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RESEARCH ARTICLE

CARBAMATE AND ORGANOPHOSPHATE RESIDUE ANALYSIS IN VEGETABLES BY RAPID BIOASSAY PESTICIDE RESIDUE

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ABSTRACT

Farmers are using pesticides without or with little knowledge about their doses, rate, waiting periods, residual health hazards, etc. It is necessary to have good knowledge of safe consumption for both producers and consumers. The purpose of this study was to analyze the residue percentage in vegetables that reach the central vegetable market, Kalimati fruits and vegetable market, Kathmandu, from where we collected a total of 80 samples with a purposive sampling method and tested in the laboratory, 40 samples were tested for organophosphate (OP), and 40 for carbamate (C) residue percentage, using the RBPR kit. The highest and lowest OP% were found in cauliflower of Dhading (77.09%C) and okra of Chitwan (0.11%C) respectively. The highest and lowest carbamate% were found in the bitter gourds of Dhading (22.82%) and okra of Chitwan (0.31%) respectively. Two samples of cauliflower (77.09% OP) and bitter gourd (50.13% OP) from Dhading were observed to have an inhibiting percentage of more than 45% which meant unsafe for consumption. This shows that among the 100% samples, 2.50% are unsafe for consumption and others are safe. For cent % safe consumption, the waiting period needs to be followed properly before harvesting and consumption.

KEYWORDS

Inhibiting percentage, Residue, Pesticide, Vegetables, Waiting period

1. INTRODUCTION

Nepal is an agrarian country with 80% of the total population living in rural areas and of the economically active population 60.4% are engaged in agriculture (Ministry of Finance, 2021). These days farmers are motivated toward vegetable cultivation and many are commercial producers. Among the total cultivated land of 3,091,000 hectares, 281,132 hectares are under vegetable crops (9.09%) and with a total production of 3,962,383 metric tons (MoALD, 2021). Agriculture and forestry contribute 27.08% and the agriculture sector solely contributes 15.44% to the national GDP and vegetables share 11.92% of the national AGDP (Krishi Diary, 2020; MoALD, 2021). Vegetables are the most important foods in the daily Nepalese diet. However, these crops receive a high amount of pesticide which is more than optimal for economic growth due to a lack of knowledge among farmers (Jha and Regmi, 2009). Vegetable farmers use the highest amount of pesticide after rice farmers in Nepal, critically as the means to control pests and diseases as these are proved to be highly efficient and quick-acting (Pokhrel and Pant, 2009).

The average consumption of pesticides in Nepal is 0.396 a.i. kg/ha (Kalauni, and Joshi, 2019). But PPD conducted research recently says that the average consumption of pesticides is almost 3 times (2.788 times) higher than the past average i.e 396-gram a.i/ha. Organophosphate pesticides inhibit an enzyme AChE at cholinergic junctions of the nervous system in humans which is even more harmful to children than to adults (Kazemi, 2012; Joy et al., 2019). Whereas carbamate pesticides do not persist longer and their application causes a major reduction in a non-target organism (Mustapha et al., 2019). According to some researchers, 24 pesticides were banned in Nepal till 2019 (Kalauni and Joshi, 2019).

Rapid Bioassay for Pesticide Residues (RBPR) method is developed by TARI to integrate the AChE test and Bt test to screen the residues of acetyl-cholinesterase-inhibiting insecticides which are considered neurotoxins (Chiu et al., 1991). In Nepal, the RBPR lab was established in Kalimati Fruits and Vegetables Market under the supervision of the plant protection directorate on 18 June 2014, which uses acetyl-cholinesterase to detect the residue or inhibition percentage of OP and carbamate. The government of Nepal has started to establish RBPR laboratories in Jhapa (Birtamod), Chitwan (Bharatpur), Sarlahi (Nawalpur), Kaski (Pokhara), Rupendehi (Butwal), Banke (Nepaljung), Kailali (Attariya) due to its positive impact in the consumer level (Adhikari, 2017). This test is rapid, taking only 15 minutes from sampling to completion.

2. MATERIALS AND METHOD

2.1 Sample Collection

The research was conducted in Kathmandu. The samples were collected purposively from the central market, Kalimati Fruits and Vegetable market where the RBPR lab is established. A total of 40 vegetable samples (8 types from every 5 districts) were collected: tomato, cucumber, brinjal, cauliflower, bean, bitter gourd, bottle gourd, and okra, and tested, each twice, for OP and carbamate pesticides residue.

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2.2 Sample Extraction

In the laboratory, the samples were cut into pieces. Nearly 1 gram sample was collected from each vegetable and then chopped into fine pieces and each sample was placed in two test tubes containing a buffer solution. Then one test tube with the sample was used for testing OP residue and was kept in fumehood and another was used for testing carbamate residue.

2.2.1 Sample Extraction for The Carbamate Test

A test tube with 1ml ethanol was taken and the sample was transferred into the test tube. Then it was vibrated on a test tube mixer for 30 seconds and left for another 3 minutes for extraction.

Similarly, for the OP test, A test tube with 2ml ethanol was taken and, the sample was transferred into it. 0.1 ml bromine water was added and vibrated on a test tube mixer for 30 seconds and left for another 20 minutes for extraction in a fume hood. Test tubes were covered with parafilm. The ethanol extract was drained into another test tube and then covered with parafilm. Test tubes were labeled properly and extracted samples were analyzed.

2.3 Sample Test

For a blank test, 3ml PBS, 20µl AChE solution, and 20µl 95% AR grade ethanol were added in a 1cm glass cuvette. For the sample test, 3ml PBS, 20µl AChE, and 20µl sample extract were added in a 1cm glass cuvette. After mixing and standing for 2 minutes and 30 seconds, 0.1ml DTNB was added and exactly at 3 minutes 20µl of ATCI solution was added and mixed for 5 seconds to start the enzyme reaction and kept in the spectrophotometer for the residue analysis. The spectrophotometer took 1 min to give the residue percentage when samples were placed inside. The data were collected from the spectrophotometer reading. When the inhibition percentage exceeds 35%, the test was repeated for confirmation.

2.3.1 Data Analysis

The data were analyzed with the help of the R-studio statistics tool and means were compared using DMRT.

Table 1: Enzyme Inhibition Percentage and Result Interpretation

Enzyme Inhibition	Result Propose
More than 45%	The commodity is not suggested for consumption.
35%-45%	The commodity needs to be kept in quarantine for a minimum of 2 days. The test is repeated after washing. Allow for the sale if the inhibition percentage is less than 35%.
Less than 35%	The commodity is approved for sale and consumption.

(Source: Ghimire et. al., 2020)

3. Result and Discussion

Table 2: Pesticides Inhibition Percentage (%) of Different Vegetables at Kalimati Market

Districts	Tomato		Cucumber		Cauliflower		Brinjal	
	OP	C	OP	C	OP	C	OP	C
Dhading	3.98[bc]	7.32[a]	1.59[e]	4.18[d]	77.09a	5.64b	6.31b	5.35c
Makawanpur	1.62bc	1.62c	16.55a	6.76b	26.46b	9.93a	4.54d	3.44d
Kavre	4.44b	3.77bc	2.67d	4.68c	5.24c	4.59b	8.18a	1.77e
Kathmandu	7.53a	5.78ab	16.10b	16.99a	4.50c	3.40bc	8.04a	12.5a
Chitwan	1.07c	1.07c	8.31c	0.86e	1.21d	1.62c	5.25c	11.7b
Mean	3.73	3.91	9.04	6.69	22.90	5.04	6.46	6.97
CV (%)	29.72	24.96	11.05	14.92	4.57	21.07	2.01	2.48
LSD (0.05)	3.08*	2.71*	0.27***	0.27***	2.91***	2.94**	0.36***	0.48***

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

The comparison of all tomato samples showed the highest residue of the OP in the tomatoes of Kathmandu valley (7.53%) and the lowest in that of Chitwan district (1.07%) which was statistically similar to the result of Makwanpur (1.62%) and Dhading (3.98%). Similarly, the highest residue of C was found in tomatoes of Dhading (7.32%) and the lowest was found in that of Chitwan (1.07%) which is statistically similar to the result of Makwanpur (1.62%) and Kavre (3.77%). Among all cucumber samples, the highest residue of the OP was found in the cucumbers of Makwanpur (16.55%) and the lowest was found in that of Dhading (1.59%). Similarly, the highest residue of C was found in the cucumber of Kathmandu valley (16.99%) and the lowest was found in that of Chitwan (0.86%).

Comparing the cauliflower samples, the highest residue of the OP was found in cauliflowers of Dhading (77.09%) and the lowest was found in that of Kathmandu valley (4.50%) which was statistically similar to the result of Kavre (5.24%). Similarly, the highest residue of carbamate was found in the cauliflower of Makwanpur (9.93%) and the lowest was found in that of Chitwan (1.62%) which was statistically similar to the result of Kathmandu (3.40%). In brinjal highest residue of the OP was found in the brinjals of Kavre (8.18%) which was statistically similar to the result of Kathmandu (8.04%) and the lowest was found in brinjals of Makwanpur (4.54%). And, the highest residue of C was found in the brinjals of Kathmandu valley (12.52%) and the lowest was found in that of Kavre (1.77%).

Table 3: Pesticides Inhibition Percentage (%) of Different Vegetables at Kalimati Mark

Districts	Bean		Bottle gourd		Bitter gourd		Okra	
	OP	C	OP	C	OP	C	OP	C
Dhading	5.30c	4.38bc	6.09b	3.65b	50.13a	22.82a	9.49b	4.82b
Makawanpur	7.62b	5.76b	0.82c	0.96c	9.51b	7.37c	0.49c	0.62c
Kavre	6.35bc	1.72c	1.07c	1.56bc	0.8d	1.06d	12.83a	11.60a
Kathmandu	11.74a	17.45a	0.79c	0.98c	0.49d	0.67d	0.4c	0.32c
Chitwan	1.17d	1.56c	8.47a	6.14a	3.82c	11.71b	0.11c	0.31c
Mean	6.44	6.17	3.45	2.66	12.95	8.73	4.67	3.54
CV (%)	12.70	17.50	17.34	32.32	3.45	6.67	8.29	8.47
LSD (0.05)	2.27**	3.00***	1.66***	2.38*	1.24***	1.61***	1.07***	0.83***

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

The results of bean samples showed, the highest residue of the OP was found in the beans of Kathmandu valley (11.74%) and the lowest was found in that of Chitwan (1.17%). Similarly, the highest residue of C was found in the beans of Kathmandu valley (17.45%) and the lowest was found in that of Chitwan (1.56%) which was statistically similar to the results of Kavre (1.72%). Comparing the results of bottle gourds, the highest residue of the OP was found in the bottle gourds of Chitwan (8.47%) and the lowest was found in that of Kathmandu valley (0.79%) which was statistically similar to the results of Makwanpur (0.82%) and Kavre (1.07%) whereas the highest residue of C was also found in the bottle gourd of Chitwan (6.14%) and lowest was found in that of Kathmandu valley (0.98%) which was statistically similar to the result of Makwanpur (0.96%) and Kavre (1.56%).

In bitter gourds of Dhading contained the highest residue of the OP (50.13%) and the lowest in the bitter gourd of Kathmandu valley (0.49%) which was statistically similar to the result of Kavre (0.81%). Similarly, the highest residue of carbamate was found in the bitter gourd of Dhading (22.82%) and lowest in that of Kathmandu valley (0.67%) which was statistically similar to the result of Kavre (1.06%). The comparison of okra samples showed the highest residue of the OP was found in the okra of Kavre (12.83%) and lowest in the okra of Chitwan (0.11%) which is statistically similar to that of Kathmandu valley (0.41%) and Makwanpur (0.49%). Similarly, the highest residue of C was found in the okra of Kavre (11.60%) and lowest in that of Chitwan (0.31%) which was statistically similar to the result of Kathmandu (0.32%) and Makwanpur (0.62%).

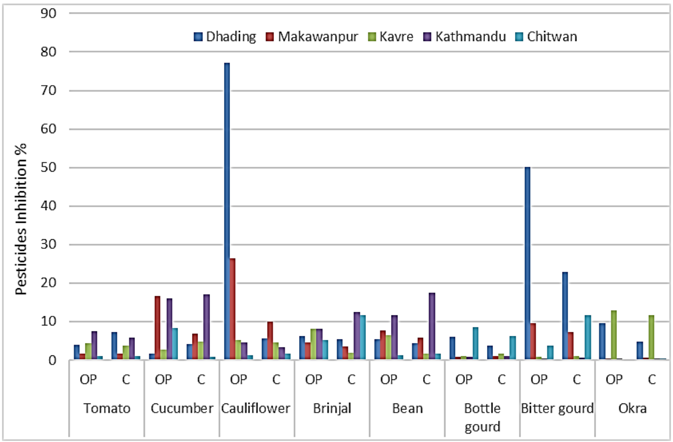

Figure 1: Vegetables from different districts with OP and C

4. Discussion

Overall, the result was significant in tomato, cucumber, cauliflower, brinjal, bean, bottle gourd, bitter gourd, and okra. The highest percentage of OP residue was found in cauliflower and a bitter gourd of Dhading district and all other vegetables were found to be safe for consumption which is supported by Rawal and his co-workers (Rawal et al., 2019). The result is also supported by the article published in the Kathmandu Post on June 19, 2020, by Prasain (Prasain, 2020). According to the article, vegetables like tomatoes, eggplants, cauliflowers, potatoes, green beans, etc arriving in the central vegetable market, from neighboring districts or farms showed no or little residue of pesticides that are safe for human consumption. Total samples of tomatoes tested in the Kalimati laboratory were found to be safe for consumption (Koirala et al., 2010).

5. Conclusion

This study aimed to analyze organophosphate (OP) and carbamate (C) residues in vegetables available at Kalimati fruits and vegetable markets. The results of the experiment show a residue level of the OP of 2 samples >45% which are unsafe for consumption and need to dispose of and follow the waiting period before the consumption of both OP and carbamate pesticide-sprayed vegetables. It may not be possible that each farmer to test his produce because the laboratory situated somewhere in his province may not be his access, the vegetable producers need to follow the waiting period before marketing and should be aware of the use, dose, types, rate, and health hazards of pesticides.

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