofuran, primicarb, and ziram, reveal the prevalence of carcinogenicity and immunotoxicity [23-24]. Exposure to chemical pesticides happens through dermal contact, inhalation, or ingestion. The possible outcome will depend on the type of chemical pesticide the person comes into contact, the duration and concentration of exposure, and the way the person is exposed [20].

Human exposure of pesticide residues can be via great variety of everyday food products. Several studies delineate that foods such as fruits, fruit juices and vegetables contain higher level of pesticide residues. Moreover, washing and peeling cannot completely remove the precarious residues [25-26]. Fruits and vegetable constitute important components of a healthy diet being good source of minerals and vitamins. However, fruits and vegetables contaminated with pesticide residues above maximum residue level pose threat to consumers.

An organophosphate pesticide, malathion has been extensively used to control pests in fruits and vegetables, such as banana, citrus fruits, mulberry, cranberry; broccoli, brinjal, beans, cauliflower, carrot, and tomato [27-28]. Dimethoate, parathion, dimethyl-dichloro-vinyl-phosphate have been applied as insecticides on different vegetable products to protect from pest [29]. Carbofuran, a milder carbamate pesticide with better insecticidal activity has also been widely employed as insecticide in carrots, lettuces and tomatoes to control soil-born pest[30].

In the context of Nepal, no extensive studies on pesticide residues in fruits, grains and vegetables has been carried out. Threat from pesticide residue is blooming, with the increasing use of pesticides on vegetables [31]. Previous studies have shown pesticide residues on vegetables [32–34]. Currently, there is very little information regarding the level of pesticide residues in food products available in different parts of the country. As a consequence, there is an urgent need to assess the pesticide residues in food products such as fruits and vegetables to ensure that the level of residues do not exceed maximum residue level.

Various analytical methods are developed to monitor pesticide residues such as Gas chromatography equipped with mass spectrometer(GC/MS), Liquid chromatography-mass spectrometer (LC/MS), High-performance liquid chromatography (HPLC), and sensors. However, all these advanced analytical techniquesare time consuming and require high initial cost per sample analysis. Therefore, the rapid bioassay for pesticides (RBPR) technique is considered to be an effective tool for the assessment of chemical pesticide residues. The RBPR technique is developed by Taiwan Agricultural Research Institute (TARI) for screening organophosphate and carbamate pesticide residues present in fruits and vegetables. It is a simple fast and low cost method based on the enzymatic reaction. The enzyme acetylcholinesterase (AChE) isolated from the head of house fly catalyses the hydrolysis of acetylthiocholine to yield acetic acid and thiocholine. The hydrolysatethiocholine or choline reacts with 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB; Ellman's reagent) to form a yellow coloured compound 5-thio-2-nitro-benzoic acid having maximum absorbance at 412nm [35-36]. Thus the basis of the method is the measurement of rate of formation of thiocholine based on Ellman's test (Figure 1). In the presence of organophophates and carbamates the activity of AChE get reduced due to phosphorylation or carbamylation of serine hydroxyl group present in the active site of the enzyme [37]. The colour reaction involved can be monitored spectrophotometrically.

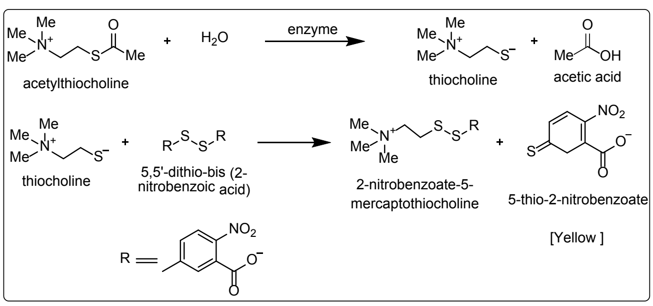

Figure 1. Reaction of enzymatic hydrolysis of acetylthiocholine and DTNB

This study aims at determining the residual levels of organophosphates and carbamate chemical pesticides present in fruit and vegetable samples available in the local markets of Attaria and Dhangadhi cities located in Kailali district of Nepal. Moreover, the study is aimed to highlight the urgent need for continuous monitoring of pesticide residue for the controlled and discriminate use of chemical pesticides.

Materials and Methods

Chemicals

Phosphate buffer, AChE, DTNB, acetylcholine iodide (ATCI), ethanol, and bromine.

Sample collection and analysis

Sample were collected from the Dhangadhi and Attariya vegetable and fruit markets located in Kailali district in January 2021. Altogether, 35 samples wererandomly collected from these markets for pesticide residue analysis. The experiments were performed at Rapid Bioassay of Pesticide Residue Analysis (RBPR) unit, at Attariain Kailali, Nepal. The laboratory is based on the bioassay kit to crisscross residues of neurotoxic pesticides organophosphates and carbamate through RBPR followed by UV-Visible spectrophotometric measurement at 412 nm [35].

Extraction Procedure

A typical sample extraction procedure used 1 gm of plant tissue collected by random sampling. The plant tissue was cut into small pieces and was transferred to test tube. Then each sample was subjected to two sort of extraction methods were for the determination of percentage inhibition from carbamates and organophosphates.

I. Test without bromine water: 1 mL of 95% ethanol was added to the test tube, shaken in a vortex mixture for 20 seconds, and was allowed to stand for 3 minutes. The supernatant extract was decanted into other test tube for inhibition assay for carbamates.

II. Test with bromine water: For organophosphates, 2 mL of 95% ethanol, 100 µL of bromine water were added to the test tube, shaken for 20 seconds in a vortex mixture, and was allowed to stand for 3 minutes. The supernatant extract was decanted into other test tube. The sample extract was allowed to stand for next 20 minutes to allow evanescence of excess bromine before inhibition assay.

Inhibition Assay

The sample extracts were subjected to inhibition assays byfollowing Standard Operating Procedure for RBPR [37-38]. The enzyme inhibition assays were performed in cuvette by taking 3.0 mL of phosphate buffer, 20 μL of AChE, and 20 μL of sample extract followed by shaking for 5 seconds at room temperature. To the resultant mixture was added 100μL DTNB followed by 20 μL ATCI solution at exactly 2.5 min and 3.0 min just after mixing with the sample extract. After performing mixing for 5 seconds the absorbance of the resultant sample solution was recorded in a spectrophotometer at 412 nm. The blank for such an assay consists of buffer, AChE, DTNB and ATCI solutions. All assays were performed in triplicate and average values are reported. The inhibition percentage of enzyme was computed according to the expression 1.

$$\text{Inhibition percentage (\%)} = \frac{\text{Absorbance}_{(control)} - \text{Absorbance}_{(sample)}}{\text{Absorbance}_{(control)}} \times 100\ \% \quad \text{............(1)}$$

Results and Discussion

AChE inhibition percentage shown by pesticides present in different fruits and vegetables

Residues present in different vegetables and fruits sample collected from local market of Attariya and Dhangadhi cities were extracted by using alcohol as extractant and detected by using the RBPR technology, i.e., AChE inhibition test. This test is used for determination of inhibition percentage of OPs and CMs pesticide residues.

Table 1. Inhibition % of AChE shown by CMs and OPs pesticides in fruit samples

Fruit sample	Inhibition percentage (%)	
	(CMs)	(OPs)
Banana	29.488	7.0668
Apple	7.4421	1.8865
Grape	2.0067	3.9085
Pineapple	0.2287	1.9967
Coconut	0.2577	1.4412
Pomegranate	9.8867	5.2365
Kiwi	5.4967	3.7649
Lemon	2.7551	0.0878
Orange	25.548	17.433
Sweet orange (Junar)	7.1889	6.8990
Tangerine	2.4544	6.3554
Plum	0.4511	2.8661

Table 1 summarizes, the AChE inhibition percentage of OPs and CMs pesticides present in the fruits samples. The highest inhibition percentage of CMs was present with the banana about 29.488% followed by orange about 25.548%. Similarly, the highest inhibition % of OPs was present with orange about 17.433, and for the other fruits sample the inhibition percentage (%) lies in the lower range of 0-10.The results demonstrated that all of the fruit samples were qualified for domestic market.

Table 2. Inhibition % of AChE shown by CMs and OPs pesticides in vegetable samples

Vegetable sample	Inhibition percentage (%)	
	(CMs)	(OPs)
Potato	12.321	17.385
Tomato	5.6741	7.4387
Cauliflower	15.280	3.1272
Cabbage	6.4985	2.1153
Brinjal	6.4986	1.9842
Capsicum	2.2453	3.6722
Onion	5.0013	1.9151
Bottle gourd	0.3192	4.7964
Carrot	0.7769	3.4215
Broccoli	10.889	2.5596
Garden pea	8.6744	1.1254
Ash gourd	0.5455	4.4349
Cucumber	11.218	5.4764
Pumpkin	1.7664	3.3788
Luffa gourd	0.4349	3.4519
Snake gourd	1.6689	0.1886
Green peeper	0.5776	0.8875
Coriander	1.2276	2.7576
Lady finger	4.6754	0.4489
Radish	1.1277	0.0599
Sting gourd	20.574	25.357
Long peeper	0.5989	1.8992
Taro root	2.4791	0.8845

The AChE inhibition percentages of OPs and CMs present in the vegetables is shown in Table 2. The highest AChE inhibition percentage of CMs was present in Sting gourd i.e. 20.574%. Similarly, the highest AChE inhibition percentage of OPs pesticides was also found to be 25.357% in sting gourd. The AChE inhibition percentage (%) in the other sample of vegetables were in the range of 0-17.

The samples above 45% inhibition contained residues above Maximum residual limits (MRL) and those samples with less than 20% inhibition [39] can be used for marketing and those with >50% should be advised to extend the interval between the pesticide application and harvest of fruits and vegetables to make sure that the residues are within the acceptable limits. The experimental data of AChE inhibition percentage (%) of OPs and CMs in the vegetables and fruits samples were below 35%, i.e., in green level. So, their quality was good and can be used for marketing and are edible.

Conclusions

The study showed that none of the fruit and vegetable samples assessedin the market of Attaria and Dhangadhi exhibited pesticide residues above maximum residual limit. It is advised that farmers be educated for better safety procedures. Moreover, pesticide residues should be monitored on regular basis.

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