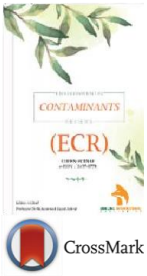
ZIBELINE INTERNATIONAL™ PUBLISHING

ISSN: 2637-0778 (Online)
CODEN: ECRNAE

Environmental Contaminants Reviews (ECR)

DOI: http://doi.org/10.26480/ecr.02.2022.69.71



RESEARCH ARTICLE

ORGANOPHOSPHATE AND CARBAMATE PESTICIDES RESIDUE ANALYSIS IN VEGETABLES PRODUCED IN FAR-WEST NEPAL

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ARTICLE DETAILS

Article History:

Received 24 July 2022
Accepted 26 August 2022
Available online 01 September 2022

ABSTRACT

This is an experimental research study carried out at the laboratory of wholesale fruits and vegetable market, Attarya, Kailali, to analyze organophosphate and carbamate pesticide residue in vegetables grown and marketed in four different districts of far-west Nepal by Rapid Bioassay of Pesticide Residue. The main goal of this study is to determine the proportion of AChE inhibition in various vegetable commodities and to compare the pesticide residue. Seven different vegetables were collected purposively from four distinct areas in Nepal's far west region. Each of the 28 vegetable samples was analyzed for both organophosphate and carbamate residues. The sample was extracted from each vegetable and examined through a spectrophotometer and the data are interpreted. The experiment's findings suggest that the Beans from Kanchanpur had the highest OP (21.69 percent) while cucumber from Dadeldhura had the lowest (0.07 percent). The bitter gourd of Kailali had the greatest C (24.39 percent), while the bottle gourd of Doti had the lowest (0.49 percent). All 56 items examined in the Attariya market laboratory were found to have low pesticide residue, with less than 35%. However, the waiting period must be strictly adhered to.

KEYWORDS

Pesticides, Residue, Sample Extraction, Vegetables, Waiting Periods.

1. INTRODUCTION

Farmers are more motivated to grow vegetables, and many of them are commercial growers. The total cultivated area under vegetable crops is 281132 hectares, with a production of 3962383 metric tons and productivity of 14.09 mt/ha and 60.4 percent of the economically active people are engaged in agriculture. Agriculture accounts for 26.98% of GDP, with vegetables accounting for 11.92% of national AGDP (MoALD, 2021; MoALD, 2021). Vegetables are the most significant foods in the daily Nepalese diet. However, due to a lack of awareness among farmers, these crops receive a significant amount of pesticide, resulting in less than optimal economic growth (Jha and Regmi, 2009). Farmers in Nepal use the largest amount of pesticides in vegetables after rice to control pests and diseases, according to because pesticides are extremely effective and quick-acting. According to the average pesticide use in Nepal is 0.396 a.i. kg/ha. At cholinergic junctions in the neurological system, the pesticide OP inhibits an enzyme called AChE (Kazemi et al., 2012; Pokhrel and Pan, 2009; Kalauni and Joshi, 2019). It is more dangerous for children than for adults (Joy et al., 2018). Carbamate spraying reduces the number of non-target organisms significantly (Mustapha et al., 2019). In Nepal, 24 pesticides have been banned till 2019. (Kalauni and Joshi, 2019).

TARI's RBPR (Rapid Bioassay for Pesticide Residues) approach combines the AChE and Bt tests to screen for acetyl-cholinesterase inhibitor (neurotoxin) pesticide residues (Chiu et al., 1991). The government of Nepal has begun using the RBPR technology to assess pesticide residues in fruits and vegetables by establishing RBPR laboratories in various parts of the country and each province. Recently, laboratories have been established in Kailali (Attariya), Banke (Nepaljung), Rupendehi (Butwal), Kaski (Pokhara), and Jhapa (Birtamod), Sarlahi (Nawalpur), and, Chitwan (Bharatpur) having a good impact on consumers (Adhikari, 2017). From sampling to completion, this test takes only 15 minutes.

2. MATERIALS AND METHODS

2.1 Research Site

The study was carried out in Nepal's far-west area. The samples were collected purposively from four different districts and examined in the laboratory of Attariya, Kailali's wholesale fruits, and vegetable market. There were 56 vegetable samples analyzed in total. Tomatoes, cucumbers, cauliflower, brinjal, bean, bottle gourd, and bitter gourd were taken from four districts: Kailali, Kanchanpur, Doti, and Dadeldhura, and each product was analyzed twice in the laboratory for organophosphate and carbamate pesticide residues.

2.2 Sample Extraction

The sample was chopped into pieces and placed in two test tubes containing buffer solution in the laboratory for sample extraction. One test-tube was used to test OP residue maintained in flumewood, and the other was utilized to test carbamate residue. Nearly 1 gram sample was collected from each vegetable and then chop into fine pieces.

The sample was put into a test tube with 1ml ethanol, vibrated on a test tube mixer for 30 seconds, then left for another 3 minutes for extraction for the carbamate test. Similarly, for the OP test, the sample was placed in a test tube with 2 mL ethanol and 0.1 mL bromine water, vibrated for 30 seconds on a test tube mixer, then left for further 20 minutes in flume

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Cite The Article: Janaki Budha, Prativa Sapkota, Archana Acharya, Rakshya Bhandari, Narendra B. Singh, Deependra Dhakal (2022). Organophosphate and Carbamate Pesticides Residue Analysis in Vegetables Produced in Far-West Nepal. *Environmental Contaminants Reviews, 5(2): 69-71.*

wood for extraction. Test tubes were covered with parafilm when a longer soaking period was expected. Before assay, the ethanol extract was drained into a new test tube and covered with parafilm. The test tubes were appropriately labeled, and the sample extracts were evaluated.

2.3 Sample Test

In a 1cm glass cuvette, 3ml PBS, 20µl AChE solution, and 20µl 95 percent ethanol(AR grade) were added for the blank or control test, and 3ml PBS, 20µl AChE, and 20µl sample extract were added for the sample testing. After mixing and standing for 2:5 minutes, the enzyme reaction was started by adding 0.1ml DTNB and 20µl ATCI solution to each solution at 3 minutes and mixing for 5 seconds. When samples were placed inside the spectrophotometer, it took 1 minute to calculate the residue. The information was gathered using spectrophotometer readings. The test was repeated when the inhibition percentage for conformation exceeded 35 percent. With the help of the R-studio statistics package, the data was evaluated and means were compared using DMRT.

Table 1: Enzyme inhibition percentage and result interpretation

Enzyme Inhibition	Result Propose
>45%	Not suggested for consumption purposes
35%-45%	Quarantine for a minimum of 2 days. Repeat the test after washing. If the inhibition percentage is less than 35%, allow it for sale.
<35%	Approve for sale and consumption

(Source: Ghimire et. al., 2020)

3. Results

Table 2: Pesticides inhibition percentage (%) of different vegetables at Attariya market

Districts	Tomato		Cucumber		Bean		Brinjal	
	OP	OP	C	OP	OP	C	OP	C
Kailali	0.49^{c}	0.66^{c}	1.41^{b}	5.46^{c}	3.24^{c}	6.53^{b}	8.60^{b}	4.08^{b}
Kanchanpur	0.89^{c}	6.32^{a}	5.19^{a}	6.26^{b}	21.69^{a}	13.45^{a}	9.21^{a}	5.39^{a}
Doti	3.35^{a}	2.91^{b}	5.41^{a}	11.19^{a}	0.09^{d}	13.80^{a}	4.28^{c}	5.69^{a}
Dadeldhura	1.55^{b}	0.79^{c}	0.07^{c}	0.57^{d}	5.17^{b}	3.33^{c}	3.00^{d}	2.55^{c}
Mean	1.57	2.67	3.02	5.87	7.54	9.28	6.27	4.43
CV (%)	11.72	4.72	5.72	2.94	4.18	3.04	0.71	7.61
LSD (0.05)	0.58**	0.40***	0.55***	0.55***	1.00***	0.90***	0.14***	1.07**

CV: Coefficient of Variation; LSD: Least Significant Difference; Mean in the columns with different superscripts differ significantly by DMRT (P<0.05).

The result in all samples was significant from tables 1 and 2. Observation recorded as tomato showed significant differences among different districts in both OP and C. In the case of tomatoes, residue percentage the highest residue of OP was found in tomatoes of Doti (3.35%) whereas the lowest was found in tomatoes of Kailali (0.49%). Similarly, the highest C residue was found in the tomatoes of Kanchanpur and the lowest in that of Kailali. In cucumber samples, the recorded results were significant. The highest residue of OP and C was found in the cucumber of Doti (5.41% and 11.19%, respectively) and found lowest in Dadeldhura (0.07% OP and 0.57%C). In beans, the result was significant. The highest residue of OP was found in the beans of Kanchanpur (21.69%) and the lowest in that of Doti (0.09%). Similarly, the highest C residue was found in the beans of Doti (13.80%) which is statistically similar to that of Kanchanpur (13.45%) and the lowest C residue was observed in the beans of Dadeldhura (3.33%). The result of the brinjal samples was significant. The highest residue of OP was found in the brinjals of Kanchanpur (9.21%) and the lowest in that of Dadeldhura (3.00%). Similarly, the highest C residue was observed in brinjals of Doti (5.69%) which is statistically similar to that of Kanchanpur (5.39%) and the lowest residue of C was observed in brinjals of Dadeldhura (2.55%).

Table 3: Pesticides inhibition percentage (%) of different vegetables at Attariya market

Districts	Cauliflower		Bottle Gourd		Bitter Gourd	
	OP	C	OP	C	OP	C
Kailali	9.31^{a}	9.06^{a}	21.38^{a}	9.48^{b}	5.80^{c}	24.39^{a}
Kanchanpur	7.48^{b}	4.45^{b}	1.01^{b}	12.33^{a}	2.60^{d}	4.36^{b}
Doti	3.48^{c}	1.29^{d}	4.57^{b}	0.49^{c}	9.46^{b}	0.58^{d}
Dadeldhura	0.91^{d}	3.35^{c}	8.24^{b}	10.30^{b}	13.40^{a}	2.82^{c}
Mean	5.29	4.54	8.80	8.15	7.82	8.04
CV (%)	4.66	6.18	30.54	3.24	1.27	5.12
LSD (0.05)	0.78***	0.89***	8.55*	0.84***	0.31***	1.31***

CV: Coefficient of Variation; LSD: Least Significant Difference; Mean in the columns with different superscripts differ significantly by DMRT (P<0.05).

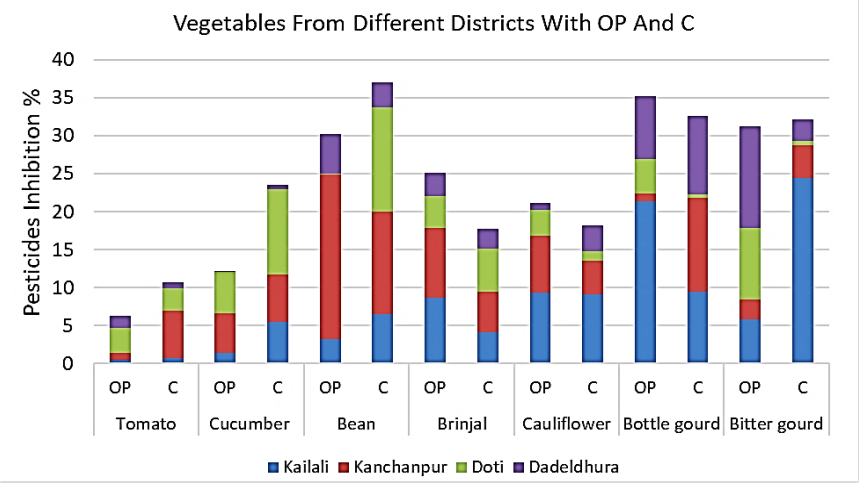

Figure 1: Pesticides inhibition percentage of different vegetables tested at the laboratory of Attariya mar

The result of cauliflower was significant. The highest residue of OP and C was found in cauliflowers of Kailali (9.31% and 9.06% respectively), and the lowest residue of OP was found in cauliflower of Dadehdhura (0.91%) and the lowest residue of C was found in that of Doti (1.29%). There was a significant result of bottle gourd. The highest residue of OP was found in the bottle gourd of Kailali (21.31%) and the lowest was found in that of Kanchanpur (1.01%) which is statistically similar to the result of Doti (4.57%) and Dadeldhura (8.24%). The highest residue of C was found in bottle gourds of Kanchanpur (12.33%) and the lowest was found in that of Doti (0.49%). The residue of C was observed in the bottle gourd of Kaiali (9.48%) and was statistically similar to that of Dadeldhura (10.30%). The results were significant for bitter gourd. The highest residue of OP was found in the bitter gourds of Dadeldhura (13.40%) and the lowest was found in the bitter gourds of Kanchanpur (2.60%). Similarly, the highest residue of C was found in the bitter gourd of Kailali (24.39%) whereas the lowest residue was observed in that of Doti (0.58%).

4. Discussion

We observed the different pesticide residues present in different vegetable commodities. Vegetable samples were collected from the different markets representing some districts purposively and tested in the laboratory by RBPR methods and compared with the pesticide residue present in the vegetables. Bean and bottle gourd contains the highest OP and carbamate residue in the Attariya market. From the test results, most of the vegetables are safe for consumption. This result was supported by published in Republika national newspaper (Sapkota, 2019). He collected random samples of vegetables such as tomato, brinjal, okra, beans, cucumber, snake gourd, luffa gourd, bitter gourd, and other fifteen vegetables from the Panchkhal and Kerabari area for surprise test of pesticide residue in Kalimati. He found that the pesticide residue of most of the vegetables was within the safety limit i.e. below 35%. In Bitter gourd, the result was significant which is in accordance with (Langyani, 2017). The found a significant result in Cauliflower and Okra which supports our result (Sahu et al., 2017). According to the article, vegetables like tomatoes, cauliflowers, eggplants, green beans, potatoes, etc. arriving from neighboring districts or farms and showed no or little residue of pesticides which are safe for consumption. In the Attariya market, a sample from Kanchanpur district contains the highest residue level supported by (Panthi, 2018).

5. Conclusion

A total of 56 samples were tested in the laboratory of Attariya, Kailali, 28 for OP and 28 for carbamate pesticide residue analysis. Seven different vegetables were sampled from 4 districts. The highest OP (21.69%) was found in beans of Kanchanpur followed by a bottle gourd of Kailali (21.38%), highest C (24.39%) was found in a bitter gourd of Kailali followed by beans of Doti (13.80%), and the lowest OP (0.07%) was found in cucumber of Dadeldhura followed by beans of Doti (0.09%), lowest C (0.49%) was found in bottle gourd of Doti followed by cucumber of Dadeldhura (0.57%). All samples tested in the laboratory of Attariya market were found to be safe for consumption with less than 35% of pesticide residue level. An awareness program related to the minimization of pesticide use, dose, types, rate, and health hazards of pesticides in vegetable production should be conducted and farmers should be motivated toward organic vegetable production making them aware of the negative health effect of pesticides or the chemical pesticides can be replaced with organic manures and in case of the use of pesticides at least follow the waiting period before marketing. RBPR test units should be established at all vegetable markets.

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