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Determination of inhabitation percentage of pesticide residue in vegetables with the help of spectrophotometer

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Abstract

The common types of pesticides such as carbamate and organophosphate are cause serious health issues for consumers. Although these have several benefits to preserve fruits, vegetables, and cereals as well as better yield. The farmers should be aware during the used of the pesticides in nature of the pesticides, ratio of pesticide to land area, and waiting period for edible vegetable. This study focused on the quantitative analysis of rapid bioassay of spectrophotometric for inhabitation of pesticides occurs in the vegetables. The nine different vegetables were collected from six places and analyzed then compared with the standard result. Eight vegetable samples provided green coloured indication and one indicated yellow colour by spectrophotometry. Furthermore, the percentage of Op and Ca pesticide residue is below 35%, which indicates such vegetables are edible. But for cucumber the Op was found in the 35-45% range and resulted in yellow colour, it indicates that the vegetables are non-edible. For cucumber, it takes to quarantine for 2-3 days after that the pesticide could be reduced and safe for consumption. It is clear from this study that no vegetable in the local market has a maximum percentage level of pesticide Op

and Ca. However, most of the vegetables available in the market of Sudurpaschim province are safe to consume.

Keywords: Pesticides, Organophosphate, Carbamate

Introduction

Organic food production is prevailing for the better health of human beings and a pollution-free environment. At present, the production of cereals and vegetables is highly dependent on the use of pesticides and herbicides which are accountable for risky health as well as better yield. Pesticides are the chemical substances widely used to control insects and pests to protect crops, and vegetables for better yield, and long-term preservation (Neufeld et al., 2015). Despite the many benefits, the most commonly used pesticides lead the contamination to the surrounding then accumulate with stratification. They are Organophosphate, Carbamate, Pyrethroid, Organochlorine, etc. (Leuprasert et al., 2014). In addition, organophosphate is considered to be an effective and efficient pesticide, especially for cereals and vegetables but the residual level of it is mandatory for rapid use (Tang et al., 2005). A pesticide like carbamate has been used all over the world for a long time but this is considered to be carcinogenic and mutagenic. The organochlorine pesticide has also been used for a long time but it has extended insistent properties, therefore, it is removed from the agriculture used in several countries (Fatunsin et al., 2020). It was mostly applied for the vegetables such as radish, potato, onion, cauliflower, chilli, tomato, and veggie. Organophosphate controls the insects by the route of the stomach and affects the nervous system of the insects and pests as well. The mainly noticeable health effects on humans are headaches, breathing problems, abdominal cramps, fatigue, and tingling by organophosphate (Ponnusamy & Arulmozhiselvan, 2019). It is commonly known as acephate simply soluble in water and uptake by the root of the plants then controlling the feeding mechanism of the insects (Elgailani & Alghamdi, 2018).

The pesticide consuming trend is increasing in Nepal as the trend going upward all over the world. Knowingly or unknowingly the Nepalese formers are utilizing pesticides, insecticides, and herbicides for cereals, fruits, and vegetable preservation

and better yield. Mostly in central development regions farmers are using around (1.5 a.i .kg/ha) or 50% of the total pesticides used in the country (Sharma, 2015). Although, the pesticides are being applied to the crops such as vegetables, cash crops, pulses, and fruits for over a decade. These are registered by their trade and common names (in number 3034 and 169 respectively) in the year 2018 based on the Nepal pesticides regulation act 1993(Sharma, 2015). At present, some advanced methods have also been applicable and they are cost-effective and efficient to detect the residue level of pesticides in crops without collecting the sample. The nanoscience and nanotechnology based tools are accountable for tracing the pesticides and heavy metals in fruits and vegetables i.e. laser induced breakdown spectroscopy and UV-Vis spectrophotometer, etc.(Zhao et al., 2019).

In this study, we have determined the percentage inhabitation of pesticides with the help of rapid enzymatic method (rapid bioassay) along with spectrophotometric instrumentation. Pesticide levels of organophosphate and carbamate in nine different sorts of vegetables collected from three different markets (Sudurpaschim province Nepal). The data was measured in percentage as indicated by a spectrophotometer in various colors such as green and yellow. Based on the colour of the sample and percentage quantity, we compare it with the standard result and decided whether the available vegetables in our market are edible or non-edible.

Materials and Methodology

Materials

All the chemical reagents used for this work were funded by Kailali Multiple Campus Dhangadhi which were analytical grade. These chemicals were used without changing the grade and some are prepared in the laboratory as required for the experiment. Bromine water, ethanol (95% pure), Acetylcholinesterase(AChE) solution, Phosphate Buffer Saline (PBS, pH 7.0), Acetylthiocholine Iodine (ACTI), and 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) solution.

Collection of vegetables samples

The vegetable collected and extracted from different shop of Dhangadhi town

(Dehoriya, Campus road and Buddha Chok) and Attariya town (Gulariya, Attariya and Shantipur) in the Sudur Paschim Province, Nepal.

Analysis of sample

The analysis of the collected sample can be tested with the help of a spectrophotometer and rapid bioassay for the Carbamate and organophosphate pesticides. The instrumentation part for this experiment was done with the help of the pesticide residue laboratory unit, Attariya Kailali(Sudur Paschim Province).

Calculation of enzyme inhabitation percentage

Level of pesticide (%) in the vegetables	Colours	Explanations	References
< 35	Green	Low pesticide level (edible)	[8,10]
35-45	Yellow	Medium level of pesticide contains (edible after quarantine for minimum 2 days where the quantity of pesticide will be degrading after holding for some duration).	
> 45	Red	Maximum level of pesticide residue (non-edible and need to destroy and dump).	

Table 1. Standard inhabitation percentage ranges of pesticides in the vegetables

The absorbance of the collected samples and control sample measure by the spectrophotometry at 412 nm based on Beers Lambert law. It was compared with the standard then the inhabitation percentage of the sample can be calculated with the help of following formula

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

Methodology

Preparation of stock Solution for carbamate and organophosphate insecticide

residue assay

First of all, cut the vegetable into small pieces (crush with the help of mortar and pastel) and weigh 1gram of it in an analytical balance then put it into two labeled test tubes A and B. The test tubes A and B are for the testing of Carbamate (Ca) and Organophosphate (Op) respectively. Adding 1 ml and 2 ml of 95% ethanol to the test tube A and B respectively and then shaking both for 5-10 seconds properly. For the carbamate test, standing the sample solution for about 3-5 minutes, decant the sample to the new test tube for final analysis. On other hand, for the organophosphate test, adding 100 µl of Bromine water into the previously prepared test tube. The test tube is shaken for 5-10 seconds until the solution is mixed properly. The solution was holding for 20 minutes then decant into the new test tube for the final analysis(Neufeld et al., 2015). The method of analyzing carbamate and organophosphate insecticides levels in vegetables, fruits, and cereals by testifying percentage inhabitation of acetylcholinesterase(AChE) also known as rapid bioassay (RBPR)(P.Ghimire, S. Baral, S.Sharma, 2020). The firstly prepared vegetable juice was used as a control for the entire experiment.

Results and Discussion

The inhabitation percentage of two pesticides such as Ca and Op in vegetables was measured with the help of UV-spectrophotometry along with a rapid bioassay. The measured percentage of carbamate and organophosphate are shown in the tables below.

Sample/ Area	Bottlegourd			Brinjal			Cauliflower		
	%Ca	%Op	Re- sult	%Ca	%Op	Result	%Ca	%Op	Re- sult
Attariya	16.18	10.46	Green	32.88	24.76	Green	14.27	32.73	Green
Gulariya	14.33	19.85	Green	16.75	26.79	Green	16.89	24.43	Green
Shantipur	16.67	18.73	Green	12.54	19.83	Green	13.02	22.72	Green

Table 2. The vegetable Sample collected from different place in Attariya

The residue level of Ca and Op in the vegetable sample (Bottle Gourd, Brinjal, and Cauliflower) were collected from the three different places in Kailali

(Dhangadhi and Attariya town) district. The percentage of both pesticides was found below the minimum level where the highest percentage of Ca and Op is 32.88%(brinjal collected from Attariya) and 32.73%(cauliflower collected from Attariya) respectively as shown in table 1. In addition, the sample was collected from the same places for the vegetables such as Bitter Gourd, Cucumber, and Tomato (P.Ghimire, S. Baral, S.Sharma, 2020). The percentage residue of Ca was found below the low level of toxicity in all the places whereas the level of Op was found in the medium toxic zone i.e. 38.07% in the Shantipur area as shown in table 2. The percentage of Ca and Op of vegetables such as Cowpea, Cucumber, and Bitter Gourd collected from the Dhangadhi area. In three (Dehoriya, Campus road, and Buddha Chok) places the percentage of both pesticides was found below the minimum toxicity level as shown in table 3.

Sample/ Area	Bittergourd			Cucumber			Tomato		
	%Ca	%Op	Result	%Ca	%Op	Result	%Ca	%Op	Result
Attariya	12.35	12.42	Green	13.89	18.15	Green	0.27	3.80	Green
Gulariya	5.68	6.06	Green	2.44	25.98	Green	1.69	3.03	Green
Shantipur	1.58	11.11	Green	3.45	38.07	Yellow Quar-antine for 2 days	9.50	2.10	Green

Table 3. The vegetable sample collected from Attariya area

Sample/ area	Cowpea			Cucumber			Bitter gourd		
	%Ca	%Op	Result	%Ca	%Op	Result	%Ca	%Op	Result
Dehoriya	3.45	13.22	Green	14.22	18.17	Green	3.09	22.7	Green
Campus Road	2.22	5.55	Green	1.45	10.78	Green	1.58	11.11	Green
Buddha Chok	16.15	22.43	Green	13.89	18.15	Green	12.35	12.42	Green

Table 4. The vegetable samples are collected from the several part of Dhangadhi Town

The nine different vegetable samples collected from six different places were analyzed by a spectrophotometer which indicates two different colours such as green

and yellow. All the samples from different zone provided green colour except the sample collected from Shantipur for the cucumber vegetable, which contains Op pesticide with yellow colour. It is clear from this study that the level of Carbamate and organophosphate pesticide is in the non-toxic zone (low residue level) for all the vegetables while Op was found in the medium toxic zone for Cucumber only.

Conclusion

Pesticides, herbicides, and insecticides are considered to be the elixir in the modern farming system due to their capability to high yields of vegetation and long-term preservation. Pesticides like Ca and Op are the most commonly used in all sorts of vegetables, cereals, and fruits but they have some side effects for the consumer. This study includes the extraction of vegetable samples (Bottle Gourd, bitterer gourd, cucumber, brinjal, tomato, cowpea, and cauliflower) and then calculates the inhabitation percentage of pesticides with the help of rapid bioassay along with the spectrophotometric technique. The percentage of inhabitation obtained from the vegetable sample compared with the standard result is then concluded. In addition, all the samples produced green colour is having pesticide residue below the low level and yellow coloured sample falls under the moderate level of toxicity. Therefore, the moderate toxic vegetables are needed to quarantine for 2-3 days to reduce the content of the pesticide. There are no vegetables found in the market taking high percentage of pesticides. From this study, it is concluded that most of the vegetables in (Sudur paschim province) the market are non-toxic for the consumers.

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