Research Article

ANALYSIS OF PESTICIDE RESIDUE ON THE LEAVES AND CURDS OF CAULIFLOWER

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ABSTRACT

Pesticide residues on the cauliflower plant were investigated quantitatively to determine the persistent residue level of each pesticide at varying days after application with different frequency. Field experiments were conducted to evaluate the residue of four types of pesticide i.e. carbofuran, chlorpyrifos, cypermethrin and mancozeb at different observation period and frequency interval in curds and leaf of cauliflower grown in farmers field at Kirtipur, Kathmandu during November 20, 2019 to April 29, 2020. Residue analysis of each pesticide was done after three days of first application of each pesticide using kit method. The residue level of each pesticide was significantly reduced with the increase in the days after application. While it was found that pesticide residue was significantly commensurate with the frequency of application. There were significant difference in the leaf and curd of cauliflower regarding the persistent of each pesticide residue. Unsafe residue level of chlorfyrifos was found persisted for more time as compare to cypermethrin and mancozeb. Present study revealed that waiting period should be considered after the application of pesticide to have the safe consumption and if possible the frequency of application should be decreased unless it is required.

Key words: *Cauliflower, frequency, RBPR, residue, pesticide*

INTRODUCTION

Nepal possesses a great opportunity in the production and marketing of vegetable crops throughout the year. The productivity and quality of vegetables greatly depend on the level of insect pests attack. Pesticides that were widely used in agriculture to increase the yield, improve the quality, and extend the storage life of food crops (Fernndez and Reyes, 2008). So, farmers were frequently using broad-spectrum synthetic chemical insecticides to manage insect pests due to their quick knock-down effect on targeted insects, easy availability and relatively cheaper. In Nepal, cauliflower is to be considered as an important

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winter seasonal vegetable crop in terms of area covered, productivity and marketable value. Different number of insects pests like diamondback moth, cutworm, aphids, cabbage butterfly etc as well clubroot, damping off, leaf spots like diseases reduce the productivity and marketable quality of cauliflower as a result of which farmers used to apply different group of pesticide in order to get rid of the harmful insect pests and diseases. Cauliflower is consumed as raw and cooked vegetable; hence chances of carrying pesticide residues to the consumers are more (Raj *et al*., 1991). Hence Pesticide residues in cauliflower are of major concern to consumers due to their negative health effects. Although, after application of particular pesticides, farmers hardly care prescribed waiting period to harvest crop (Maharjan *et al*., 2004). As the farmers harvest the crops at close frequency, there might be toxic residues remained in vegetable produce which might have been causing toxic problems in human health, as the produce is being consumed immediately or within few days of harvest. Pesticides cause the acute and chronic health effects; organophosphate and carbamate groups are more important on this health effects. These insecticides inhibit cholinesterase, an enzyme critical for normal functioning of the nervous system (Soares and Porto, 2012). Carbosulfan, Dimethoate, Chlorpyrifos, Cypermethrin and Mancozeb are the major pesticides used in vegetable in-terms of their effectiveness and preference among farmers.

Although different chemical analysis method has been developing for pesticide residue analysis, an enzyme inhibition method is considered as an effective tool for the quantitative measurement of pesticide residues as the pressing demands of our economy force us to have a rapid method for timely monitoring of pesticide residues in vegetables. An enzyme inhibition procedure involves the measurement of the uninhibited activity of an enzyme which is the simple and fast method and doesn't require expensive apparatus. The effect of pesticides on the inhibition of an enzyme in a reaction system is dependent on their concentration and own chemical property. Also, PY and DTC kit method can only measure the level content of the pesticide residue in a sample, and it is unable to analyze individual pesticides (Kao, 2010 and PPD, 2017).

The study regarding the quantitative determination on residue level of different group of pesticide at different days of interval and frequency of their application on the vegetable like cauliflower under farmer practices in Nepal is still lacking. Although, residue levels are generally compared to maximum residual level (MRL) and average daily intake (ADI), nowadays availability of different test kit help in quantitative determination of different group of pesticide within short period of time and with ease. Therefore the study was carried out to determine the persistent residual level of different insecticide and fungicide in cauliflower curd and leaf after different days of interval and frequency of their application using the kit method.

MATERIALS AND METHODS

Field experiment

Field experiment were carried out with twelve plot for four different pesticide in which three plots with the size of 2x2 m were separated. Three frequency of each pesticide were sprayed at seven days interval at farmers field located at Kirtipur, Kathmandu during 20 November 2019 to 29 April 2020. Cauliflower variety Snow Mystique was planted at the rate of 20 seedlings per plot with a spacing of 45*45 cm. Agronomic practices were carried as recommended for cauliflower cultivation. First application of insecticide carbofuran 3%G (Encapsulated) @1 kg a.i/ ha, chlorpyrifos 20% EC@ 2ml/l, cypermethrin 10%EC @ 2ml/l and fungicide mancozeb 75% WP @ 2.5 gm/l on cauliflower plot was carried out at one week after curd setting takes place while other second and third application of pesticide were done at 7 days interval.

In order to avoid the drifting of pesticide from one plot to another while spraying the plant with pesticide, border plant is used as trap which were not used for analysis as well plastic sheet were placed in between plot as barrier and reducing the height between spray nozzle and application part.

Sample collection

Representing sample from each plot was collected after 2 days of application till the sample was found with no residue or safety level. Sample was collected in the polythene bags and tested on the same day of sample collection.

Sample analysis

The analysis of the sample collected for the tested pesticide was done in the Rapid Bioassay for Pesticide Residue Laboratory Unit, Kalimati, Kathmandu under the Research and Development program.

1. Carbamate and organophosphate group of insecticide residue assay

Sample preparation, sample processing and extraction was done to obtain analytical portions before visible deterioration occurs using RBPR kit method. Rapid bioassay for pesticide Residue analysis (RBPR) has also been adopted by the Republic of Korea, Vietnam, The Philippines, Panama and many Southeast Asian countries and 11 international RBPR training workshops were held during 1993-2010 (Kao *et al.*, 2010). Although not as reliable as GC-MS/ LC-MS, the RBPR is considered sensitive enough to meet the FAO–WHO regulations for pesticides in vegetables (Chiu *et al.*, 1991). This test assesses the toxicological effect of two common types of insecticides (carbamates and organophosphates) by measuring the percentage of inhibition of the acetylcholinesterase (AChE), a key enzyme in the nervous system of animals. More than 65% of the most dangerous pesticides (i.e WHO toxicity class I or II) used in the research area belongs to these categories (Bosch *et al.*, 2005).

Quantitative analysis of the pesticide residue using the kit method was done as described by Standard Operating Procedure for RBPR Laboratory (PPD, 2017).

Calculation of enzyme inhibition percentage

The reduction of absorbance for the sample to control (blank) test was compared and inhibition percentage of enzyme was calculated and grouped as follows:

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

Below 35%	Approved for sell and consumption as pesticide residue was found low.
35%-45%	Kept at Quarantine for minimum 2 days as presence of pesticide residue was found in considerable amount that may be degraded with holding for certain period. After the sample was hold for certain period, test was repeated and as the inhibition percentage was found less than 35%, then sample was allowed for sale.
Above 45%	It was not suggested for sale and consumption purpose since significantly high amount of pesticide residue was found in the sample. As a result, such sample were destroyed and dumped.

2. Synthetic pyrethroid group of insecticide residue assay

Sample extraction, Cleanup and testing was done as a procedure for quantitative determination of pyrethriod insecticide using PY test kit method as described in Standard operating Procedure for RBPR Laboratory (PPD, 2017).

Result was interpreted as described below comparing the color developed in the sample tube and blank test tube

Evaluation of color developed	Interpretation
Test samples that has blue color or close to blank	Not Finding (0)
The test sample that shows stronger blue color than blank up to deep purple	Finding Residue at safety level (1)
Test sample with no color	Finding Residue at Hazardous level(2)

3. Dithiocarbamate (DTC) fungicide residue analysis

In the procedure of residue determination of Mancozeb (Dithiobarcamate fungicide), extraction of sample and testing was carried using Dithiobarcamate, DTC test kit as described in Standard operating Procedure for RBPR Laboratory (PPD, 2017).

Comparison of color developed in the blank tube and sample extract tube evaluate the residue level as described below.

Evaluation of color developed	**Interpretation**
Test samples with no color/equal to blank	Not finding (0)
The test samples that show stronger blue color than blank up to deep purple	Finding dithiocarbamate at safety level (+1, +2)
The test samples that show grayish purple to green indicate finding residue at hazardous level.	Finding dithiocarbamate at hazardous level (+3)

Statistical analysis

The obtained data were analyzed using regression model and ANOVA by SPSS Software (IBM®, version 16).

RESULTS AND DISCUSSION

Residue level of Carbofuran and Chlorpyrifos

Residue of carbofuran could not be determined on both leaf and curd of cauliflower after three days of application during the period of study.

While in case of residue analysis of chlorpyrifos, it was found that increase in the time period of application decreases the insecticide residue level. The result showed that chlorpyrifos residue in curd of cauliflower at the safety level (level 1) was found at 10th days after last first spray while it was found at 18th and 28th days respectively after 2nd and 3rd spray. Similarly, it was clear from the Figure 1 that the residue of chlorpyrifos in cauliflower leaf at the safety level was achieved at 14th days, 24th and 33th days after 1st, 2nd and 3rd spay respectively.

Thus, the result suggests that the residue persistent in leaf would take more time to decrease than curd of cauliflower with the increase in the time period of last spray. Similarly, the study also found that increase in the frequency of chlorpyrifos application also increases the residue persistent. The residue level of chlorpyrifos in cauliflower curd decreased with the regression coefficient of -0.201, - 0.125 and -0.083 with the increase in time exposure from 3rd day to 33rd days after 1st, 2nd and 3rd spray respectively. While in case of leaf, residue level decreased with the regression coefficient of -0.162, -0.079 and -0.043 with the increase in days after application at 1st, 2nd and 3rd spray respectively (Fig. 1).

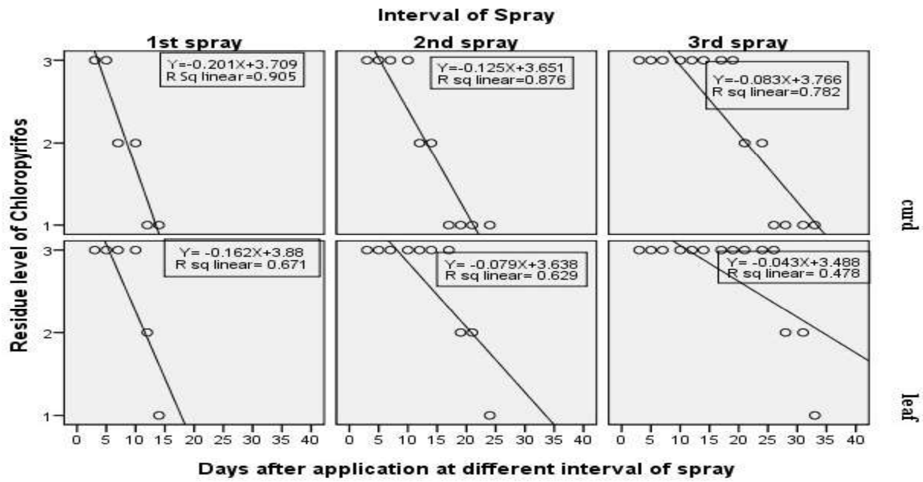

Fig. 1: Graph showing the relationship of insecticide residue level in curd and leaf of cauliflower with the observation period and frequency of application.

Residue level of Cypermethrin

From the result, it was found that with the increase in the days of cypermethrin insecticide application the residue level of cypermethrin in both leaf and curd was found decreasing. Although the residue level of cypermethrin on leaf of cauliflower was found for more days after application as compared to curd. It was clear from the Figure 2 that with the increase in the frequency of spray, increases the residue level.

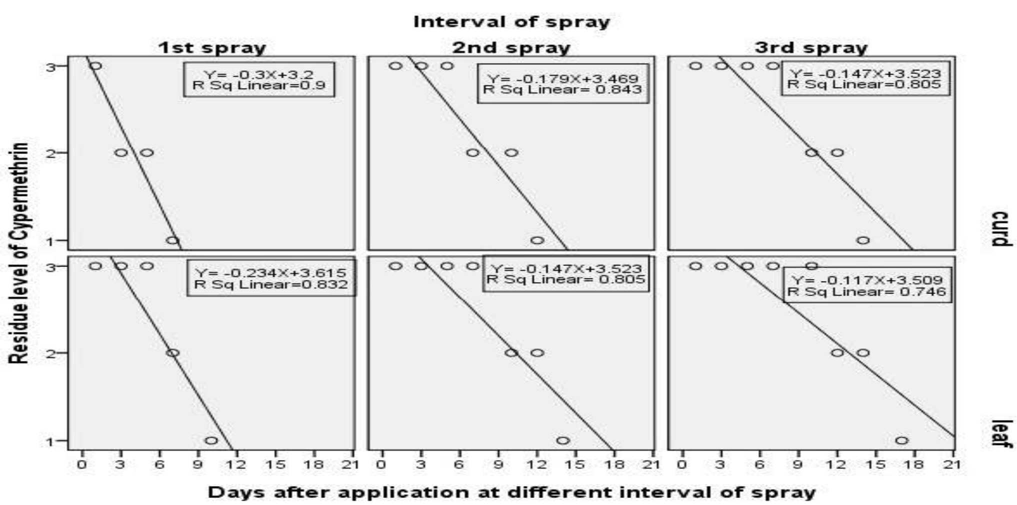

Fig. 2: Graph showing the relationship of residue level of cypermethrin in curd and leaf of cauliflower with the observation period and frequency of application.

The residue level of cypermethrin in cauliflower curd was found decreasing with the regression coefficient of -0.3, -0.179 and -0.147 with the increase in time exposure from 1st day to 14th days after 1st, 2nd and 3rd spray respectively. Similarly, it was found that the residue level of cypermethrin in leaf of cauliflower decrease with the regression coefficient of -0.234, -0.147 and -0.117 after 1st, 2nd and 3rd spray respectively (Fig. 2).

Residue level of Mancozeb fungicide

During the analysis of mancozeb residue level, it was found that with the increase in time exposure the residue level of mancozeb was found decreasing in curd with the regression coefficient of -0.250, -0.150 and -0.098 while in leaf with regression coefficient of -0.250, -0.098 and -0.48 after 1[st], 2[nd] and 3[rd] spray respectively (Fig. 3).

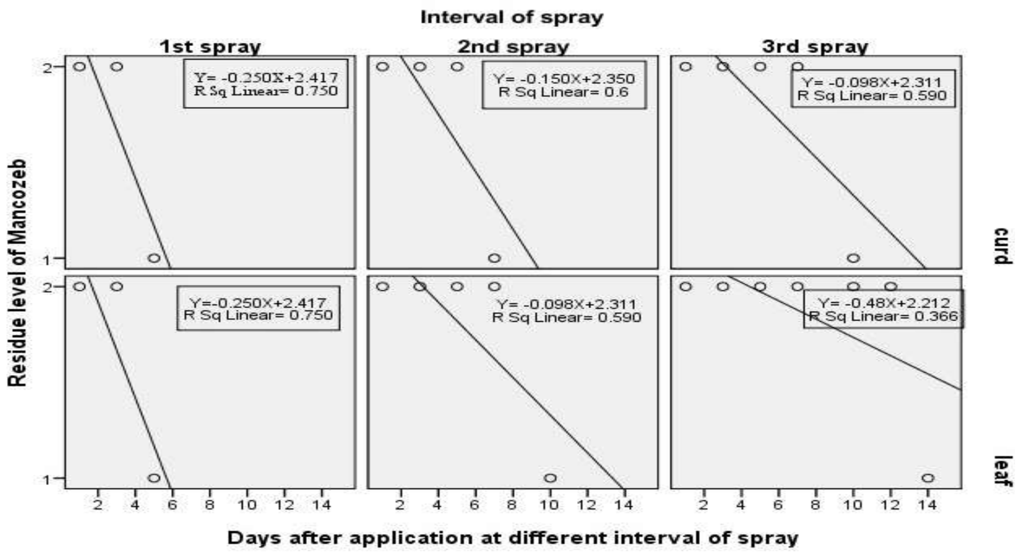

Fig. 3: Graph showing the relationship of residue level of Mancozeb in curd and leaf of cauliflower with the observation period and frequency of application.

From the Figure 3, it was found that increase in the spray frequency is directly proportionate with the residue level of mancozeb.

During the data analysis, it was found that the overall in multiple regression model, all the predictor i.e. frequency of spray, days after application and plant parts (leaf, curd) accounts significant unique amount of variance in the residue analysis of chlorpyrifos,cypermethrin and mancozeb. From the result of the experiment, the unsafe residue level of chlorpyrifos was found persisted for more time as compare to cypermethrin and mancozeb. Similarly decrease in the residue to safety level of mancozeb was found within short time period as compare to chlorpyrifos and cypermethrin.

Haque and Freed (1974) found that most of the organic pesticides decompose in the presence of sunlight or ultraviolet radiation. Adsorption also reduces the biological activity and chemical degradation of carbofuran in soil (Ogawa *et al.*, 1976), hence the residue is retained for longer in soil. Photodecomposition studies in the laboratory confirmed more rapid degradation in the field, giving a half-lives for carbosulfan and carbofuran of 1.4 hours and 3.2 days, respectively (Tejada, 1983). The maximum conversion of carbosulfan to carbofuran occurred one day after spray application with the residue level declining thereafter (Varca and Tejada, 1998). These different studies support the result of unidentified residue of carbofuran during the study period.

Tran Van An *et al*. (1998) suggested that the samples above 43% inhibition contained residues above MRL and hence those samples with less than 20% inhibition can be used for marketing and those with > 50% should be advised to extend the interval between the pesticide application and harvest of the vegetable which support the result with the assumption of achieving the safe level of pesticide residue that is safe for consumption with the extension of time interval.

The residue level of different pesticide on cauliflower was found decreasing with the increasing time period of their last application is supported by the study conducted by Paneru *et al*. (2012).The residual level of same pesticide was found different in leaf and curd of cauliflower, which is consistence with the finding of Akbar *et al*. (2020). This can be explained by differences in surfaces which received these insecticides. Especially the surface area and surface characteristics e.g. presence or absence of wax and spines affect the amount of insecticide which retain after spray (Freeman and Beattie, 2008).Carbofuran residue was concentrated on the leaves, followed by the stems, and was least on the grains (Varca and Tejada, 1998).

The residue level of chlorpyrifos was found on safety level at 14-33 days after its last application at different frequency of spray, that of cypermethrin was found at 7–17 days after its last application and residue level of mancozeb at safety level after its application was found at 5-14 days. This finding was supported by the information about the waiting period of different pesticide on different crop published from Plant Quarantine and Pesticide Regulation Center, 2018. Chlorpyrifos residues on rice leaves were detectable at up to the 15th day after application (Varca and Tejada, 1998).

CONCLUSION

Pesticide Residue analysis on the vegetable crops is an alarming issue for the assurance of food safety to the consumers. The study reveals that the residue of chlorpyrifos persist for longer duration as compared to mancozeb and cypermethrin, as well persistent of pesticide differ on the parts of the crops sprayed. It was concluded that the waiting period varies for different pesticide applied and edible parts of the crops intended to harvest. The study affirms that increase in the frequency of pesticide application, increase the residue persistent in the crops applied. It is recommended for farmers to consider the waiting periods before harvesting crops after application of pesticides. Farmers should be able to choose the pesticide that has less waiting periods and should reduce the frequency of application.

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