RESIDUE ANALYSIS OF SOME SYNTHETIC CHEMICAL INSECTICIDES ON CAULIFLOWER CURDS IN KHUMALTAR, LALITPUR, NEPAL

Ram B Paneru, Sunil Aryal and Yagya P Giri
Entomology Division, Khumaltar, Lalitpur

ABSTRACT

Toxic residue level of chemical insecticides in cauliflower curds treated with dichlorovos (Nuvan 76% EC), endosulfan (Thiodane 35% EC), fenvalerate (Fen-fen 10% EC), and cypermethrin (Super Killer 10% EC) was analyzed through gas-chromatography (GC) in the Entomology Division during 2008/09. The cauliflower plants at curd formation stage were treated with four consequent sprays (at 12 days interval) of above insecticides each using recommended dose. The curd samples were taken for the analysis from the treated plots at 3, 7, 15 and 25 day after last treatment. Standard procedures were followed for residue extraction, purification and concentration of targeted insecticides for analysis through GC. The analysis showed that the residual level of dichlorovos, endosulfan, fenvalerate and cypermethrin varied from 0.0027 to 0.0004, 1.3918 to 0.0096, 4.8292 to 0.8585 and 2.7401 to 0.1650 ppm respectively from 3 to 25 days. Residue of Nuvan was < maximum residue limit (MRL) at 24 hours of its application, and it was < average daily intake (ADI) level at 3 days onward. The residue of Thiodane was < MRL in all days but it was > ADI up to 25 days. Likewise, residue of Fen-fen was > its MRL and ADI level up to 25 days. The residue of cypermethrin was > MRl level up to 3 days and > its ADI value up to 25 days. The analyzed data indicated that the consumption of cauliflower treated by Thiodane, Fen-fen and Superkiller is not safe up to 25 days if four consequent sprays of these insecticides are given. Amongst the insecticides, rate of degradation of Nuvan was the highest, and that of Fen-fen was the least. Residual level of insecticides in the curds of commercial production areas could be much more than this because farmers used to apply such insecticides frequently in higher doses than recommended, and give little attention to waiting period for harvesting after application of insecticides.

Key words: Cauliflower curds, Degradation, Insecticide, Residue, Toxicity

INTRODUCTION

General public is becoming increasingly concerned in the recent days with the use of chemical pesticides in vegetable crops in the sense that chemical pesticides do have several ill effects on human health and environment. The use of chemical pesticides, however, debatable, has been a necessary tool for the control of insect pests and will continue to be so for some time till the farmers will be assured of an effective and realistic alternative method. The only concern at present context is to suggest the use of safer and less toxic insecticides on the concept of as and when needed basis while taking necessary safety precautions. Majority of farmers so far have been using insecticides and have considered it as the only means of insect pest management. In summer vegetables, pesticides are applied 6 – 25 times and sometimes even more than 25 times particularly in egg plant and tomato. Majority farmers apply 11 – 25 times in a crop period. In winter vegetables, majority of farmers are applying pesticides 6 – 15 times especially in cabbage whereas few of them apply even up to 20 times (Giri *et.al.* 2005). The case of misuse, overuse and improper use of pesticides is commonly observed in field situations. In recent years, the practice of cocktail spray is getting popular. Over dose and frequent application of insecticides has a direct impact on degree of insecticide residue problem.

Major chemical insecticides used are dichlorovos (Nuvan 76 % EC), dimethoate (Rogor 30 % EC), cypermethrin (Super killer 10 % EC), fenvalerate (Fen-fen) and chlorpyriphos (Dursban 20% EC). It is observed that farmers do not hesitate to use synthetic chemical insecticides due to its quick knock down effect on targeted insects, easy availability and relatively cheapness nature. And, they hardly care waiting period to harvest crop after application of insecticides as prescribed period for particular insecticide. There are enough reasons to believe the fact that there might be certain level of toxic residues on the produce the farmers harvest at close frequency. The frequent use of chemical insecticides on vegetable crops can cause

severe problem of toxic residue on vegetables as the produce is used immediately or within few days of harvest. Human body can tolerate only a certain level of toxicity of different pesticides that is up to tolerance limits. The quantitative information regarding residue level of chemical insecticides to be remained in the insecticide treated vegetables at different days after application in particular crop in Nepalese farm conditions is lacking. With this background, an experiment was carried out with an objective to study the persistence of dichlorovos, endosulfan, fenvalerate, cypermethrin in cauliflower curds at different days of their application.

METHODOLOGY

Cauliflower (var. Kathmandu Local) was grown at Entomology Division premises at Khumaltar, Lalitpur during January – June, 2009 in order to collect and analyze the insecticide treated curd samples. The cauliflower was planted in 15 plots with each plot of an area of 3 m length x 2 m breadth. Every plot was set at 1.5 meter apart from each other. A total of 20 seedlings were planted in each plot with a spacing of 70 cm RR x 40 cm PP. At curd formation stage, cauliflower plants were treated with four consequent sprays of four insecticides i.e. dichlorovos (Nuvan 76% EC), endosulfan (Thiodane 35% EC), fenvalerate (Fen-fen 10% EC), cypermethrin (Super killer 10% EC) at recommended dose together with sole water spray as control treatment. There were five treatments with three replications in randomized complete block design. The cauliflower plants were treated at 12 days interval. During treatment period, a plastic sheet barrier of 2 meter height was placed in between two plots to avoid spray drift from one another. The insecticide application was usually repeated next day in the case of heavy rainfall immediately after treatment. The curd samples were collected at 3, 7, 15 and 25 days after last treatment for the analysis. The treatments and their dose were as followings;

T1: dichlorovos (Nuvan 76% EC) @ 1 ml / L water
T2: endosulfan (Thiodane 35% EC) @ 1.5 ml / L water
T3: fenvalerate (Fen-fen 10% EC), @ 1 ml / L water
T4: cypermethrin (Super killer 10% EC), @1 ml / L water
T5: Control (Water spray)

Sample Collection

The sample were collected and prepared on 1st, 3rd, 7th and 15th and 25th day after application of the insecticides. 1st day sample was taken only from Nuvan treated plots. Altogether 3 curds were randomly harvested once from each treatment @ 1 curd per plot as a cauliflower sample. The samples were kept in polythene bag and then stored for few days in the refrigerator (at 4°C) until initiation of process of extracting insecticide residues from sample for analysis.

Sample Preparation

As in the normal pesticide extraction process, three separate methods such as insecticide residue extraction, purification and concentration process were used for sample preparation for gas-chromatography analysis as suggested by KK Sharma, ICAR, New Delhi, in Pesticide Residue Analysis Manual (June, 2007 ISBN 81-7164-064-8). The sample was analyzed through Gas-chromatography to determine residue level of targeted insecticides.

The procedure followed for sample preparation for analysis of fenvalerate, cypermethrin and dichlorovos was similar. The fenvalerate treated samples of particular date were separately chopped into small pieces. The chopped pieces of individual treatment were mixed thoroughly to make homogeneous sample. A fifty gram of homogenous sample was then dipped into 50 ml of acetone HPLC grade and blended into high-speed blender jar for 1 minute. The mixture was filtered with suction through sintered glass filter. The volume of filtrate was concentrated into 5 ml, and it was mixed with 100-ml of 10% NaCl solution and the mixture was transferred into a 1000 ml separatary funnel, in which, 50 ml of dichloromethane (DCM) added, and then the mixture was vigorously shaken for 1 minute and then the mixture was allowed to separated into layers. The lower layer (DCM layer) was collected into conical flask after passing it through an-hydrous sodium sulphate powder filled on cotton plugged glass funnel. The upper layer (saltwater layer) was further separated adding 30 ml of DCM with it and shaking vigorously and allowing separating layers. Similarly, salt layer was further washed with 20 ml of DCM. In both cases the lower DCM layer was passed through sodium sulphate powder. The

salt layer was discarded and the solvent layer was concentrated using rotatory evaporator and the volume of sample was adjusted up to 10 ml of mark volume with 1:9 Acetone-Hexane. The process was same for the control samples and those other dates. Similar procedure was followed for all samples treated with cypermethrin and dichlorovos i.e., Sample extracted with Acetone, partitioned with DCM, filtered with an-hydrous sodium sulphate powder, and concentrated at required mark of volume for sample preparation.

The procedure followed for sample preparation for the analysis of endosulfan was slightly different than that of fenvalerate, cypermethrin and dichlorvos. Endosulfan treated cauliflower sample of particular date was chopped into small pieces and they were thoroughly mixed for making homogeneous sample. A fifty gram of homogenous sample was then dipped into 50 ml of Acetone HPLC grade and blended into high-speed blender jar for 1 minute. The mixture was filtered with suction pump through sintered glass filter. The volume of filtrate was concentrated into 5 ml using rotatory evaporator, and it was mixed with 100-ml of 10% NaCl solution and the mixture was transferred into a 1000 cc separatary funnel, in which, 50 ml of Hexane HPLC grade added, and then the mixture was vigorously shaken for 1 minute and then the mixture was allowed to separated into layers. The upper layer (Hexane layer) was collected into a conical flask after passing it through an-hydrous sodium sulphate filled on cotton plugged glass funnel. The lower layer (salt-water layer) was further separated adding 30 ml of hexane with it and shaking vigorously and allowed separating layers. Similarly, salt layer was further washed with 20 ml of hexane. In all cases the upper hexane layer was passed through sodium sulphate powder. The salt layer was discarded and the solvent layer was concentrated using rotatory evaporator and the volume of sample was adjusted up to 10 ml of mark volume with hexane. The process was same for the control samples and those other dates. Similarly, all the endosulfan treated cauliflower were extracted with Acetone, partitioned with Hexane, filtered with an-hydrous sodium sulphate, and concentrated at required mark of volume for sample preparation.

,Chemicals, Reagents and Analytical Conditions

All the solvents used for the sample extraction and purification during preparation of sample for analysis was of the HPLC grade. Some chemicals such as sodium chloride and sodium sulphate were of analytical reagent grade.

All the samples prepared through extraction, purification and concentration as mentioned on above procedures were analyzed through gas chromatographic method and the residue level of insecticides in each sample was estimated. The general analytical conditions in the GC applied for the detection and quantification are as followed;

Table 1. Analytical Condition used for Insecticide Residue Analysis through GC

Descriptions	Analytical Conditions			
	Endosulfan	Cpermethrin	Fenvalerate	Dichlorovos
Instrument	GC	GC	GC	GC
Model	GC-1000	GC-1000	GC-1000	GC-1000
Detector	ECD	ECD	ECD	TID
Column	BPx5	BPx5	BPx5	BPx50
Injector Temperature	250 °C	250 °C	250 °C	200 °C
Detector Temperature	300 °C	300 °C	300 °C	300 °C
Column Temperature	220 °C	240 °C	240 °C	150 °C
Volume injected	1 µl	1 µl	1 µl	1 µl

Standardization of Sample Extraction and Analysis Procedure

To ascertain the accuracy of the produced data, analysis of the spike samples were also carried out during this study. Known quantity of analytes were added into the sample or control sample and then treated and analyzed following same analytical procedures as applied for the sample analysis. The obtained residue quantity was then compared with the added quantity of each analyte to assess the recovery performance of the method.

RESULTS AND DISCUSSION

The analysis of spiked samples and control samples indicated that the procedures adopted were accurate and error free. The recovery tests carried out depicts that very good recovery was obtained for all analytical components. The recovery percentage was found to be varied from 82.9 – 92.6, which seemed quite satisfactory.

Table 2 shows mean value of insecticide residue level (in ppm) detected on 1, 3, 7, 15 and 25 days of their application in cauliflower. The residue level of test samples revealed that the toxic residue is decreased with increasing time period of insecticide application. The prepared control sample is also analyzed along with the samples to identify the possible levels of contamination that may occur either from the solvents and reagents or from the workplace environment. Pesticide residue is not detected from the respective control samples. Table 3 shows the maximum residue level and acceptable daily intake level for dichlorovos, endosulfan, cypermethrin and fenvalerate in cauliflower curds. The detail is presented below.

Table 2: Mean value of pesticide residues level (ppm) in cauliflower curd treated with different insecticides at different day's interval in 065/66

Treatments	Mean value of pesticides residues level in cauliflower (in ppm) (n = 3)				
	1st Day	3rd Day	7th Day	15th Day	25th Day
Dichlorovos (Nuvan 76%)	0.0249	0.0027±0.001	0.0004±0.000		
Endosulfan (Thiodane 35% EC)	NA	1.3918±0.05	1.1239±0.066	0.1854±0.02	0.0096±0.0009
Fenvalerate Fen-fen 10% EC)		4.8292±0.11	2.4785±0.127	2.1677±0.082	0.8585±0.088
Cypermethrin (Superkiller 10% EC)	NA	2.7401±0.37	1.3099±0.10	0.3835±0.018	0.1650±0.015
Control	NA	ND	ND	ND	ND

ND: Not Detected, NA: Not Analyzed

Table 3: Maximum residue level (MRL) and acceptable daily intake (ADI) level of insecticides in cauliflower /Vegetables

	Dichlorovos	Endosulfan	Cypermethrin	Fenvalerate
MRL in mg/ kg	0.15	2.0	2.0	2.0
ADI (mg/kg body weight/day)	0.004	0.006	0.05	0.02

Source: Pesticide Residue Analysis Manual (June, 2007 ISBN 81-7164-064-8) by KK Sharma, ICAR, New Delhi

The analysis revealed that the residue level of dichlorovos is found varied from 0.0249 to 0.0004 ppm from 1 to 7 days of its application. The higher level of residues is observed in the sample collected after 24 hours of dichlorovos application and then the level of residue is continually decreased to 0.0004 ppm in 7 days. The residue level is found lower than its maximum residue limit (MRL) after 24 hours. But residue level is higher than its acceptable daily intake (ADI) level till 3 days. From the analyzed data it can be concluded that the cauliflower curds treated with dichlorvos is safe for consumption after 3 days only. Likewise, the analysis revealed that the residue levels of endosulfan are found varied from 1.3918 to 0.0096 ppm from 3 to 25 days of its application. The higher level of residues is observed in the sample collected at 3 days of treatment and the lowest level in the sample collected at 25 days. The data revealed that all the residue levels are found lower than its MRL, and higher than its ADI level. The residue level of fenvalerate is 4.8292 ppm at 3.days of treatment, then residue level is decreased to 0.8585 ppm at 25 days. The residue level is found higher than its MRL up to 15 days and also higher than its ADI level up to 25 days. Similarly, the analysis showed that the residue levels of cypermethrin is found varied from 2.7401 to 0.1650 ppm from 3 to 25 days of its application. The residue level at 3 days onward is found lower than its MRL, and higher than its ADI level up to 25 days of its application. It indicated that the cauliflower curds treated with Thiodane, Fen-fen and Super killer are not safe for consumption up to 25 days of their application.

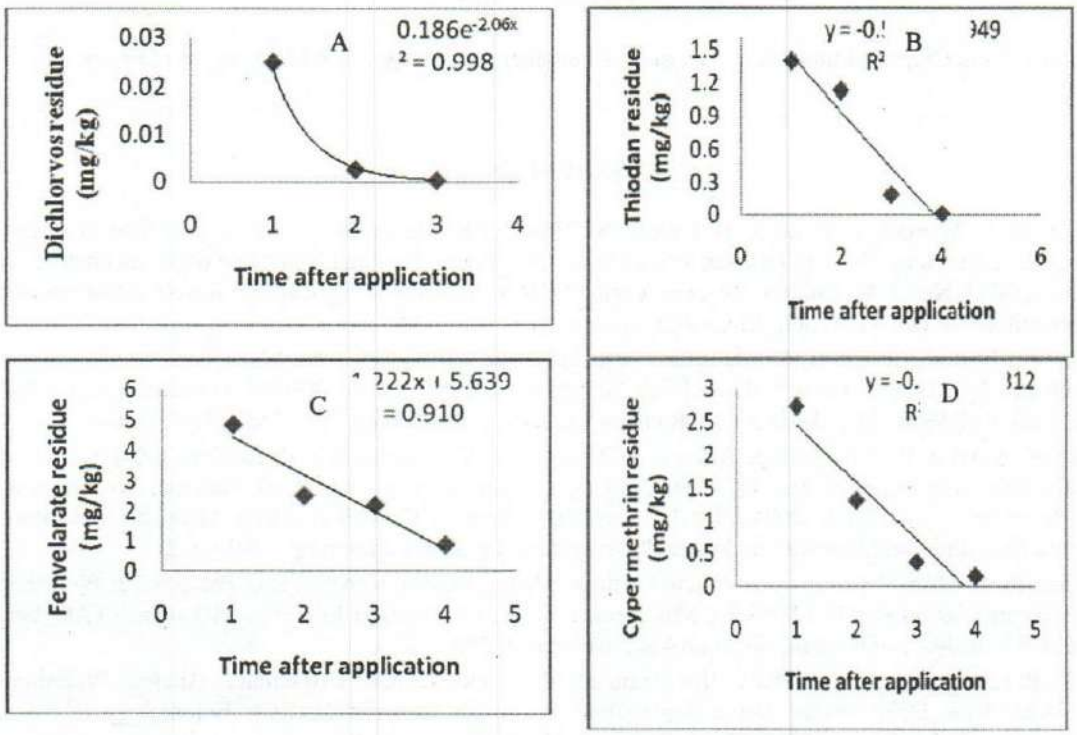

Figure 1 Regression curve between residue level and time after application (A-Exponential regression of dichlorvos with exposure to time; B, C and D are linear regression of endosulan, fenvalerate and cypermethrin respectively with exposure to time).

Figure 1 show regression curve between residue level of dichlorvos, endosulfan, fenvalerate and cypermethrin and time exposure from pesticide application. The number of X axis in A, denotes 1 = 1 Day, 2 = 3 Day and 3 = 7 Day, similarly the number of X axis in B, C & D denote 1 = 3 Day, 2 = 7 Day, 3 = 15 Day & 4 = 25 Day. Residue of the dichlorvos is found decreasing with exponential regression coefficient of $0.186e^{-206x}$ with time exposure from 1day to 7th days of application. Similarly, residue of the endosulfan, fenvalerate and cypermethrin is found decreasing respectively with the regression coefficient of - 0.508, -1.22 and -0.865 with time exposure from 3rd day to 25th days of pesticide application. The regression equation for dichlorovos, endosulfan, fenvalerate and cypermethrin has described 99%, 92%, 91% and 91 % of the variability in the data respectively. With the increasing time period of application the residue level of above insecticides is continually decreased. Fenvalerate is found to be the least degradable as compared to cypermethrin, endosulfan and dichlorovos.

Above findings are based upon the four consequent spray of pesticides at recommended dose which is not common for commercial vegetable farming condition of Nepal. Generally, the farmers used to spray pesticides more frequently and in higher doses than that of this study. Majority farmers of commercial vegetable growing area of hill conditions used to spray pesticides 6 – 15 times at higher doses than recommendations in a crop cycle. The literature revealed that farmers are not aware of waiting period for harvest after pesticide application due to various reasons for not maintaining waiting period, such as they had no idea about waiting period, problem in marketing, no need to wait because that was for selling, they have applied safe pesticides on crop, they do not care for waiting period. Even the day after application of pesticides, the crop is some time harvested for sale. That is why; use of these pesticides on vegetable crops could be detrimental to consumers' health in Nepalese condition.

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