PESTICIDE RESIDUE ASSESSMENT ON VEGETABLE CROPS THROUGH RAPID BIOASSAY OF PESTICIDE RESIDUE IN NEPAL

Dipak Khanal[*], Sadhana Poudel, Meena Shrestha and Navaraj Upadhyaya

Paklihawa Campus, Institute of Agriculture and Animal Science, T.U., Nepal
[*]Correspondence: dipakbabu@hotmail.com; dipak.khanal@pakc.tu.edu.np

ABSTRACT

The increasing use of pesticides has also raised the risk of higher pesticide residues in food. Also, the food commodities imported from India are considered to be very unsafe to consume because of their indiscriminate pesticide applications on crops. Sometime, this situation also occurs in Nepal food commodities. The research was carried to evaluate the residue level of carbamate and organophosphate pesticides in different vegetable commodities of Nepal origin and regularly imported from India. Pesticide residue was evaluated through Rapid Bioassay of Pesticide Residue analysis in 285 samples obtained from Bagmati Province, Lumbini Province and Sudurpaschim Province of Nepal and bordering areas of Koshi, Lumbini and Sudurpaschim Provinces. In laboratory test, most of vegetable crops were found to have pesticide residue in them. In Bagmati province, brinjal (17.29%), capsicum (16.6%), and chilli (18.12%); in Lumbini, brinjal (17%), cauliflower (15.8%), and potato (16.68%); in Sudurpaschim, broadleaf mustard (15.21%) and cauliflower (41.64%) were found relatively higher pesticide residues while potato (18.38%), tomato (29%), bean (32.52%), brinjal (39.25%), bitter gourd (14.37%) imported from India were found relatively higher pesticide residues.

Key words : Carbamate, crops, organophosphate, risk, samples

INTRODUCTION

Malthusian theory of population indicates that the world population is growing exponentially while the food supply is growing arithmetically. The gap is increasing every day. Farmers are using agricultural chemicals synthetic fertilizers, pesticides, plant growth regulators etc. to meet the food demand of this increased population (Upadhyaya and Bhandari, 2022). Among the agricultural chemicals, pesticide is the most toxic one and is used indiscriminately in large amount in crops against pests. These pesticides are used with an intention of reducing pest load in crops but, certainly, this pest protection activity is increasing the health hazard of the agricultural products consumers. It is causing harmful impacts to every single organism prevalent in the ecosystem, where these pesticides are used (Igbedioh, 1991; Jeyaratnam, 1981). The increase in production is nothing as compared to the increase in social cost. Over 98% of the sprayed insecticides and 95% of herbicides reach a destination other than their target, including non-target species, air, water, bottom, sediments, and food (Miller, cited in Sharma *et al.*, 2012). It is reported that every day around the world almost 700 people die from the poisoning and several thousand more are affected by pesticide poisoning (Kumar *et al.*, 2011). Adverse health effects are more common in less developed countries because of weak regulation, the low hazard awareness of users, inadequate use of personal protective equipment, lack of proper care during application, and the use of highly toxic pesticides. Certain pesticides termed as endocrine disruptors are known to have adverse effects by mimicking or antagonizing natural hormones in the body and it has been postulated that their long-term, low-dose exposure is increasingly linked to human health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Brouwer *et al.*, 1999; Crisp *et al.*, 1998; Hurley *et al.*, 1998).

The use of chemical pesticides in Nepal is 396 g a.i/ha (PPD, 2015). National survey on the pesticide consumption statistics in Nepal revealed the highest load of pesticide 3.34 g a.i. kg/ha being used on eggplant followed by tomato crops (1.95 g a.i. kg/ha), cotton (1.5 g a.i. kg/ha), potato (1.03 g a.i. kg/ha) and cole crop (0.70 g a.i. kg/ha) (PPD, 2014). Nepalese people remained at alarming threat of pesticides in their diets and the use of pesticide is increasing every year (Ghimire and GC, 2018; Khanal *et al.*, 2021).

There are different methods of pesticide residue analysis on foods viz. Gas Chromatography (GC), Liquid Chromatography (LC), High Performance Liquid Chromatography (HPLC), Rapid Bioassay of Pesticide Residue (RBPR). GC, LC, HPLC methods of residue analysis are complicated, expensive and time consuming techniques. However, RBPR technology is simple, quick in detection and less expensive. It is more useful for residue analysis in fruits and vegetables products which are highly perishable that need quick testing. It can detect residue of pesticide toxicity of organophosphate, carbamate, pyrethroid and dicarbamate groups. This technique is based on the inhibition of acetyl-cholinesterase (AChE) enzyme responsible for controlling stimuli in the nervous system in animals and human beings (CAL, 2017). It includes AChE test and Bt (*Bacillus thuringiensis)* test to screen the residue of fungicide and insecticide, respectively, to segregate the crop products which exceeds the pesticide tolerance limits and withdraw such products from the market before reaching the consumer. When AChE reaction solution is mixed with samples from healthy produce, it slowly turns yellow as the level of 5-thio-2-nitrobenzoateanion increases. If an insecticide is present, the enzymatic reaction slows down or stops. The rate at which color development is inhibited indicates the quantity of chemical present as well as their toxicity. The main objective of the research is to identify the current residue status of pesticides available in the marketed vegetables and, at the same time, their consumption status.

MATERIALS AND METHODS

The research is completely based on the primary data collected through Rapid Bioassay on Pesticide Residue. The test was carried out at Rapid Bioassay of Pesticide Analysis Lab Unit, Butwal, Rupandehi, Central Agricultural Laboratory, Ministry of Agriculture and Livestock Development.

Site and Crop Selection: Koshi, Bagmati, Lumbini and Sudurpaschim provinces of Nepal (Fig. 1) were selected for pesticide residue analysis on year 2019. The province was selected randomly in ways that it would represent the sample of whole country. Experiment was based on CRD design with three replications in each. Different types of crops were selected for different selected sites. Crop season, availability of vegetable in the market and availability of secondary data were considered during crop selection.

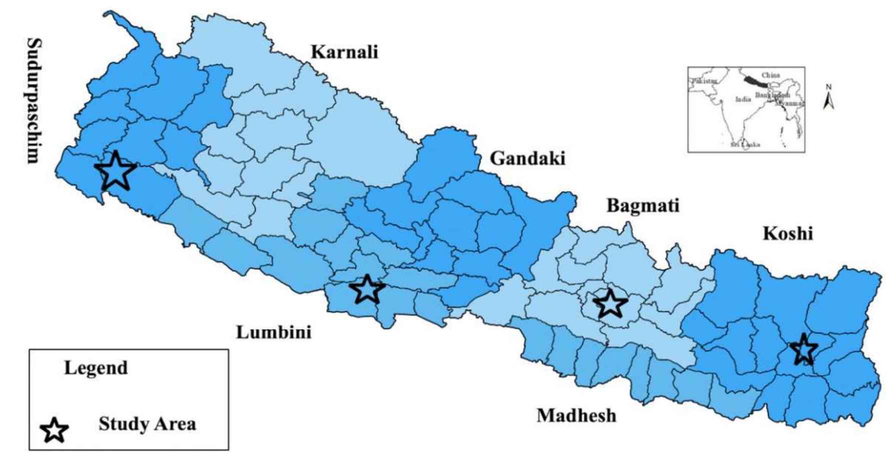

Fig. 1. Provincial map of Nepal showing the study area.

Sample Collection: Vegetables produced within the selected sites were sampled for the laboratory experiment. One sample of each vegetable crop was collected from Bagmati (15 crops), Lumbini (15 crops) and Sudurpaschim (20 crops) Provinces of Nepal and bordering areas of Koshi (9 crops), Lumbini (18 crops) and Sudurpaschim (18 crops) Provinces. For the collection of samples, polyethene bags of size 23 cm*19 cm (one bag for each sample) were used. A unique identification code was given for each sample and the region of collected sample of corresponding code was noted (Table 1). A sample quantity of vegetables for one sample was different for different vegetables.

Table 1. Sample quantity for vegetables

SN	Crops	Sample quantity for one sample		
		Number	Part used	Weight
1.	Fresh bean (simi)	20	Whole pod	
2.	Brinjal, capsicum	5	Fruit	
3.	Potato	7	Tuber	
4.	Tomato	13	Fruit	
5.	Broad leaf mustard	5	Leaves	1 g or 4 leaf discs of plant tissue (PPD, 2017)
6.	Cauliflower, cabbage	2	Head	
7.	Chilli	13	Fruit	
8.	Cucumber	3	Fruit	
9.	Carrot	7	Root	
10.	Radish	4	Root	

(CAL, 2017)

Equipment and Chemicals Used: For sample extraction, knife, chopping board, forceps, wash bottle, cotton, test tubes along with test tube racks, micropipettes of different volumes (20 µl,100 µl,1000 µl), pipette tips, vortex mixture (Touch-type, optics technology) and fume hood were used. Similarly, for sample analysis additional equipment like cuvettes (5 ml), para films, scissors, and visible spectrophotometer (412 nm, 220-240 EC, UV-1280, Shimadzu company) were used. Visible spectrophotometer was used to measure the colour inhibition rate which finally detected the presence or absence of pesticide residue. Measuring cylinder, beakers, conical flasks, stopwatch, marker, air conditioner/electric fan and personal protective equipment like mask, goggles, apron, hand gloves, etc. were also used during the laboratory test. Chemicals, used for the sample extraction and analysis were, Acetylcholinesterase (AChE), Acetylthiocholine Iodine (ATCI), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), Phosphate buffer solution (PBS), bromine water (0.4%), Ethanol (95%), and distilled water.

Preparation of Solution from Reagent: Reagents that come in raw form were mixed with distilled water to make a solution for a laboratory test. Methods for the preparation of solution from reagent, and its storage are described as followed.

Approx. 30 mg of AChE, 216 mg of ATCI and 19.8 mg of DTNB, each was dissolved separately in 10 ml,10 ml and 50 ml of distilled water, respectively, as prescribed in a bottle and stored at below 0º C after dividing it into proportions. 0.4% bromine concentration was prepared and stored at room temperature. Bromine ampoule must be kept away from heat sparks, flame, direct sunlight, and contact with oxidizing materials. Buffer solution was stored at room temperature. After preparation of the reagent solution, the date of preparation and volume was written clearly in a label.

Sample Extraction Process: For the extraction of sample, the following procedure was performed.
The sample was cut into fine pieces and its 1-2 g of it was taken in a test tube. And 1 ml and 2 ml of 95% ethanol were added to the test tubes containing samples for testing carbamate and organophosphate, respectively. It was shaken properly with the help of vortex mixture for 20 seconds and allowed to stand for about 3 minutes. 0.1 ml (100 µl) of 1% Bromine water was added into a test tube for organophosphate test inside the fume hood. Additional standing was done to organophosphate sample for 20 minutes to evaporate excess of bromine water. Then the supernatant solution of sample extract was drained into another test tube for further tests.

Incubation/Analysis of Sample Extract: Before testing the sample, a control test was carried out to determine a standard value. Followed by control test, sample tests were carried out and the difference between two test was considered as the enzyme inhibition caused by the pesticide residue present in the sample.

Control Test: 3 ml PBS (Phosphate Buffer Solution) was added in a 5 ml cuvette followed by 20 µl AChE solution and 20 µl ethanol. It was mixed thoroughly for 5 seconds and allowed to stand for 2.5 minutes, and 100 µl DTNB solution was added. After 3 minutes 20 µl ATCI solution was added and mixed for 2 seconds. Then the cuvette was placed into a spectrophotometer at 412 nm to read absorbance change. When the inhibition percentage exceeded 35% then the test was repeated with the second test tube for confirmation.

Sample Test: 3 ml PBS was added in a 5 ml cuvette followed by 20 µl AChE solution and 20 µl sample extracts. It was mixed thoroughly for 5 seconds and allowed to stand for 2.5 minutes and 100 µl DTNB solution was added. After 3 minutes 20 µl ATCI (Acetyl thiocholine iodine) solution was added and mixed for 2 seconds. Then the cuvette was placed into a spectrophotometer at 412 nm to read absorbance change. When the inhibition percentage exceeded 35% then the test was repeated with the second test tube for confirmation.

Result Interpretation: Enzyme inhibition percentage was observed on spectrophotometer which was calculated as followed:

$$\text{Enzyme inhibition (\%)} = \frac{\text{Absorbance change (control) - Absorbance change (sample)}}{\text{Absorbance change (control)}} \times 100$$

The enzyme inhibition percentage obtained was interpretated as shown in Table 2.

Table 2. Interpretation of inhibition percentage

Enzyme Inhibition	Result Propose
≥ 45%	Not suggested for consumption purpose.
35%-45%	Quarantine for a minimum of 2 days. Repeat the test after washing; if inhibition is less 35%, it is safe for sale.
≤35%	Safe for sell and consumption.

Source: (Chiu *et al.*, 1991)

RESULTS AND DISCUSSION

Pesticide Residue on Vegetables Produced in Bagmati, Lumbini and Sudurpaschim Provinces

Fig. 2 reflects the inhibition percentage against carbamate. In case of Bagmati province, among the tested vegetables the pesticide content was highest for brinjal (17.29) followed by capsicum (16.65) and lowest in bottle gourd (1.9) followed by sponge gourd (2.25) and cauliflower (2.4). Fig. 3 depicts the inhibition percentage of organophosphate (OP). The highest inhibition percentage was observed in chilli (18.12) and lowest was observed in case of capsicum (2.5). Highest individual sample inhibition was exhibited by chilli (30.78) for organophosphate and followed by capsicum (28.40) for carbamate.

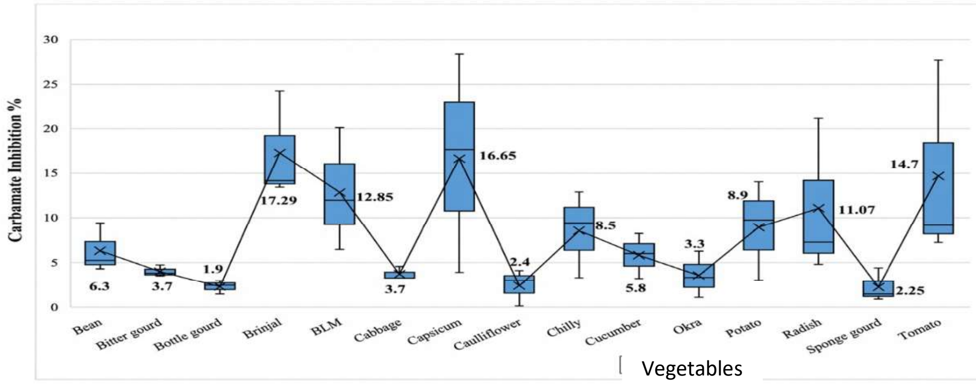

Fig. 2. Average inhibition percentage of carbamate exhibited by different vegetable samples in Bagmati Province.

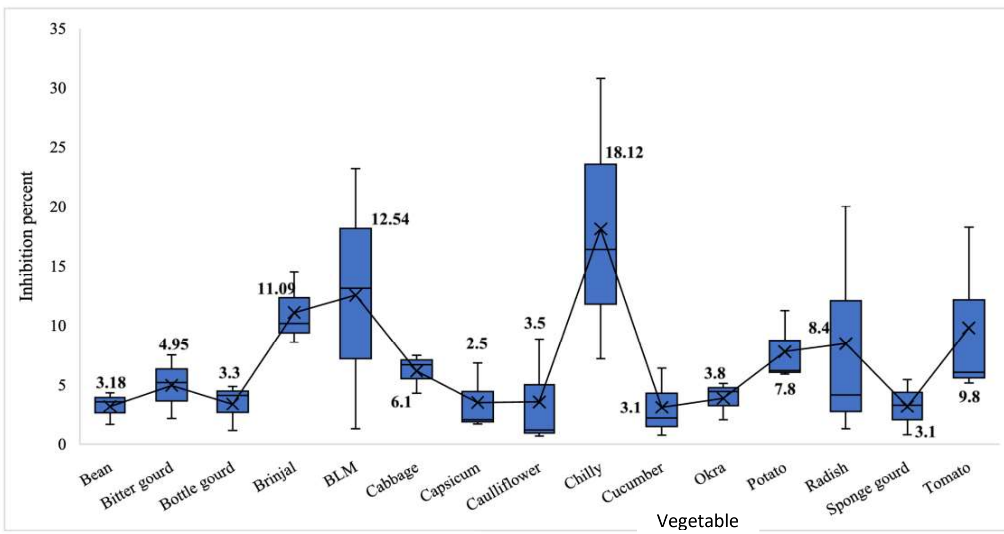

Fig. 3. Average inhibition percentage of organophosphate exhibited by different vegetable samples in Bagmati Province.

For Lumbini Province, the mean inhibition percentage for carbamate pesticide was found highest in brinjal (17.07) and followed by potato (15.95) and lowest in bottle gourd (2.98), and thus followed by sponge gourd (3.44) while that for organophosphate was found higher in potato (16.68) and followed by cauliflower (15.88) and lowest was observed in tomato (3.21), and thus followed by bottle gourd (5.8) as shown in Fig. 4. The highest individual value was shown by radish (29.44) for carbamate and by cauliflower (26.8) for organophosphate (Fig. 5).

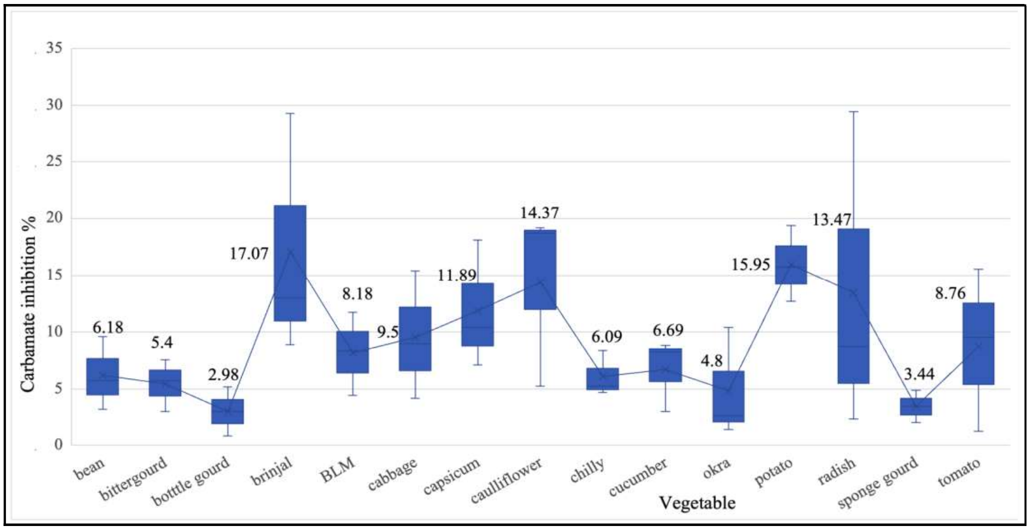

Fig. 4. Average inhibition % of carbamate exhibited by different vegetables in Lumbini Province.

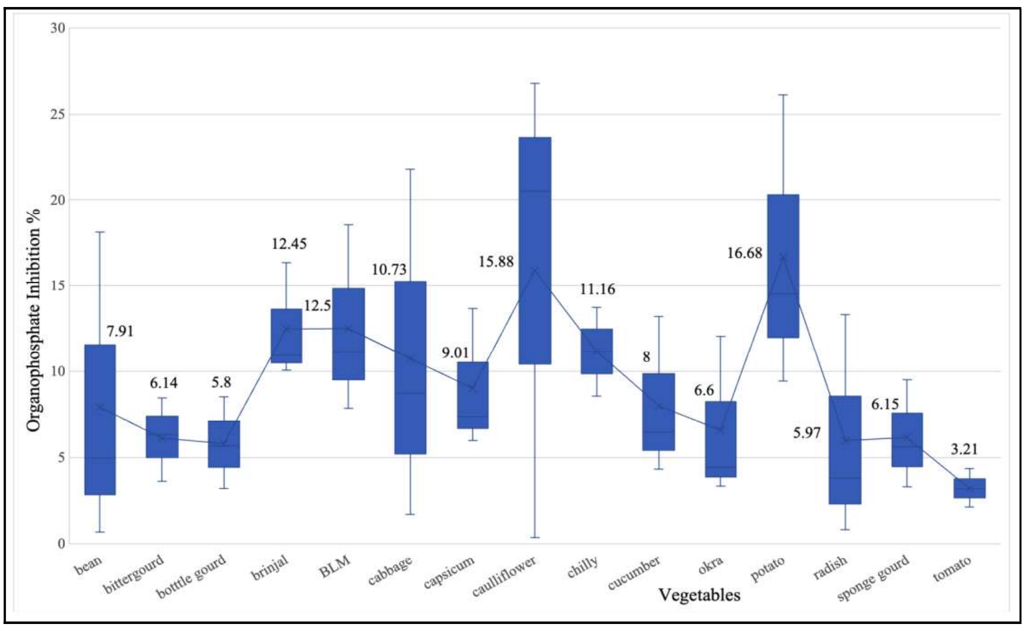

Fig. 5. Average inhibition % of organophosphate exhibited by vegetables in Lumbini Province.

In case of Sudurpaschim Province, the mean inhibition percentage for carbamate pesticide was found higher in broadleaf mustard (13.85) followed by tomato (13.12) and lowest by spinach (2.16) followed by snake gourd (2.24) and okra (2.78) as shown in Fig. 6. For organophosphate highest inhibition was exhibited by cauliflower (41.64) followed by pumpkin (16.68) and lowest by spinach

(1.38) followed by snake gourd (1.97) (Fig. 7). The highest individual value was shown by BLM (27.04) for carbamate and by cauliflower (96.63) for organophosphate.

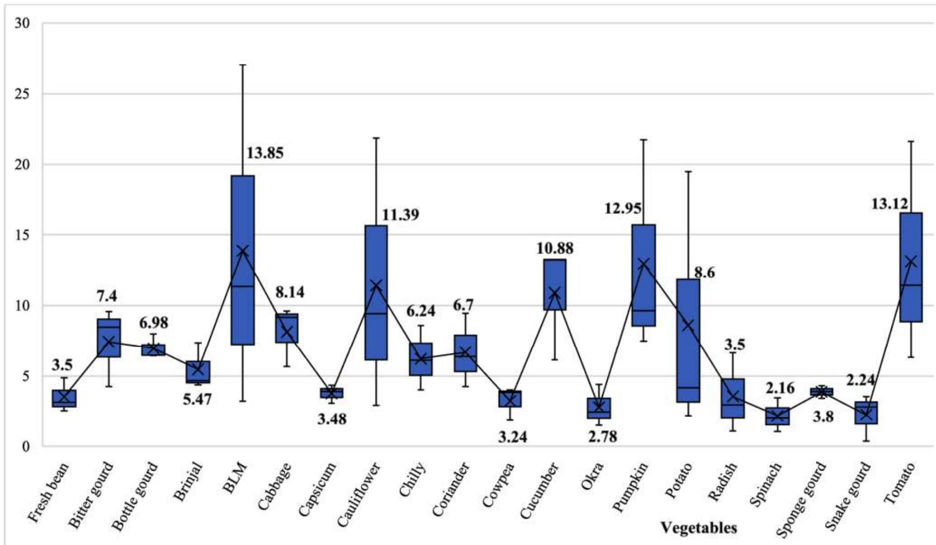

Fig. 6. Average inhibition % of carbamate exhibited by different vegetables in Sudurpaschim Province.

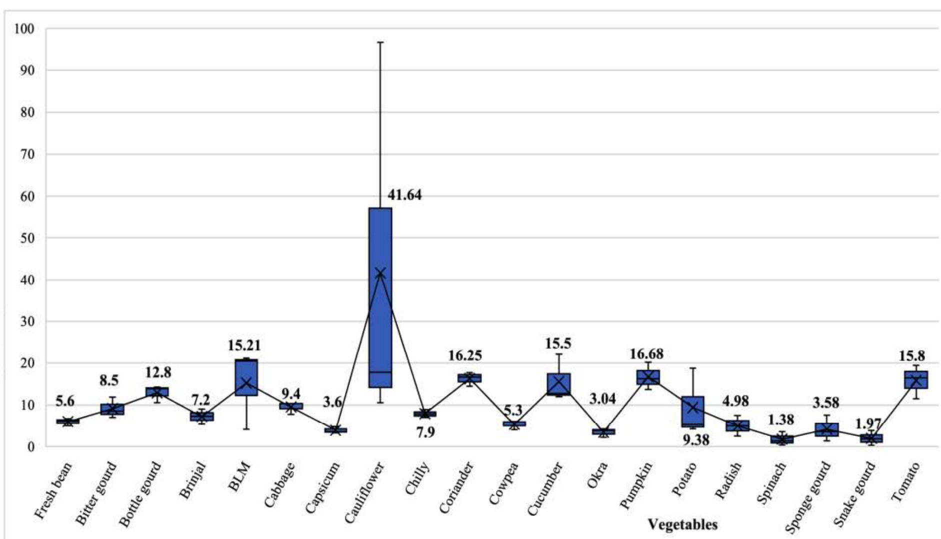

Fig. 7. Average inhibition % of organophosphate exhibited by different vegetables in Sudurpaschim Province.

Pesticide Residue on Vegetables Imported from India via Sunauli, Kanchanpur and Kakarvitta Border (Lumbini Province, Sudurpaschim Province and Koshi Province)

There is a huge import of vegetables through the borders of Lumbini province, Sudurpaschim and Koshi Province and the consumptions are also massive. So, the vegetables supplied from India in these border areas were also analysed for both carbamate and organophosphate. In case of Sunauli border, for carbamate the average inhibition percentage was highest in case of potato (17.71) followed by tomato (16.12) and BLM (14.23) whereas least was exhibited by coriander (6.48) followed by fresh bean (6.9) (Fig. 8). In an assessment for organophosphate pesticide, potato (18.38) had highest inhibition percentage followed by tomato (17.92), and the least by radish (4.42) followed by broad leaf mustard (4.75) (Fig. 9). The highest individual inhibition was shown by bitter guard (34.24) for carbamate and by potato (34.95) for organophosphate.

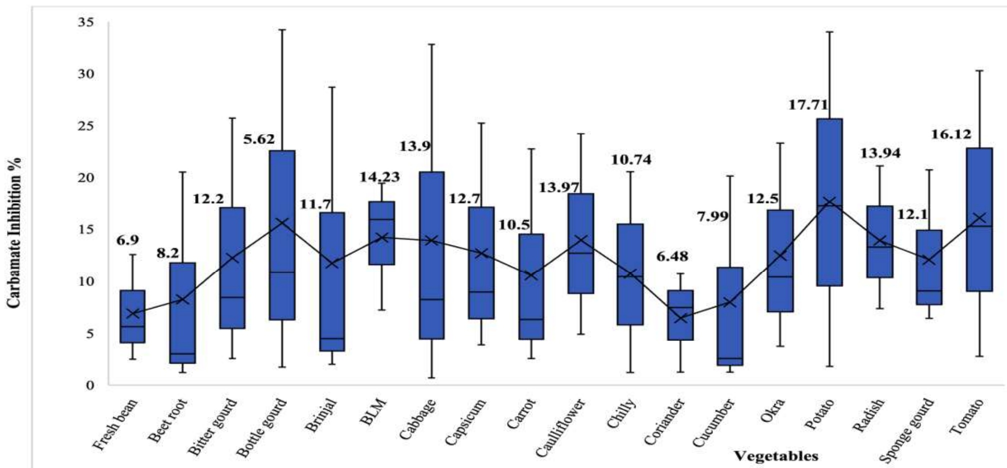

Fig. 8. Average inhibition % of carbamate exhibited by vegetable from Sunauli (Indian border Lumbini Province).

For Kanchanpur Border, the mean inhibition percentage for carbamate pesticide was found highest in fresh bean (32.52) and lowest in pointed gourd (1.94) followed by beet root (5.29), pumpkin (5.4) (Fig. 10). The mean inhibition percentage for organophosphate was found higher in brinjal (39.25) followed by tomato (29) and least in beet root (6.47) followed by radish (7.6). For carbamate highest individual inhibition was shown by fresh bean (60.46) and for organophosphate by brinjal (98.18) (Fig. 11).

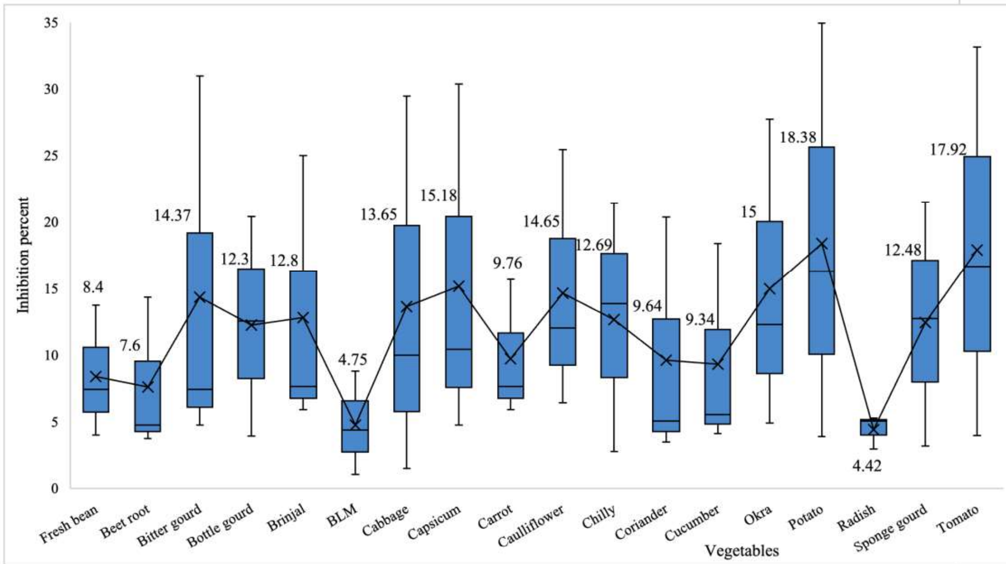

Fig. 9. Average inhibition % of organophosphate exhibited by vegetables from Sunauli.

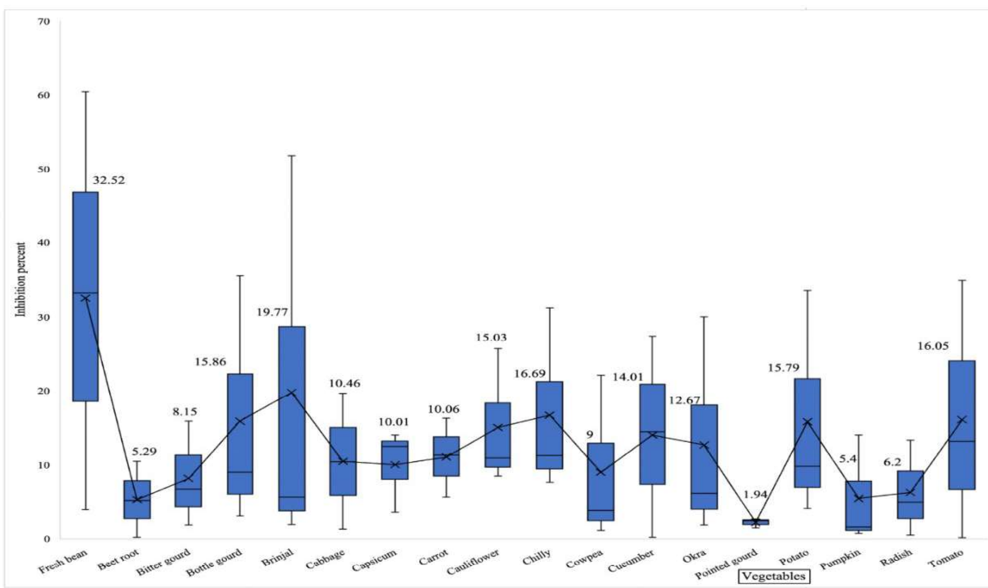

Fig. 10. Average Carbamate inhibition % exhibited by vegetable (India-Kanchanpur border).

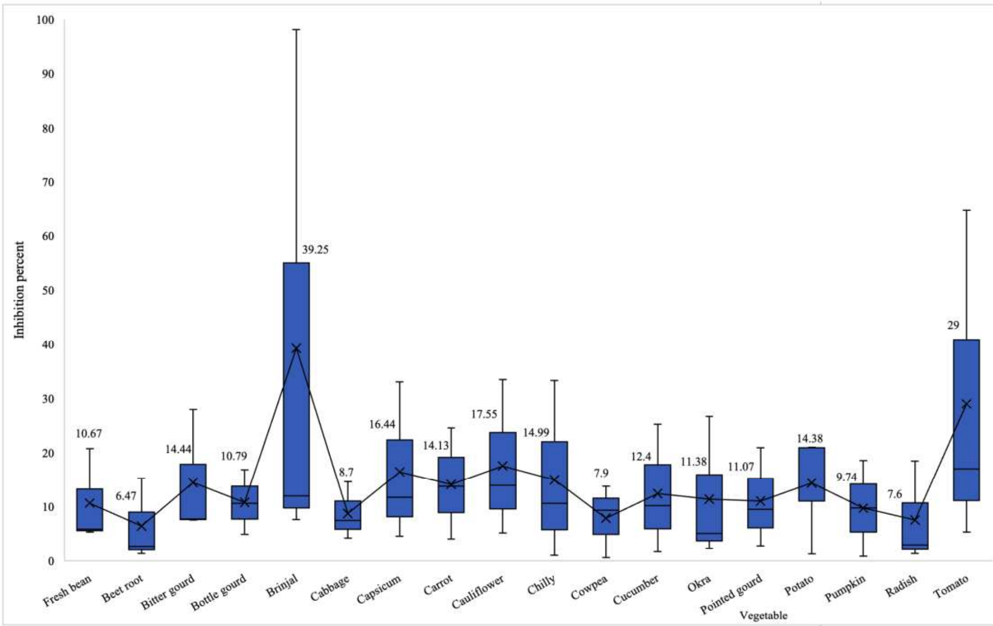

Fig. 11. Average organophosphate inhibition%
(India- Kanchanpur border, Sudurpaschim Province).

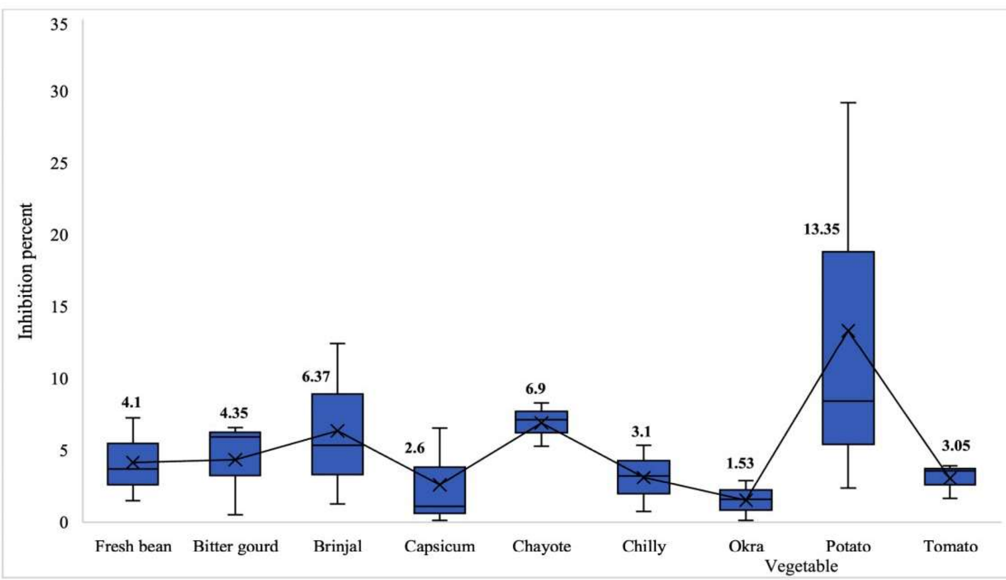

Fig. 12. Average carbamate inhibition % exhibited by different vegetables
(India-Kakarvitta border, Koshi Province).

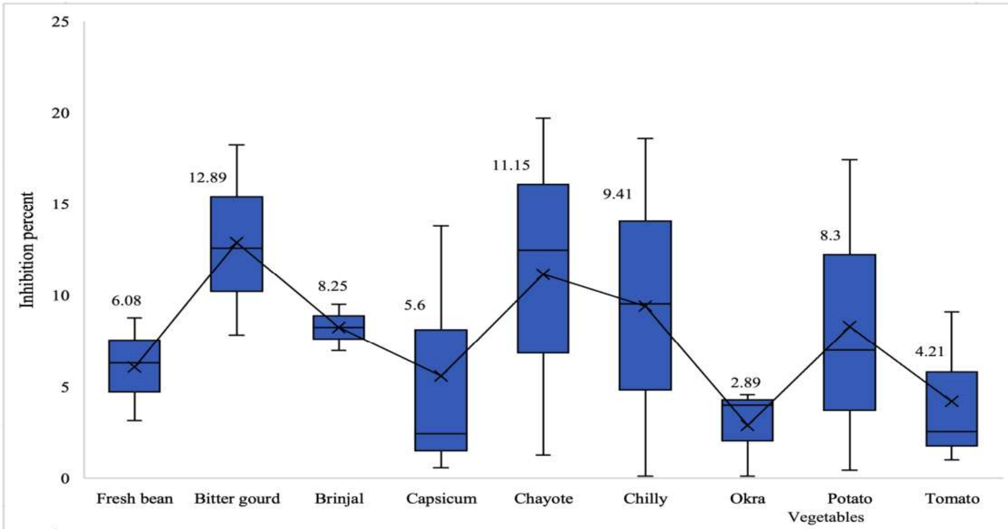

Fig. 13. Average organophosphate inhibition % exhibited by different vegetables (India- Kakarvitta border, Koshi Province).

For Kakadvitta border, the mean inhibition percentage for carbamate pesticide was found highest in potato (13.35) and lowest in okra (1.53) followed by capsicum (2.6), tomato (3.05), chilli (3.1) (Fig. 12). For organophosphate, Fig. 13 exhibited the mean highest inhibition percentage in bitter gourd (12.89) followed by chayote (11.15), and least in okra (2.89) followed by tomato (4.21). The maximum individual inhibition was exhibited by potato (29.22) for carbamate and by chayote (19.71) for organophosphate.

The residue analysis showed the presence of residue in most vegetables which inferred the non-judicious use of pesticides especially use of pesticides without proper knowledge on pre-harvest interval (waiting period), indicating the need of awareness about judicious use of pesticides to farmers and consumers, regular monitoring of marketable crops all over Nepal, and strong policy implementation on import and use of less hazardous pesticides. After RBPR test on the vegetables samples of Nepal origin, brinjal (17.29%), capsicum (16.6%), and chilli (18.12%), potato (16.68%), broadleaf mustard (15.21%) and cauliflower (41.64%) were found to have comparatively higher pesticide residue in them. Study by Kodandaram *et al.*, (2013) also reported massive use of pesticide in brinjal in comparison to other vegetables. Vegetables imported from India, potato, tomato, bean, brinjal, and bitter gourd were found relatively higher pesticide residues.

Reports from the study of also reported a large pesticide application in vegetables like potato (Vilvert *et al.*, 2022), brinjal and tomato (Lozowicka *et al.* 2015) in India. Higher frequencies of pesticide applications were reported in many crops like potato, chili; 93% of the farmers sprayed pesticides 2-6 times/season in potato field in Kavrepalanchowk district while 6% farmers used pesticides more than 10 times on potato crops in a season (Sapkota *et al.*, 2020). Farmers applied pesticides 9 times on crucifers and 6 times on green chillies in Andaman, India (Swarnam and Velmurugan, 2013).

The highest inhibition percentage was observed for organophosphate in brinjal (98.18%) imported from India through Kanchanpur border followed by cauliflower (96.64%) produced in Sudurpaschim Province of Nepal, and tomato (64.66%) imported from India through Kanchanpur. Inhibition percentages of 60.46%, 51.8% and 35.55%, respectively, were observed for carbamate in fresh bean, brinjal and bottle gourd imported from India.

Among inhibition percentages of individual samples, 2% (6 samples) had inhibition percentage above 35%. Mostly, the vegetables found in Nepal were within the safe consumable limits except in case of Sudurpaschim province, where the average inhibition % in cauliflower (41.64%) was above the acceptable range (<35%) and not fit for fresh consumption. Some sample values beyond 90%, exhibiting the lack of knowledge on waiting period in producers. This was the case of organophosphate residue as most commonly used pesticides in Nepal are organophosphates.

Bhandari *et al.*, (2019) reported residues in 93% of total brinjal samples, 56% of total brinjal samples were found with multiple pesticides residues and triazophos [25.5 µg/kg] in 4% of the total brinjal samples exceeded EU MRL(European Union Maximum Residue Limit). Potatoes were highly contaminated as 70% of the potato samples were found with pesticide residue (Khandekar *et al.*, 1982). 44% of tomato samples [10.6-1772 µg/kg] exceeded the EU MRL for chloropyrifos whereas 25% exceeded the Nepalese foodstuff MRL. Triazophos in 6% [237-685 µg/kg] and omethoate in 3% [27.9 µg/kg] of tomato samples exceeded the EU MRL (Bhandari *et al.*, 2019). Similarly, Rawal *et al.* (2013) found chilli with higher methyl parathion residue (0.025 ppm), exceeding MRL value. Chlorpyrifos residue was observed in 81% of chilli samples of Rupandehi district whereas 19% [10.5-491 µg/kg] exceeded the EU MRL and 4% exceeded the Nepalese MRL [50 µg/kg]. Also, quinalphos and dichlorovos, respectively, were detected in 19% [1.17-5.96 µg/kg] and 11% [1.35-2.76 µg/kg] of chilli (Bhandari *et al.*, 2019)

CONCLUSIONS

The use of pesticides in agricultural commodities is increasing day by day in an alarming rate. However, most of the vegetable samples in Nepal were found well below the safe limits. Vegetables like cauliflower, potato and brinjal were found mostly with higher pesticide residues. The use of organophosphate chemicals was found to be a commonly occurrence and more frequently used, and thus the residue level of organophosphate was higher compared to the carbamate residue. The pesticide residue level within Nepal was higher in the vegetables grown in Sudurpaschim province, which exhibited that farmers in that province were not very aware of the observable waiting period of pesticide after its application on crops. Comparatively, the pesticide residue levels in the imported vegetables from India were higher compared to those from Nepal. The pesticide residue, in many occasions, was significantly higher in an unacceptable rate. This is a big matter of concern that government should make specific filtration technique where the vegetable which does not meet the criteria of safe pesticide level should not be allowed to enter into the Nepal markets through border.

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