NEPALESE JOURNAL OF AGRICULTURAL SCIENCES,
July, 2025, volume 29
e-ISSN: 2091-0428; p-ISSN 2091-041X; esjindex ID =6279
Published by HICAST, Purbanchal University, Kathmandu

Analysis of Carbamate and Organophosphate Insecticides Residue by Rapid Bioassay Pesticide Residue in Vegetables in Bagmati Province Nepal

BHUSAL, Anish [1]*, REGMI, Sheershak[1], GAIRHE, Shristi[1], & KHANAL, Dipak[1]

[1]Institute of Agriculture and Animal Sciences, Tribhuvan University, Kathmandu, Nepal
Corresponding Author's Email: anishbhusal2057@gmail.com

ABSTRACT

The excessive use of chemicals, specifically the carbamates and organophosphates, results in pesticide residues in vegetables posing a significant risk and issue of causing both acute and chronic diseases. This study aimed to analyse the pesticide residue levels in vegetables grown in seven districts of Bagmati Province, of Nepal, using Rapid bioassay of pesticide residue (RBPR) technique. The acetyl cholinesterase (AChE) inhibition percentage of spinach, potatoes, and sword beans displayed consistently high inhibition percentages across 888 vegetable samples tested. One of spinach sample turned out to be unsafe for consumption since it exceeded a 45% inhibition threshold. Furthermore, higher pesticides residues were observed in some of the major vegetables like potato, capsicum, sword beans as compared to others. Samples from Bhaktapur district showed the highest carbamate and organophosphate inhibition percentage level, while those from Lalitpur displayed the lowest as compared to the other districts. As per the findings, producer must stick to follow proper pesticide use and follow the practices such as waiting period, in order to safeguard the consumers. This study demonstrates the significance of RBPR as a cost-effective and easy method for timely evaluation of pesticide residues in easily perishable commodities.

Keywords: Acetyl cholinesterase, acute diseases, cost effectivity, inhibition percentage, inhibition threshold

INTRODUCTION

Nepal is an agricultural country, with almost 57.3% of the population engaged in the farming of food and cash crops. A total area of 302,135 hectares (8.97%) of the 3,091,000 hectares of cultivated land are being utilized for the production of vegetable crops that yields 4,376,077 metric tons. Out of 24.10% of GDP that is contributed through agriculture, vegetables make up 3.9% of AGDP (MoALD, 2023/24). Pesticides are broad spectrum substances that are used to control pests and disease carriers. However, they are being used haphazardly without the rational consideration of their doses, rates, waiting period, etc. on crops- that leads the commodities unsafe for consumption. The world's population is increasing substantially, while there is the arithmetic increase in the food production that has resulted in disparity between supply and demand (Upadhyaya & Bhandari, 2022). Agriculture is forced to increase the output due to this imbalance. To fulfil the increasing food demand farmers have shifted to use chemicals such as synthetic fertilizers and pesticides and produce the desired crop yields (Kumar et al., 2011). Pesticides act as the poisons, play a significant role in pest control but also possess substantial hazards. This problem is widespread around the world, including Nepalese food commodities too. Pesticide poisoning is an emerging health issue, especially in the developing countries. Around 700 people each day around the world suffer from the exposure. There are numerous problems linked with the long-term such as immunological suppression, hormone disturbance, and cancer (Brouwer et al. 1999). The use of pesticides in Nepal has steadily increased, attaining 396 g a.i./ha and is 1.60469 a.i.kg/ha in vegetables (PPD, 2015). Eggplant and tomatoes are the most pesticide-used crops, that has been problematic to both consumers and the surroundings (PPD, 2014). Pesticide application is increasing every year, and Nepalese people continue to face a serious risk from the chemicals in their food (Ghimire and GC, 2018; Khanal et al., 2021).

The list of Organophosphate class that are used in Nepal are Acephate, Azamethiphos, Chlorantraniliprole, Chlorpyrifos, Dimethoate, Ethion, Malathion, Phenthoate, Profenofos, Quinalphos, Temephos and Carbamates class are Propoxur, Thiodicar. In contrast banned pesticides of organophosphate are Chlorpyrifos and in use but prohibited in tea fields are Ethion, Quinaphos (Nyaupane, 2021). Due to inadequate rules and regulations, limited user understanding of hazards, improper use of personal safety devices, inappropriate handling and the usage of highly toxic pesticides developing countries are more likely to have adverse health consequences. Conventional method of detecting the residue of pesticides like Liquid Chromatography (LC) and Gas Chromatography (GC) are the precise method but they are expensive and tedious. These approaches are not so effective for very perishable crops that require quick and rapid testing (Sharma et al., 2012). Rapid Bioassay for Pesticide Residues (RBPR) is an efficient, economical, and effective procedure for the identification of pesticide residue of organophosphate and carbamate pesticides especially in the vegetables and fruits. Since, it determines the reduction of acetylcholinesterase (AChE) enzymatic activities, being an invaluable method for perishable goods that require rapid evaluation (CAL, 2017). The RBPR technique utilises acetyl-cholinesterase to calculate and determine the residue or inhibition percentage of Organophosphate (OP) and Carbamate. AChE, the molecule used in this method, was taken out from the housefly's brain. The combination of AChE and healthy food sample gives a yellow reaction due to increase in 5-thio-2-nitrobenzaoateanion. The enzymatic activity becomes slows or ceases when insecticide is present. The speed of inhibition of colour development is reflected by quantity and the toxicity present in the chemical (Khanal et al., 2023).

The objective of the research was to use Rapid Bioassay Pesticide Residue (RBPR) to analyse the carbamate and organophosphate inhibition percentage in different vegetable commodities, and pesticide inhibition percentage across different districts to assure compliance with the recommended levels and protect consumer's health.

MATERIALS AND METHODS

Site and crop selection

For the pesticide residue analysis, seven districts of Bagmati Province of Nepal; Chitwan, Dhading, Makwanpur, Kavrepalanchok, Kathmandu, Bhaktapur and Lalitpur (Fig. 01) were chosen on the year 2024. The districts were chosen randomly so that it represents the sample of Bagmati province as a whole various crops were selected for various selected districts. Season of cultivation, availability of different vegetables in the market and the obtained secondary data were taken into account while selecting the crops. The study was based on the primary data obtained from Rapid Bioassay of Pesticide Residue (RBPR) Laboratory unit Kalimati, Kathmandu, under the Central Agricultural Laboratory.

Sample collection

The vegetable samples for the study were collected from 7 districts, Chitwan (21 crops), Dhading (18 crops), Makwanpur (16 crops), Kavrepalanchok (18 crops), Kathmandu (10 crops), Bhaktapur (11 crops), Lalitpur (8 crops). A single, representative sample was taken of each crop type from the district. Each sample was then carefully labelled with a unique identification code (sample identification included the crop name and the district of collection), to ensure that there could be no confusion or loss of identity. The vegetable samples that were collected for the study included different plant parts depending on the crop type. With respect to each crop type, the selected samples were as follows: fruit were collected from balsam apple, bitter gourd, bottle gourd, sponge gourd, brinjal, capsicum, cucumber, green chilli, green peas, pumpkin, tomato; head (whole) were from broccoli, cabbage, cauliflower; leaves from broad leaf mustard; roots from carrot and radish; tubers from potato; whole pod - kidney bean. One gram (1g) sample was taken from each selected plant part.

Chemicals and reagents used

Acetylcholinesterase (AChE), Acetylthiocholine Iodine (ATCI), 5,5' dithio-bis-2-nitrobenzoic acid (DTNB) and sodium phosphate buffer, bromine water (0.4%), ethanol, and distilled water were the chemicals and reagents used for sample extraction and analysis.

Preparation of solution from reagents

The powder form of regents was mixed with the distilled water and stored in the bottle to create a solution for the test. Approximately, 30mg of AChE and 216mg of ATCI was then dissolved in 10ml and after dividing into the proportions, 19.8mg of DTNB was dissolved in 50ml of distilled water or equivalent or following the bottle's direction and preserved at **below 0°C**. Buffer solution was placed at room temperature. One percentage of bromine concentration was made. The date of preparation and volume was mentioned in label after preparation of solution from reagent.

Sample extraction process

The sample extraction was carried out in two consecutive test tubes each for carbamate and organophosphate. At first, plant tissue of 1-2 grams was cut into small pieces and placed in the test tubes. Following this, in the test tubes 1 ml and 2 ml with 95% ethanol (AR grade) were added respectively and after that it was shaken for 20 seconds using a vortex mixer and left to stand for three minutes in case of carbamates while for organophosphate analysis, it was left to stand for 20 minutes, after adding 100 µl of 1% bromine water in order to remove the excess bromine water. Then, for analysis, extracted sample's supernatant solution was transferred to the new test tube. When a longer soaking period was anticipated the test tube was shielded with parafilm to avoid evaporation of solvent.

Incubation or analysis of sample extract

Prior to the analysis of extracted sample, a control test and sample test were conducted. The difference between these two tests was regarded to be the enzyme inhibition percentage that is resulted by the residual pesticide present within the sample.

Control test

To conduct the control test, first in 4ml cuvette, 3ml of Phosphate Buffer Solution (PBS) was filled. Then, blank $20\mu l$ of solution of AChE and 20 $\mu l$of 95% ethanol (AR grade) was added. After that, the mixture was mixed for 5 seconds and 2.5 minutes of waiting was done followed by addition of $100\mu l$ of DTNB solution. Finally, 20 µl of ATCI was placed exactly 3 minutes later and it was mixed for 5 seconds in order to initiate the reaction of enzymes.

The control test must show an absorbance range between 0.250 to 0.320 at spectrophotometer(412nm) to ensure validity of enzyme activity. This test is significant because as it represents 100% AChE activity, assist the baseline for calculating pesticide inhibition in sample tests. If this reading falls outside the range, then the test should not be proceeded, or it may lead to faulty results.

Sample test

As in the test, 3ml of PBS was added in 4ml of cuvette along with 20 µl ACHE solution and 20 µl of sample extract. Then, it was mixed well for 5 seconds. 100 µl of DTNB solution was added after 2.5 minutes and waited for 2.5 minutes. Exactly at 3 minutes, when the last reaction was ready, 20 µl of ATCI solution was added and stirred for 5 seconds, then the enzyme reaction begun. By placing the cuvette in spectrophotometer, the absorbance change was read at 412 nm wavelength, and compared with the reading of the control test, and when the inhibition percentage exceeded 35%, the same process was repeated two times for the confirmation.

Data collection

The sample was placed in the spectrophotometer (412nm) and after running the test it took one minute to give the inhibition percentage.

Interpretation of results

Using a spectrophotometer, enzyme inhibition percentage was observed and calculated as follows:

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

For the inhibition percentage of enzyme that was obtained was interpretated as shown in Table 2
Source: (Chiu et. al., 1991)

Limitation of the study

This research was bounded to the detection of organophosphate and carbamate insecticide residues using the RBPPR technique, groups of pesticides like pyrethroids and neonicotinoids doesn't detect. Based on enzyme activity, RBPR technique only provides partially quantitative output. furthermore, no any other chemicals confirmations GC, MS, or HPLC was performed. Samples were collected from selected districts in Bagmati province during particular period of time without considering seasonal variations and geographical range. There may have been limitations with this study in terms of its sample handling, storage, and the number of different vegetable types evaluated and the human health risk assessment models were not included. These factors could have limited the generalizability and accuracy of the study's findings.

Table 2. 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

RESULTS AND DISCUSSION

PESTICIDE RESIDUE ANALYSIS ON VEGETABLES PRODUCED IN CHITWAN DISTRICT

The average inhibition percentage of carbamate and organophosphate is displayed in Fig. 02. Among all the vegetables examined from Chitwan district the pesticide residue as indicated by the ACHE inhibition percentage, was highest for broccoli (8.0) followed by brinjal (7.36) and the lowest was found in cauliflower and green peas (2.3).

NEPALESE JOURNAL OF AGRICULTURAL SCIENCES,
July, 2025, volume 29
e-ISSN: 2091-0428; p-ISSN 2091-041X; esjindex ID =6279
Published by HICAST, Purbanchal University, Kathmandu

The inhibition percentage of organophosphate (OP) in Kidney beans (8.6) followed by capsicum (7.8) had the highest inhibition percentage of organophosphate and green chilli (1.3) exhibited the lowest. Capsicum (21.1) exhibited the greatest individual sample inhibition percentage for organophosphate whereas brinjal (20.4) showed the highest for the Carbamate.

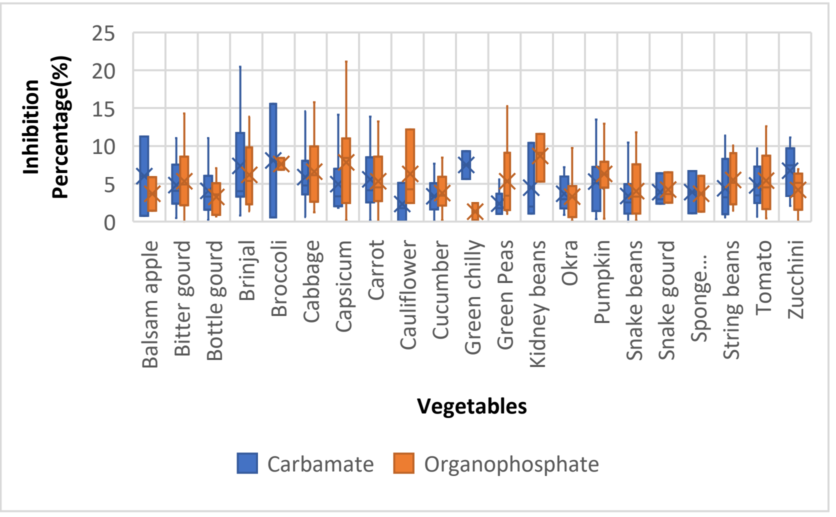

Figure 1. Average carbamate and organophosphate inhibition percentage in Chitwan district across different vegetable samples

PESTICIDE RESIDUE ANALYSIS ON VEGETABLES PRODUCED IN DHADING DISTRICT

For Dhading district, as shown in Fig. 03, sword beans (21.3), followed by green chilly (7.1) showed the highest average inhibition percentage for carbamate and lowest in bottle gourd (2.6). However, for organophosphate residue test, the average inhibition percentage was seen higher in sword beans (22.2) followed by green chilli (9.8) and lowest was depicted in snake beans (3.5). For both the carbamate, and organophosphate the highest individual inhibitory percentage was displayed by sword beans (30.3) and (29.8), respectively.

The residue analysis showed that residue was present in most vegetables, indicating the non-judicial use of pesticides, especially when pesticides were used without adequate understanding of the pre-harvest interval (waiting period). This

NEPALESE JOURNAL OF AGRICULTURAL SCIENCES,
July, 2025, volume 29
e-ISSN: 2091-0428; p-ISSN 2091-041X; esjindex ID =6279
Published by HICAST, Purbanchal University, Kathmandu

suggests that farmers need to be taught about proper use of pesticides, and that marketable crops should be constantly tracked throughout the nation. At the same time consumers should also be awared about the residues of pesticides in vegetables. After the conduction of RBPR test on vegetable samples of Bagmati region it was found that Brinjal (20.48%) and sword beans (22.2%) from Dhading, potato (16.7%) from Kathmandu, spinach (27.5%) from Bhaktapur were found to have comparatively higher pesticide residue which was in line with the findings of Kodandaram et al., (2013) which demonstrated that brinjal was grown using high amount of pesticide in brinjal comparing to other vegetables.

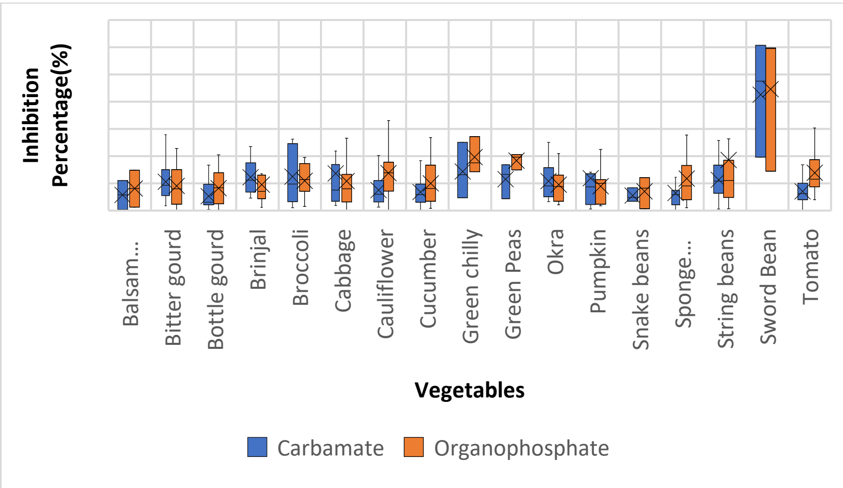

Figure 2. Average carbamate and organophosphate inhibition percentage in Dhading district across different vegetable samples

Pesticide residue analysis on vegetables produced in makwanpur district

As shown in Fig.04, for the case of Makwanpur district, the average inhibition percentage for carbamate was observed the highest in potato (14.5) followed by broccoli (12.5) and lowest in radish (1.2) followed by hatch chilli (2.0).

The highest organophosphate inhibitory percentage was observed in potato (12.6) followed by broccoli (10.2) and lowest was recorded in balsam apple (2.4) subsequently followed by string beans (3.6). Potatoes exhibited the highest individual inhibition percentage for both carbamate (20.7) and organophosphate (19.3).

Pesticide residue analysis on vegetables produced in kavrepalanchok district

The inhibition percentage of carbamate is shown in Fig. 05. Among all the vegetables examined in Kavrepalanchok district the inhibition percentage was highest for carrot (15.2) followed by the potato (13.0) and the lowest in string beans (2.8) and bottle gourd (3.0) subsequently followed by hatch chilly (3.4). The inhibition percentage of organophosphate (OP) pumpkin (12.6) followed by cabbage (11.1) had the highest inhibition percentage of organophosphate and of green capsicum (3.0) exhibited the lowest. Potato displayed the greatest individual sample inhibition percentage for carbamate 29.4 and organophosphate 23.6.

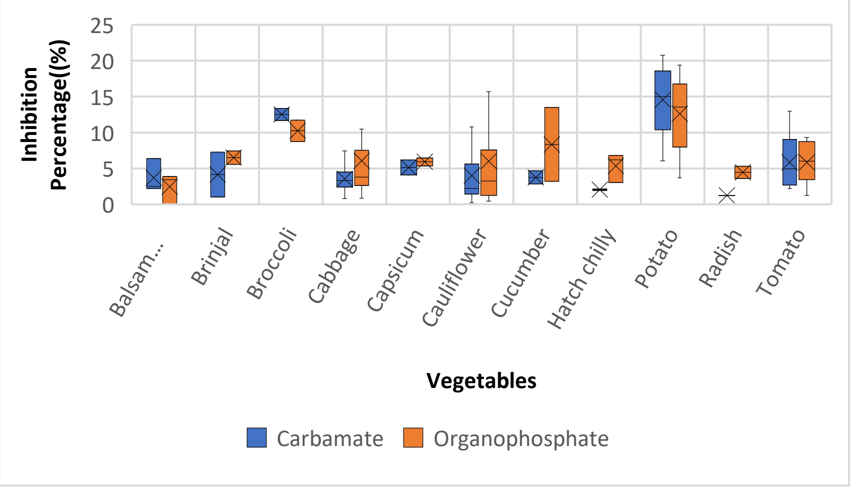

Figure 3. Average carbamate and organophosphate inhibition percentage in Makwanpur district across different vegetable samples

Pesticide residue analysis on vegetables produced in Kathmandu district

Fig.06 demonstrates the average inhibition percentage for carbamate and it was found highest in potato (16.7) which was followed by sword beans (9.4) and least in pumpkin (2.2). Likewise, for the organophosphate the average inhibition percentage was observed highest in potato (13.4), followed by sword beans (12.5) whereas the least was seen in basil (3.0) as shown in the figure. Potatoes exhibited the highest individual inhibition percentage for both carbamate (30.06) and organophosphate (24.03).

NEPALESE JOURNAL OF AGRICULTURAL SCIENCES,
July, 2025, volume 29
e-ISSN: 2091-0428; p-ISSN 2091-041X; esjindex ID =6279
Published by HICAST, Purbanchal University, Kathmandu

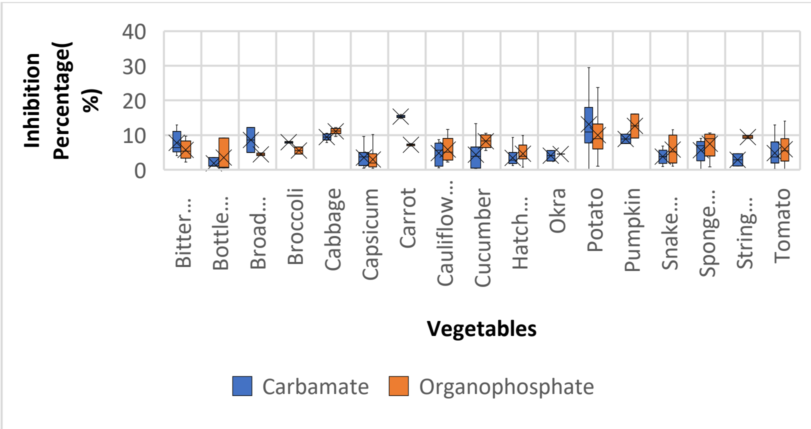

Figure 4. Average carbamate and organophosphate inhibition percentage in Kavrepalanchok district across different vegetable samples

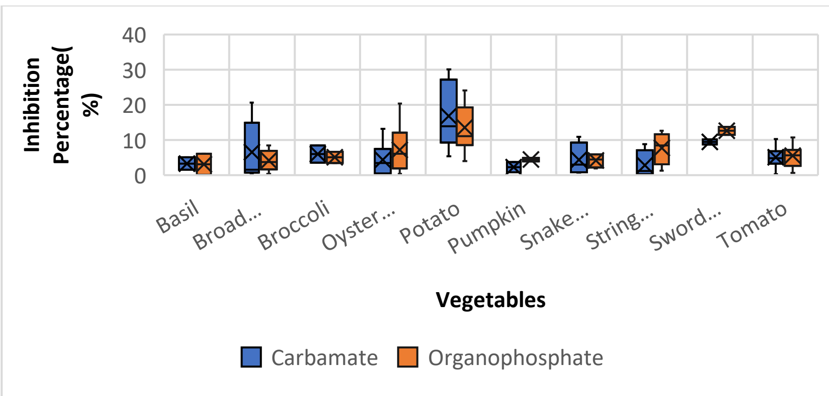

Figure 5. Average carbamate and organophosphate inhibition percentage in Kathmandu district across different vegetable samples

Pesticide residue analysis on vegetables produced in Bhaktapur district

As seen in Fig. 07, the average inhibition percentage for carbamate pesticide in the Bhaktapur district has been found to be greater in spinach (17.5), subsequent to potatoes (15.4), and least in broccoli (4.0), followed by cucumber (4.2). While, spinach (27.5) shows the greatest organophosphate inhibition percent, followed by potatoes (13.4), and cucumber (4.0) shows the least inhibition, followed by cabbage (5.0). Spinach had the highest inhibition percentage (46.4 and 76.2) for carbamate and organophosphate, respectively, which are very harmful for consumption and sale.

Spinach produced in Bhaktapur district showed highest inhibition percentage for organophosphate (76.22%) followed by sword beans (29.86%) of Dhading district. Inhibition percentages of (20.48%) brinjal from Chitwan, (30.33%), sword beans from Dhading, (46.41%) spinach from Bhaktapur and (29.4%) potatoes from Kavre district of Bagmati province were observed for carbamate. Numerous crops, including potatoes and chili, were found to have higher rates of pesticide applications; in the Kavrepalanchowk area, 93% of farmers sprayed pesticides on potato fields two to six times every season, while 6% applied pesticides to potato crops more than ten times in a period of a season (Sapkota et al., 2020).

Two samples (0.22%) delivered more than 35% inhibition percentage, out of permissible levels. Most vegetables cultivated in different districts of Bagmati Province excluding Bhaktapur, seem to have lower traces of organophosphate (OP) or carbamate pesticides. However, the mean inhibitions percentage of spinach (76.22) was greater than the permissible range (<35) and unsafe for consumption. Some sample showed residues were over 70%, indicating that producers were unaware of the recommended waiting period, but could also outcomes from the unacceptable use of carbamate and organophosphate pesticides, overdoses or the mixing of various pesticide formulations. This exercise reflects a failure to ensure pesticides practices by products as well as lack of extension support.

Pesticide residue analysis on vegetables produced in Lalitpur district

As shown in the Fig. 7, the average inhibition percentage for carbamate pesticide in the Lalitpur district was highest in string beans (8.4) and subsequently followed by broad leaf mustard (5.9) and lowest by cauliflower (2.1) followed by button mushroom (2.7). While Tomato (6.2) showed the highest average inhibition percentage for organophosphate followed by broad leaf mustard (5.7) and least by snake beans (0.4) followed by string beans (1.6) as shown in the figure. The most significant individual values for carbamate and organophosphate (12.09) and (10.04) have been shown by broad leaf mustard, which is safe for consumption and sale purpose.

NEPALESE JOURNAL OF AGRICULTURAL SCIENCES,
July, 2025, volume 29
e-ISSN: 2091-0428; p-ISSN 2091-041X; esjindex ID =6279
Published by HICAST, Purbanchal University, Kathmandu

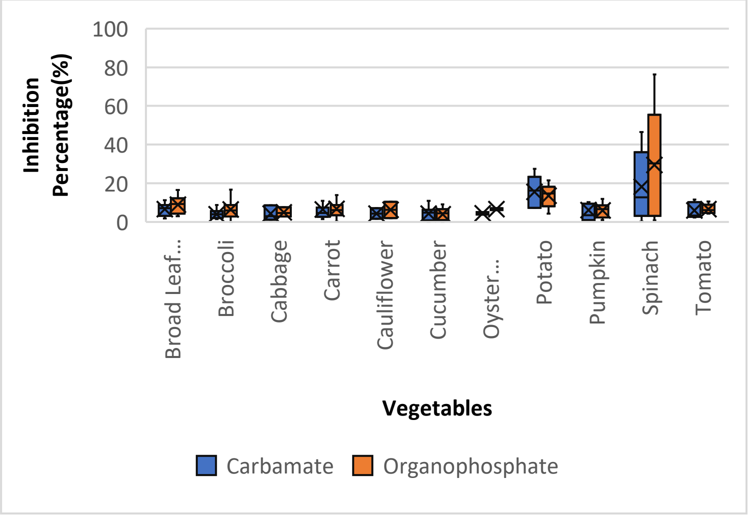

Figure 6. Average carbamate and organophosphate inhibition percentage in Bhaktapur district across different vegetable samples

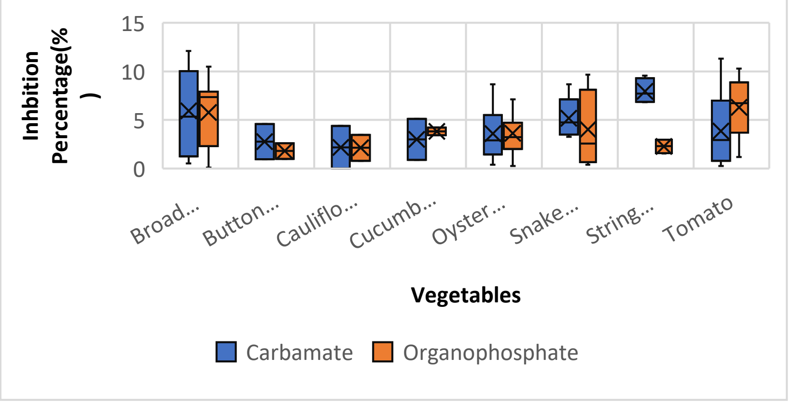

Figure 7. Average carbamate and organophosphate inhibition percentage in Lalitpur district across different vegetable samples

According to Bhandari et al. (2019), 99 % of the brinjal samples had residues, 56 % had several pesticide residues, and 4 % had triazophos [22.5µg/kg] across the EU MRL (European Union Maximum Residue Limit). Crops like brinjal and tomatoes are highly vulnerable to pests, which encourage the farmers to apply pesticides with a great effort to ensure the market quality and quantity. 70% of the potato sample contained pesticide residue, suggesting that the potatoes were highly contaminated (Khandekar et al.,1982). In contrast,44% of tomato samples [10.6-1772µg/kg] had levels of chlorpyrifos beyond the EU MRL was surpassed by triazophos in 6% [237-685µg/kg] and omethoate in 3% [27.9µg/kg] of tomato samples (Bhandari et al.,2019). Similarly, Rawal et al. (2013) discovered chilies with more methyl parathion residue (0.025 ppm) than the MRL threshold. The few reasons behind higher pesticide may be overuse of pesticides, failure to observe the preharvest interval as well as high pest pressure in the field.

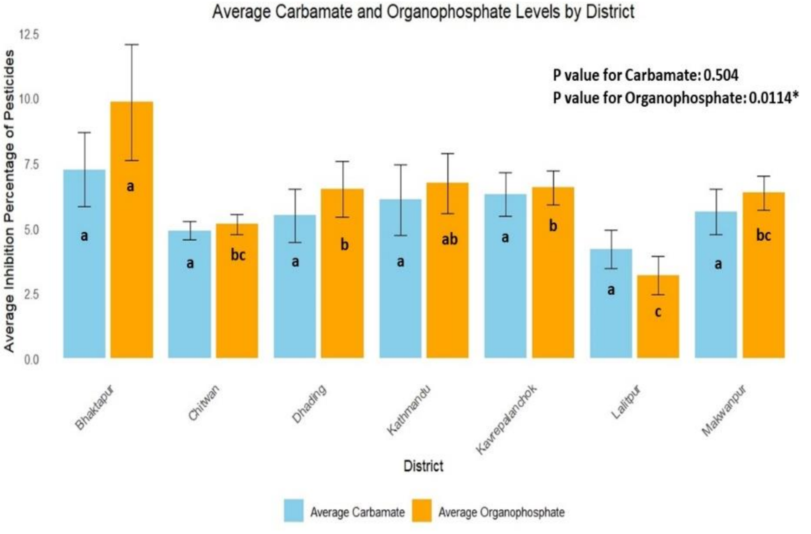

Figure 8. Average inhibition percentage of Carbamate and Organophosphate in seven districts

Chilly samples from Kavre, Bhaktapur, and Rupandehi included methyl parathion, carbendazim, and chlorpyrifos (Bhandari et al., 2019; Rawal et al., 2012). Cucumber samples from Chattradeurali, Dhading, were treated with metalaxyl. Potato samples from Chitwan, Bhaktapur, and Indian Bangali had traces of mancozeb and DDT (Manandhar, 2006; IPEN, 2005). Spinach from Kavre and Bhaktapur had dichlorvos and chlorpyrifos (Rawal et al., 2012; Giri, 2010), while tomato samples from Kavrepalanchowk, Dhading, and Rupandehi shows residues of cypermethrin, mancozeb, carbendazim, and chlorpyrifos (Manandhar, 2006; Giri, 2010; Bhandari et al; 2019).

Similar results were seen on comparison with other studies. Ghimire et al. (2020) executed the dangerous use of pesticide residues in cauliflower leaves and curds showing the unsuitable use of pesticide is still widespread in Nepal. Similarly, Brouwer et al. (1999) highlighted risk associated with the human health with pesticide exposure, including cancer and hormone disruption. These results align the current study's conclusion of high pesticide residue levels in specific vegetable, making it evident that stronger laws enforcement and farmer education is must needed. The vegetables like brinjal, cauliflowers, tomatoes, potatoes, green beans, and many more that were brought from the neighboring districts or the farms to the central vegetable market were considered safe for the consumption since it displayed little or no use of pesticides (Koirala et al., 2010).

The average inhibition percentage of Carbamate and Organophosphate across seven districts is displayed by fig.8. The highest carbamate inhibition percentage was seen in Kavrepalanchok district (7.69), while the lowest was seen in Lalitpur (4.1). For the organophosphate, Bhaktapur (8.44) demonstrated the greatest inhibitory percentage whereas Lalitpur showed the lowest (4.46). Organophosphate continuously exhibited higher average inhibition percentages than Carbamate across all the districts.

CONCLUSION

The study concluded that nearly all the vegetable samples tested from Bagmati province had residue levels of organophosphate and carbamate pesticides that were below the Maximum Residue Limits (MRL), while a few commodities specifically spinach, sword beans, and potatoes were consistently found to have pesticide level higher than the acceptance level. One of Spinach sample exceeded the 45% limit, make it unsafe for consumption. A main drawback of this study is that it was limit of only carbamate and organophosphate pesticide groups through the RBPR technique. Given the wide range use of other pesticide classes such as synthetic fungicides, pyrethroids, and neonicotinoids, further research should aim to widen the scope of pesticide residue analysis using more systematically multi-residue detection technique.

In countries with scant laboratory resources RBPR approach is crucial as it is cost effective and efficient, is essential for rapid screening approaches. But to cover farmers throughout the nation, broader implementation is needed. This can be accomplished by educating people on the proper use of pesticides, waiting periods, and testing options. This will reduce health risks and improve food safety, which should worry lawmakers. Along with referencing agriculture and public health, this reinforces the growing need for a more integrated strategy to pesticide usage.

ACKNOWLEDGEMENTS

Authors are grateful to Rapid Bioassay of Pesticide Residue Analysis Lab Unit situated in Kalimati, Kathmandu, Central Agricultural Laboratory, Ministry of Agriculture and Livestock Development for procurement of the laboratory equipment and required supplies. There was no involvement of any outside fundings; the researchers themselves covered all the expenses required for the study.

REFERENCES

Bhandari, G., Zomer, P., Atreya, K., Mol, H. G., Yang, X., & Geissen, V. (2019). Pesticide residues in Nepalese vegetables and potential health risks. *Environmental research*, *172*, 511-521.

Brouwer, A., M. P. Longnecker, L.S. Birnbaum, J. Cogliano, P. Kostyniak, J. Moore, S. Schantz and G. Winneke. (1999). Characterization of potential endocrine related health effects at lowdose levels of exposure to PCBs. Environmental Health Perspectives, 107(4), 639-649.

CAL. (2017). Standard Operating Procedures for Rapid Bioassay of Pesticide Residue Analysis Laboratory. Central Agricultural Laboratory (CAL), Hariharbhawan, Lalitpur, Nepal.

Chiu, C. S., Kao, C. H., & Cheng, E. Y. (1991). Rapid Bioassay of Pesticide Residues (RBPR) on Fruits and Vegetable.

Ghimire, K., Arun, G.C. (2018). Trend of Pesticides Uses in Nepal. J. Plant Prot. Soc., 5, 32 42.

Ghimire, P., Baral, S., Sharma, J., & Ghimire, A. (2020). Analysis of pesticide residue on the leaves and curds of cauliflower. *Journal of the Plant Protection Society, 6*, 161-170.

Giri, N. (2010). *Pesticide use and food safety in Kathmandu Valley, Nepal*. na..

IPEN. (2005). Identification of a POPs hotspot - examination of DDT and lindane (BHC) residues in potato and farm soil. The International POPs Elimination Project, Pesticide Watch Group, *Nepal Forum of Environmental Journalists.*

Khanal, D., Neupane, S. K., Poudel, S., & Shrestha, M. (2021). An overview of chemical pesticide import in Nepal. *The Journal of Agriculture and Environment*, *22*, 121-134.

Khanal, D., Dhital, A., Neupane, A., Paudel, K., Shrestha, M., Upadhyaya, N., Bhandari, R., & Pandey, P. (2023). Insecticide residue analysis on vegetable crops through Rapid Bioassay of Pesticide Residue (RBPR) technique in Nepal. *Journal of King Saud University - Science*, *35*(5), 102671. https://doi.org/10.1016/j.jksus.2023.102671

Khandekar, S. S., Noronha, A. B. C., & Banerji, S. A. (1982). Organochlorine pesticide residues in vegetables from Bombay markets: a three year assessment. *Environmental Pollution Series B, Chemical and Physical*, *4*(2), 127-134.

Kodandaram, M. H., Saha, S., Rai, A. B., & Naik, P. S. (2013). Compendium on pesticide use in vegetables. *IIVR Extension Bulletin*, *50*, 133.

Koirala, P., Tamrakar, A. S., Bhattarai, B. P., Yadav, B. K., Humagain, S., & GC, Y. D. (2010). Use and handling practice of pesticides in vegetables: A case study on some selected districts of Nepal. *Journal of Food Science and Technology Nepal*, *6*, 105-109.

Kumar, S., Pathak, A., & Mangal, H. M. (2011). Trends of fatal poisoning in Saurashtra region of Gujarat (a prospective study). *Journal of Indian Academy of Forensic Medicine*, *33*(3), 197-199.

Manandhar, D. N. (2006). Pesticides in Nepal. The Rising Sun Printer, Teku, Kathmandu, Nepal.

MoALD, 2024. Statistical Information on Nepalese Agriculture, 2023/24. Ministry of Agricultural Development, Singh Durbar, Kathmandu.

Nyaupane, S. (2021). Use of insecticides in Nepal, its impact and alternatives of insecticides for Nepalese farmers. In Insecticides-Impact and Benefits of Its Use for Humanity. *IntechOpen*.

PPD. (2014). Survey on National Pesticide Consumption Statistics in Nepal. Plant Protection Directorate (PPD), Hariharbhawan, Lalitpur, Nepal.

PPD. 2015. Survey on National Pesticide Consumption Statistics in Nepal., Plant Protection Directorate, Hariharbhawan, Lalitpur.

Rawal, D. S., Rana, N., Shrestha, S., & Sijapati, J. (2012). Assessment of pesticide residues in some vegetables grown in Kavrepalanchok and Bhaktapur districts. *Nepal Journal of Science and Technology*, *13*(2), 45-50.

Rawal, D. S., N. Rana, S. Shrestha and J. Sijapati. (2013). Assessment of Pesticide Residues in Some Vegetables Grown in Kavrepalanchok and Bhaktapur Districts. *Nepal Journal of Science and Technology*, 13(2), 45-50.

Sapkota, K. R., Sapkota, S., Sapkota, S., & Katuwal, K. (2020). Pesticides handling practices among potato growers in Kavrepalanchok, *Nepal. Journal of Agriculture and Natural Resources, 3(1)*, 77-87.

Sharma, D. R., Thapa, R. B., Manandhar, H. K., Shrestha, S. M., & Pradhan, S. B. (2012). Use of pesticides in Nepal and impacts on human health and environment. *Journal of Agriculture and environment*, *13*, 67-74.

Upadhyaya, D., & Bhandari, R. (2022). Malthusian theory and food security: A comparative analysis. *Journal of Agricultural Sustainability*, 10(2), 15-21.