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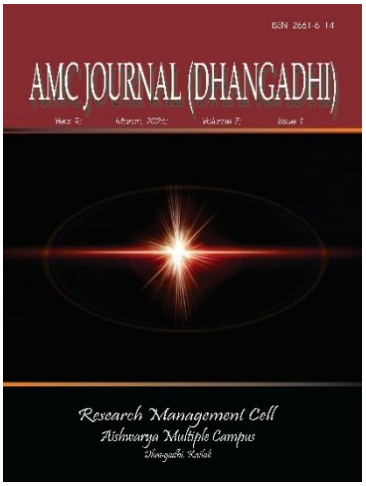
Analysis of Pesticide Residue in Mango by RBPR Technique in Dhangadhi, Kailali



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Abstract

Mango is one of the most abundant and economically valued fruits of Nepal and is widely treated with organophosphate and carbamate insecticides for quality and pest control. But in its non-scientific use and bulk applications, toxic residues are created that affect human health as well as the environment. Mango fruits used for this investigation were bought from four major bazaars Haatbazar, Purano Sabjimandi, Chatakpur Haatbazar, and Bhat-Bhateni and analyzed for quality through RBPR process, a pesticide residue screening system technology derived from AChE enzyme inhibition. The main objective of this study is to find the existence of pesticide residues in mango fruits sold in local markets of Dhangadhi Sub-Metropolitan City, Kailali, through the Rapid Bioassay of Pesticide Residue (RBPR) method. RBPR method was used because it is easy, cheap, and convenient to use, hence particularly ideal for developing countries like Nepal where access to advanced laboratory equipment might not be readily available. Spectrophotometric detection showed variation in residues with the highest inhibition at Chatakpur Haatbazar (17.514% carbamate, 12.123% organophosphate) and lowest at Bhat-Bhateni. All were below the 35% inhibition national safety limit and hence safe for consumption. Variability in residues on markets reflects variability in post-harvest and pesticide treatment. It requires vigilant monitoring, mass consciousness, training for farmers, and adoption of safer agricultural technologies like Integrated Pest Management (IPM). The study concludes that RBPR is an affordable, cost-saving, and practical approach for local food safety assessment and must be implemented at the national level policy for making Nepalese fruit production exportable and sustainable.

Key words: Pesticide Residue, RBPR technique, Mango, Food Safety, Carbamate

Introduction

Organic farming is becoming more common as people look for healthier lifestyles and cleaner, safer environments. In contrast, the way we currently grow most grains vegetables and fruits depends heavily on chemical pesticides and herbicides. While these chemicals can boost harvest and help to preserve crops for longer periods, they also carry serious health concerns. Pesticides, in particular are used to kill pests and insects that threaten crops, but their widespread use raises growing worries about long-term impacts on both our well-being and the environment (Neufeld et al.,2015).

Of the numerous agricultural chemicals, pesticides are the most dangerous and are illicitly applied to crops to control pest infestation. Although the main aim of applying these chemicals is to reduce pest levels to the minimum, their widespread usage is heavily amplifying health risks to consumers of agriculture products. Besides, the pesticides are causing adverse effects on nearly all living organisms in the ecosystems where they are being utilized (Igbedioh, 1991; Jeyaratnam, 1981).

Studies have established that even low doses of pesticide exposure over time can result in respiratory diseases like asthma, and low sperm count and quality, affecting reproductive health (Bhandari Govinda, 2014).

The mango is a tropical fruit. Its scientific name is *Mangifera indica* L. It is reported for its sweetness and wonderful fragrance. It is highly nutritious and is eaten worldwide as a healthy, nutritious fruit (Yahia et al., 2023). It is cultivated in most of the Terai districts and the crops grown there are the same as in India; commercially worthy varieties are Dasheri, Maldaha, Bombay, krishnabhog, and Amarpali (PMAMP, 2022/23). The mango fruit is getting attention for the biochemical and pharmacognosy research because of its antioxidant properties and therapeutic potential (Masibo & He, 2008). As more people become health-conscious, they are starting to see how eating the right foods can help prevent and manage metabolic problems (Burton-Freeman et al., 2017).

The field setting of this research was a 19th Jestha 2082 activity where pesticide residue testing was conducted at Shahid Gate Agricultural Wholesale Market of Dhangadhi Sub-Metropolitan City. During the drill, Indian-imported mangoes kept in Jai Shree Ma Janaki Store had pesticide residues beyond the permissible limit. While other fruits and vegetables were found to have a regular pesticide composition, mango samples were made unfit for human use. Accordingly, 2,260 kilograms of mangoes were destroyed in association with Dhangadhi Sub-Metropolitan City and with assistance provided by the Food Technology and Quality Control Office, Pesticide Residue Rapid Analysis Laboratory Attariya, District Administration Office, Nepal Police, and other concerned stakeholders.

This event had generated a massive concern regarding food safety and consumer health, and this greatly encouraged me to select this topic—to evaluate the safety of mangoes found in local markets of Dhangadhi according to the RBPR approach. The main objective of this study is to evaluate the presence, concentration, and potential health risks of pesticide residues in mangoes sold in Dhangadhi using the Rapid Bioassay of Pesticide Residue (RBPR) technique.

Materials and Methods

Research Design

This study employs descriptive and experimental research design in determining the composition of pesticide residues in the mango fruits obtained from Dhangadhi, Kailali. Its descriptive aspect is record-keeping of storage and origin of mango samples from farms and markets. Meanwhile, its experimental aspect employs the Rapid Bioassay of Pesticide Residue (RBPR) method in performing experiments for contamination. The bioassay employs *Musca domestica* larvae to measure toxic pesticide residues in terms of percentage inhibition determination, which represents a value of the percentage lost in biological activity due to pesticide exposure. The research being systemic was ensured so that data collection, laboratory analysis, and results interpretation are carried out scientifically. Finally, the design aims at triggering potential knowledge on food safety concerns and stimulate safe consumption and production in the subject area.

Study Area

Dhangadhi in the Terai belt of Nepal is located in Kailali district and is composed mostly of gently sloping to flat plains with highly fertile alluvial soils formed by massive Mohana River sediment deposits. Such fertile plains offer highly conducive conditions to grow mangoes, a commercial high-value fruit crop in the area. The subtropical climate of the region, with exceedingly hot summers, adequate monsoon rains, and fairly cold winters are all of vital importance to the cultivation of mango plants as well as to the fate of dairy animals upon which these pesticides are sprayed.

Field Location

For the research purposes, mango samples were gathered from prominent fruit bazaars of Dhangadhi Sub-Metropolitan City of Kailali district. Sampling sites chosen were Bhat-Bhateni (Boradadi area), Haatbazar (Hasanpur locality), Chatakpur Haatbazar (Chatakpur area) and Purano Sabjimandi (middle Dhangadhi Bazaar). These markets were selected because of their high volumes of fruit transactions, massive sources of mango supply, and high consumer traffic. Dhangadhi is situated at around 28.7075° North latitude and 80.5937° East longitude.

Sampling

Samples of mangoes were collected from the study region. About half kilograms of mangoes were collected from each point and placed in separate polythene bags, each of which was properly labeled with the respective name of the market. The samples collected were transported to the laboratory for analysis with maximum speed. Prior to analysis, all the necessary equipment and containers were properly labeled to avoid any confusion. The process of extraction was initiated without delay to ensure that the original quality of the samples was preserved.

Materials:

- Acetylcholinesterase (AChE): 30 ml dissolved in 10 ml distilled water (Part I and Part II assays)
- Acetylthiocholine Iodide (ATCI): 216 mg per bottle, dissolved in 10 ml distilled water (Part I and Part II assays), or in 500 ml phosphate buffer saline (PBS) (Part III assay)
- 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB): 19.8 mg/ml in PBS (pH 7.0)
- Ethanol 95%
- 0.1 M phosphate buffer saline (PBS), pH 8.0
- Converting solution: 0.4% bromine water
- Fruit samples (mango): 1–2 grams, collected in labeled polythene bags

Instruments:

- Cuvette, Test tubes, Mixer, Spectrophotometer, Micropipettes
- Fruit sampler, forceps, stopwatch, measuring cylinders, filter paper, stopwatch and other glassware

RBPR Procedure

Sampling and Extracting:

- Cut 1gm of plant tissue into pieces and put into two test tubes.
- Add 1ml and 2ml 95% ethanol to test tube A and B, respectively.
- Add 100µl bromine water into tube B. Shake for 20 sec and drain the sample extract to a new test tube at 3 min sharp.
- Allow 20 min standing for tube B to evaporate excess bromine.

Incubation of Sample Extract and AChE:

- Add 3ml PBS buffer in the cuvette.
- Add 20µl AChE solution and 20µl sample extract (or 20µl 95% ethanol for blank).
- Mix for 5 sec. Wait till 2.5 min and add 100µl DTNB solution.
- Add 20µl ATCI solution at 3 min, mix for 5 sec to start enzyme reaction.
- Read absorbance change by Spectrophotometer at 412nm.

Spectrophotometric Principle

A spectrophotometer is a device to determine the amount of light a sample will absorb. A beam of light is passed through the sample and both light intensities before and after traveling through the sample are measured.

Calculation of inhibition

Read absorbance change in 412 nm, and compare reduction of absorbance for sample to normal AChE reaction in a fixed reaction period. The percent inhibition was calculated as follows:

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

Results

This research effectively calculated the percentage inhibition of pesticide residue in the mango samples through the RBPR (Rapid Bioassay of Pesticide Residues) method. The experiment measured the absorbance difference between the mango extract and the standard AChE activity over a specified reaction time. This absorbance difference indicated the level to which the pesticide residues had inhibited the enzyme activity in the mangoes being analyzed.

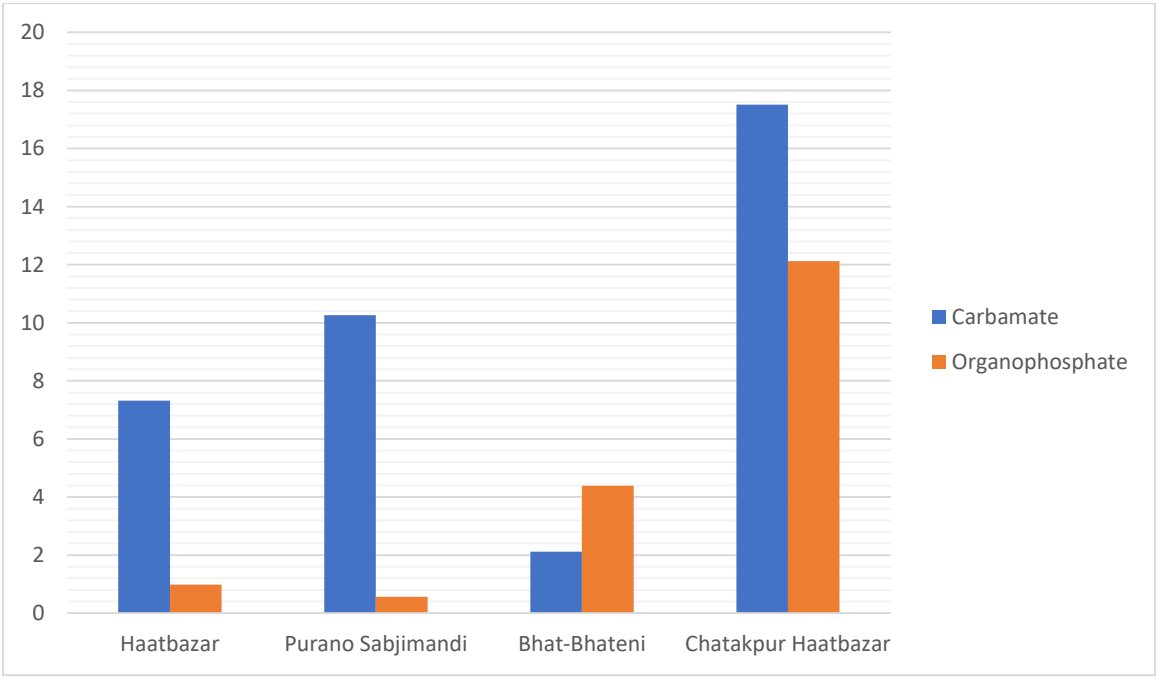
According to set safety standards, samples containing inhibition levels of 35% or less are safe and acceptable for consumption, those between 35% and 45% require quarantine and thorough washing before consumption, and samples above 45% are marked as unsafe and recommended for disposal due to the high concentration of pesticide residues.

Table 1: The inhibition percentage of carbamate and organophosphate pesticides in mango Samples which was determined in Kailali (Attariya) laboratories by RBPR Technique.

S.N	Sample (Market)	Carbamate (%)	Organophosphate (%)	Result
1.	Haatbazar	7.3182	0.9808	consumable
2.	Purano Sabjimandi	10.261	0.5619	consumable
3.	Bhat-Bhateni	2.1202	4.3918	consumable
4.	Chatakpur Haatbazar	17.514	12.123	consumable

Graphical representation of data

Comparison of carbamate and organophosphate pesticide residues in mango samples collected from various markets within Dhangadhi Sub-Metropolitan City, Kailali District.



The findings of the test revealed that samples from Chatakpur Haatbazar contained the highest pesticide residues, namely 17.514% carbamate and 12.123% organophosphate inhibition percentages. Samples taken from Purano Sabjimandi contained moderate pesticide residues of 10.261% carbamate and 0.5619% organophosphate, while samples from Haatbazar contained 7.3182% carbamate and 0.9808% organophosphate. The lowest pesticide residues were present in samples from Bhat-Bhateni at 2.1202% carbamate and 4.3918% organophosphate.

These results suggest that all the mango samples under test possess pesticide residues in the safety level, thereby being mostly safe for consumption. However, the disparity in levels of pesticide residues in markets highlights the importance of constant surveillance and promotion of good agricultural practice toward enhancing safety in food and consumer health. Cleaning of mango washing, especially for

mangoes from high residue level markets, is recommended to also reduce any risk that may be caused by pesticide application.

Utilization of the Rapid Bioassay of Pesticide Residues (RBPR) technique in this study provided an easy and effective method of detecting and quantifying pesticide residues, enabling the timely assessment of food safety and support for appropriate risk management measures.

Discussion

Result of this study indicates huge variation in residue amount of mango fruits of various market places of Dhangadhi Sub-Metropolitan City of carbamate and organophosphate pesticides. Though all the levels of inhibition are within the safe level of 35%, i.e., the composite fruits are safe to consume, they vary due to the variability in the farmer's and trader's post-harvest handling practices and inputs used.

Maximum enzyme inhibition was found in Chatakpur Haatbazar mangoes. This could be due to overuse of chemical pesticides, inadequate pre-harvest waiting periods, or non-compliant crop and wash controls. On the other hand, Bhat-Bhateni mangoes contained low residues, which can be due to greater levels of control compliance, cleaner and safer production systems, or procuring suppliers who used controlled and clean practices.

Evidence is supported by other national and regional studies that record levels of pesticide contamination of farm-origin produce purchased in markets (e.g., Syed et al., 2014; Bohara et al., 2022). Evidence unequivocally records the necessity for decentralized chains of food inspection and pesticide residue examination in urban places such as Dhangadhi, where fruit and vegetable consumption are rising but food safety regulation does not exist or is non-functional.

The RBPR method was the best and effective method in such a case. It was economical, rapid, and simple method of determination of the pesticide residues, particularly neurotoxic groups like organophosphates and carbamates. With the help of the enzyme inhibition assay of acetylcholinesterase (AChE) enzyme, the significant biomarker of neurotoxicity, RBPR allows early detection in bulk even without the pre-advance technology such as GC-MS and HPLC, if not yet centralized in the city but still to be extended to the rural locales.

Lastly, the argument not only alludes to the ubiquity of pesticide residue in locally exported mangoes but also betrays inconsistency in abiding by sound agronomical conduct. It promotes the conducting of regular farmer training sessions, compliance with safe and controlled use of pesticides, incorporation of pre-market washing of fruit, and enhancement of food monitoring systems in the hope of protecting consumers and communities' health.

Conclusion

The study successfully employed Rapid Bioassay of Pesticide Residue (RBPR) technique to ascertain the level of pesticide pollution in mango fruit samples that were obtained from four large local markets of Dhangadhi, Kailali. The test sample guaranteed that the pesticide residue level in all of the samples was less than the set safe limit, and thus it indicates that such fruits are safe for human consumption.

However, the seeming volatility of inhibition percentages across the involved markets is an indication of inconsistency in pesticide use, harvesting, and post-harvest handling of crops — all priorities in the

determination of food safety. RBPR was a cost-effective, efficient, and user-friendly analytical technique for analyzing pesticide residues in the field.

Its user-friendliness, non-requirement for high-technology hardware, and quick turnaround time make it ideally placed in environments such as Dhangadhi and other Nepalese semi-urban or rural environments where limited resources are available. The fact that the method can determine the enzyme inhibition by the neurotoxic classes of pesticides, the carbamates and the organophosphates, says a lot about its application in cases where one would not have access to high-technology laboratory tools such as GC-MS or HPLC or even where it is not viable.

Apart from that, the results lean towards greater coordination among consumers, market vendors, farmers, and policy makers. Improved education regarding the risks of pesticides, promoting sustainable use of pest management practices like Integrated Pest Management (IPM), and enforcing better washing and grading of fruits before sale can reduce the risk of exposure and food safety level effectively.

Lastly, although the mangoes under study in this research fell within safe eating limits, the guarantee of different levels of residues present verifies the necessity of ongoing monitoring and control. Mass utilization of low-cost screening techniques like RBPR, supplemented with strengthened policy adherence and greater public consciousness, can assist in going a considerable distance in improving the consumers' protection. Protection of good market practice and sustainable agricultural practice will not only provide health, but also raise the credibility, quality, and marketability of Nepalese fruit exports within and outside the nation.

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