Diazinon and Dursban residue in Soil at Different Applied Doses and Response of Cabbage at Different growth stages

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Submission: August 13, 2017; Published: September 06, 2017

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Abstract

An experiment was conducted at Experimental Field of BCSIR, Dhaka during winter season 2008 to assess the responses and residual effects of different doses of applied Diazinon and Dursban on cabbage cultivation at different growth stages. During experiment five treatments with three replications of two pesticides were applied in the experimental field. The plot size was 2m X 2m and total plots were 45 for the above mentioned treatments. According to experimental procedure, at early growth stage after application of two pesticides, the growth of Cabbage was enhanced but at middle and mature growth stages were slowed down the growth. At varying growth stages after spraying of both pesticides, nutrient uptake were increased and growth was simply enhanced, at that time the concentration of different doses of Diazinon ranges from 2.159 μg l-1 to 9.284 μg l-1 (Diazinon doses: 1.50 L/ha, 5.00 L/ha, 8.00 L/ha and 12.00 L/ha); in second stage it ranges 2.823 μg l-1 to 12.140 μg l-1 and at mature stage the concentration ranges from 2.936 μg l-1 to 12.626 μg l-1 at the respective applied doses, which is statistically significant at 0.001 level (t > 12.941). On the other hand, the residual effects of Diazinon at different doses at different time line were found, which were ranges from 2.767 μg l-1 to 7.284 μg l-1 at varying time and doses of application, which is statistically significant at 0.001 level (t > 12.941). Again, about Dursban application at different doses, where the responses of uptake by Cabbage ranges from 0.373 μg l-1 to 5.715 μg l-1 at rate of 0.50 L/ha; 1.00 L/ha; 2.00 L/ha and 4.00 L/ha throughout the growth stages, which is statistically significant at 0.004 level (t > 3.182). On the other hand, the residual effects of Dursban at different doses at different time line were found, which were ranges from 0.799 μg l-1 to 5.818 μg l-1 at varying time and doses of application, which is statistically significant at 0.001 level (t > 12.941). The intangible of this experiment concluded that there was a positive response of plant uptake and residual effect on soil clearly occurs in both cases.

Keywords: Pesticide; Diazinon; Dursban; Cabbage; Residual effect and Plant uptake

Introduction

Cabbage is an important and nutritious winter leafy vegetable and is widely grown in Bangladesh mainly in Robi season. It contains a range of essential vitamins, ascorbic acid and minerals as well as small amount of protein and good caloric value Haque et al. [1]. In recent years, vegetable consumption has been increased in our country. However, the productivity of Cabbage per unit area is quite low as compared to developed countries of the world FAO [2]. The response of Cabbage is high to nitrogen application and moderate to phosphorus application Mallik & Charya [3]; Vice & Polach [4]. For the higher productivity, pesticides are also other major agro-chemicals that controlling pest to destroy or decaying the vegetable growth of Cabbage but unfortunately, the application of pesticides are heavily sprayed on Cabbage field as this vegetable is more prone to pest infestation. But indiscriminate use of pesticides on vegetables are considered to be a serious health hazard to human as the residues and it also affect the yield and mineral content of Cabbage Reddy et al. [5].

Organophosphorus (OP) and organochlorine (OC) pesticides are widely used in agriculture as insecticides and leave residues to varying extents in agricultural produce such as vegetables and fruits. Due to their toxic properties and potential risk to consumers, their residues in food commodities is an issue of public concern and controlled by legislation Dimitra [6]. Indiscriminate application of inorganic and organic pesticides has led to an accumulation of heavy metal and metalloid residues in many agricultural soils, dramatically reducing agricultural productivity. Soils with low levels of trace elements are frequently used for vegetable growing; accumulation of these trace elements in the edible portion of these crops can occurred and pose significant health risks once entered into the human food chain Meagher [7]; Moreno et al. [8].

The sources of these elements vary and the propensity for plants to accumulate and translocate them to edible and harvested parts depends to a large extent upon plant genotype;
Materials and Methods

Location of Experimental site

An experiment was conducted at Experimental Field of BCSIR, Dhaka during winter season 2008. The soil of BCSIR is belongs to Tajgaon Series, and there was no pesticide concentration found before after analysis. In this experiment *Brassica Olearis* L. variety of Cabbage was used.

Experimental Design

The total plot size was 12 m X 24 m which required 45 small unit plots. The per unit plot size was 2 m X 2 m which accommodated 16 plants. The experiment was carried out in a randomized Block Design (RBD) with three replications for Diazinon and Dursban pesticides. All plots were treated with basal Fertilizers for supplying plant nutrition. These fertilizers were applied during land preparation and as per standard procedures. The doses of Diazinon and Dursban pesticides were sprayed on and around plants were 1.50 L/ha, 5.00 L/ha, 8.00 L/ha and 12.00 L/ha for Diazinon and 0.50 L/ha; 1.00 L/ha; 2.00 L/ha and 4.00 L/ha for Dursban in a respective rates. Thus the different intercultural operations were applied whenever necessary.

Collection, Preparation and Storage of Soil and Plant Samples

The time of soil and plant samples were collected from experimental sites or plots due to 6 hours later after different doses of Diazinon and Dursban applied to the field then second sampling was done 30 days after first sampling and third or last sampling was done 45 days after second sampling. After each sampling time, soil and plants brought back to the Analytical Laboratory, Department of Soil, Water and Environment, University of Dhaka. The samples were taken into sun light exposing and contamination or alteration of organic properties. The blended Cabbage sample (75 g) was cut into small pieces and homogenized by means of a kitchen blender and kept in a protected non-polythene bag with well labeled to prevent contamination or alteration of organic properties. Soil samples were collected at the depth of 15 cm from surface.

Reagents

The organic solvents, acetonitrile, ethyl acetate used were HPLC grade and were purchased from E. Merck. Technical grade pesticide standards were obtained from Center for Advanced Research, University of Dhaka with a purity of 95-99%. The standards were stored in a freezer at -5°C. Ultra high quality water was obtained from Milli-Q water purification system (Millipore, Bedford, MA, USA). Milli-Q Water and acetonitrile were degassed by vacuum suction. All samples and solvents were filtered through Millipore membrane filters (Polysulfone membrane and 0.45 μm pore size) before injection on the column. Anhydrous sodium sulphate for residue analysis, 12-60 mesh, was maintained at 300°C overnight. A source of pure nitrogen was used for evaporation to dryness in the extraction step.

Standard Preparation

For preparation of stock solution, standards were dissolved in acetonitrile and four levels of intermediate standard solutions of each pesticide were prepared maintaining the same matrix concentration for the preparation of calibration curve and stored at 4°C in the dark. Working solutions were prepared daily by appropriate dilution with acetonitrile.

Sample Preparation

After brought to the laboratory, soil and plant samples were weighted in a field moisture condition and then kept them to air dry. Then they were mashed into 2 mm sized grain and they were subjected to analyses.

Extraction

Triturate a sample of 25 gm, with Sodium Sulphate to dry, powdery mixture, with the aid of an extraction thimble; extract the mixture exhaustively with Petroleum Ether in Soxhlet apparatus. Concentrate just to dryness the extract solution by a concentrator and dilute to 25 ml with Petroleum Ether saturated with Dimethylformamide Peter & Zeumer [12]. Edible part of each vegetable sample (75 g) was cut into small pieces and homogenized by means of a kitchen blender and kept in a freezer by wrapping with clean airtight polythene bag (zip lock) at temperature below -15°C. The blended Cabbage sample (75 g) was mixed with anhydrous sodium sulphate (50 g) and extracted with ethyl acetate Islam et al. [13] in a 200 mL conical flask using an Ultra-Turrax (IKA-WERK) for 4-5 min.

The content was allowed to settle down for about half an hour and the ethyl acetate extract was then filtered through a Buchner-funnel fitted with a filter paper covered by 20 g of anhydrous sodium sulphate. After filtration, the extract was evaporated to dryness and re-dissolved in 5 mL of acetonitrile (MeCN) and finally the volume was made up to 2 mL using rotary vacuum evaporator. The extract was then transferred to a graduated test tube and the final volume was adjusted at exactly 2 mL by adding a few drops of acetonitrile. Solutions were then centrifuged and filtered. The clean organic layers were taken and were analyzed by a high performance liquid chromatography having UV/visible detector Mendham et al. [14], De [15].

HPLC Systems

A Shimadzu SCL-10A VP, Version 5.22 High performance liquid chromatography having UV/visible detector was used for identification and quantification of pesticides. Separation was...
Identification and Quantification

The compound was identified by comparing its retention time with respect to technical grade reference standard. The quantitative determination was carried out with the help of a calibration curve drawn from chromatographic experiments with standard solution. For quantification an external calibration curve with four different concentrations of each pesticide, with matrix matching were made. The standard solutions for the calibration curves were prepared in control matrix because samples may possess co extractants in the matrix which may affect the peak area of the unknown samples Mendham et al. [14].

Table 2: Amounts of residues detected in cauliflower samples treated with the respective pesticide.

| Dose             | Diazinon | Dursban |
|------------------|----------|---------|
| Recommended dose | 1.085    | 1.628   |
| Double of the    | 1.64     | 2.243   |

ND = Not detected i.e., below detection level of 0.02 mg kg⁻¹

Recovery

Recovery studies were performed to examine the efficacy of extraction and clean up. Untreated cauliflowers were spiked with known concentration of the pure pesticides standard solution of each type of pesticide and extraction and clean-up were performed as described earlier. The concentration of each pesticide in the final extracts was calculated Mendham et al. [14].

Statistical Analysis

The response and residue results were the means from three replicates of each treatment and all data’s were analyzed using descriptive statistics such as regression and correlation using SPSS version 12 for windows.

Result and Discussion

Limit of Detection (LOD) was calculated from the peak intensity at 0.1 mg kg⁻¹ and blank levels in recovery tests. LOD was defined as S/N > 4 so that it is in the linear range of the standard calibration. The LOD of Diazinon and Dursban was 0.02 mg kg⁻¹. Recoveries which were obtained by triplicate analysis of cauliflower sample spiked with each type of pesticide at one fortification level were satisfactory for response and residue analysis and are of the same order as those obtained by using more complicated methodologies. The percent recoveries for Diazinon and Dursban were 106.0 and 81.7, respectively. Residues were corrected according to the average of recovery. Linear calibration curves were found between peak areas and analyte concentration in the whole range studied. The linear regression (y = ax + bx) parameters for method calibration are shown in Table 1. The determination coefficients (R²) of analytical curves were near 0.99, with linearity for each compound, which allows the quantitation of these compounds by the method of external standardization.

Diazinon and Dursban were detected in all samples (Table 2). According to MRL Status Report [16], it is found that Diazinon was detected above Maximum Residue Limit (0.01 µg kg⁻¹ of sample) in the samples where Diazinon was sprayed at the recommended dose then double of the recommended dose and higher on. The amount of the residues of Diazinon detected at 1st sampling time were 2.768 µg l⁻¹ for 1.50 L/ha application whereas the Diazinon content in 1.50 L/ha was detected directly 6.59 µg l⁻¹, 6.455 µg l⁻¹ for 5.00 L/ha application whereas in this application rate was detected directly 15.37 µg l⁻¹; for 8.00 L/ha application, the residual effect was found 8.694 µg l⁻¹ whereas 20.70 µg l⁻¹ detected and 11.903 µl⁻¹ residual content was found at 12.00 L/ha application whereas in this application rate was detected 28.34 µg l⁻¹ (Figure 1). The amount of the residues of Diazinon detected at 2nd sampling time were 1.882 µg l⁻¹ for 1.50 L/ha application; 4.390 µg l⁻¹ for 5.00 L/ha application; for 8.00 L/ha application, the residual effect was found 5.912 µg l⁻¹ and 8.094 µg l⁻¹ residual content was found at 12.00 L/ha application (Figure 1).
The amount of the residues of Diazinon detected at 3rd sampling time were 1.694 µg l⁻¹ for 1.50 L/ha application; 3.951 µg l⁻¹ for 5.00 L/ha application; for 8.00 L/ha application, the residual effect was found 5.321 µg l⁻¹ and 7.285 µg l⁻¹ residual content was found at 12.00 L/ha application (Figure 1). The amount of the uptake response of Diazinon detected at 1st...
sampling time were 2.159 µg l\(^{-1}\) for 1.50 L/ha application; 5.035 µg l\(^{-1}\) for 5.00 L/ha application; for 8.00 L/ha application, the uptake response was found 6.781 µg l\(^{-1}\) and 9.284 µg l\(^{-1}\) uptake response was found at 12.00 L/ha application (Figure 2). The amount of the uptake response of Diazinon detected at 2nd sampling time were 2.82 µg l\(^{-1}\) for 1.50 L/ha application; 6.584 µg l\(^{-1}\) for 5.00 L/ha application; for 8.00 L/ha application, the uptake response was found 8.867 µg l\(^{-1}\) and 12.140 µg l\(^{-1}\) uptake response was found at 12.00 L/ha application (Figure 2). The amount of the uptake response of Diazinon detected at 3rd sampling time were 2.936 µg l\(^{-1}\) for 1.50 L/ha application; 6.847 µg l\(^{-1}\) for 5.00 L/ha application; for 8.00 L/ha application, the uptake response was found 9.222 µg l\(^{-1}\) and 12.626 µg l\(^{-1}\) uptake response was found at 12.00 L/ha application (Figure 2).

Correlations for Diazinon Treatment against Residual and Uptake in all cases were statistically significant at 1% level (r = 1.00 & 0.99; (Tables 3a, 3b & 3c) and the regression for same treatment against residual and uptake were statistically significant at 0.1% level (t \(3.12.941\)).

**Table 3a:** Correlations for Diazinon Treatment against Residual and Uptake 1st sampling.

| Treatments | Residual | Uptake |
|------------|----------|--------|
| Treatments | 1        | 0.990**| 0.990**|
| Residual   | 1        | 1.000**|
| Uptake     | 1        | 1      |

**Correlation is significant at the 0.01 level (2-tailed).**

Similarly According to FAO/WHO Standards [2], it is found that Dursban was detected above Maximum Residue Limit (0.05 mg kg\(^{-1}\) of sample) in the samples where Dursban was sprayed at the recommended dose then double of the recommended dose and higher on. The amount of the residues of Dursban detected at 1st sampling time were 0.799 µg l\(^{-1}\) for 0.50 L/ha application whereas the Dursban content in 0.50 L/ha was detected directly 1.08 µg l\(^{-1}\), 2.131 µg l\(^{-1}\) for 1.00 L/ha application whereas it was detected directly 2.88 µg l\(^{-1}\); for 2.00 L/ha application, the residual effect was found 5.638 µg l\(^{-1}\) whereas 7.62 µg l\(^{-1}\) detected and 8.17 µg l\(^{-1}\) residual content was found at 4.00 L/ha application whereas in this application rate was detected 11.05 µg l\(^{-1}\) (Figure 3).

**Table 3b:** Correlations for Diazinon Treatment against Residual and Uptake 2nd sampling.

| Treatments | Residual | Uptake |
|------------|----------|--------|
| Treatments | 1        | 0.990**| 0.990**|
| Residual   | 1        | 1.000**|
| Uptake     | 1        | 1      |

**Correlation is significant at the 0.01 level (2-tailed).**

**Table 3c:** Correlations for Diazinon Treatment against Residual and Uptake 3rd sampling.

| Treatments | Residual | Uptake |
|------------|----------|--------|
| Treatments | 1        | 0.990**| 0.990**|
| Residual   | 1        | 1.000**|
| Uptake     | 1        | 1      |

**Correlation is significant at the 0.01 level (2-tailed).**

Figure 3: Dursban residue content (mg l\(^{-1}\)) at different time against different treatment doses.
The amount of the residues of Dursban detected at 2nd sampling time were 0.689 µg l⁻¹ for 0.50 L/ha application; 1.837 µg l⁻¹ for 1.00 L/ha application; for 2.00 L/ha application, the residual effect was found 4.861 µg l⁻¹ and 7.049 µg l⁻¹ residual content was found at 4.00 L/ha application (Figure 3). The amount of the residues of Dursban detected at 3rd sampling time were 0.568 µg l⁻¹ for 0.50 L/ha application; 1.516 µg l⁻¹ for 1.00 L/ha application; for 2.00 L/ha application, the residual effect was found 4.012 µg l⁻¹ and 5.818 µg l⁻¹ residual content was found at 4.00 L/ha application (Figure 3). The amount of the uptake response of Dursban detected at 1st sampling time were 0.373 µg l⁻¹ for 0.50 L/ha application; 0.996 µg l⁻¹ for 1.00 L/ha application; for 2.00 L/ha application, the uptake response was found 2.636µg l⁻¹ and 3.82 µg l⁻¹ uptake response was found at 4.00 L/ha application (Figure 4).

![Figure 4: Dursban uptake response (mg l⁻¹) at different time against different treatment doses.](image)

| Treatments | Residual | Uptake |
|------------|----------|--------|
| Treatments | 1        | 0.979**|
| Residual   | 1        | 1.000**|

**Correlation is significant at the 0.01 level (2-tailed).

| Treatments | Residual | Uptake |
|------------|----------|--------|
| Treatments | 1        | 0.979**|
| Residual   | 1        | 1.000**|

**Correlation is significant at the 0.01 level (2-tailed).

The amount of the uptake response of Dursban detected at 2nd sampling time were 0.430 µg l⁻¹ for 0.50 L/ha application; 1.149 µg l⁻¹ for 1.00 L/ha application; for 2.00 L/ha application, the uptake response was found 3.040 µg l⁻¹ and 4.408 µg l⁻¹ uptake response was found at 4.00 L/ha application (Figure 4). The amount of the uptake response of Dursban detected at 3rd sampling time were 0.558 µg l⁻¹ for 0.50 L/ha application; 1.489 µg l⁻¹ for 1.00 L/ha application; for 2.00 L/ha application, the uptake response was found 3.941 µg l⁻¹ and 5.715 µg l⁻¹ uptake response was found at 4.00 L/ha application (Figure 4). Correlations for Dursban Treatment against Residual and Uptake in all cases were statistically significant at 1% level r = 1.00 & 0.97; (Tables 4a, 4b & 4c) and the regression for same treatment against residual and uptake were statistically significant at 0.4% level (t > 3.182).

| Treatments | Residual | Uptake |
|------------|----------|--------|
| Treatments | 1        | 0.979**|
| Residual   | 1        | 1.000**|

**Correlation is significant at the 0.01 level (2-tailed).
Discussion

Diazinon may decompose in plants in two directions. One of them may be oxidation of the phosphorothioate to the corresponding phosphate (diazinon) followed by hydrolysis of the P-X bond with the formation of non toxic diethylphosphoric acid and 2-isopropyl-4-methyl-6-0xypyrimidinamide and the other direction of the decomposition of diazinon may be the oxidation of the side isopropyl group of the ring with the subsequent hydrolysis of the phosphorus halogen bond with decomposition of the heterocyclic ring and the liberation of carbon dioxide gas. Diazinon is highly toxic to humans and animal. So the recommended dose which is applied by the farmer in the field to control the pests in cauliflower should be lower. Dursban may decomposes in plants and may produce chlorpyrifosoxon and 3, 5, 6-trichloro-2-pyridinol, which is further degraded to 3, 5, 6-trichloro-2-methoxypyridine and carbon dioxide Racke [17]. Dursban are highly toxic to human and animal. So, the recommended dose of the Dursban in Cabbage should be lower.

Since the organophosphorus and pyrethroid pesticides residues are not degraded into non toxic products in short period of time. They still persisted in vegetable. So the recommended dose, which is applied by the farmer in the field to control pests in cauliflower, should be lower or pre-harvest interval should be longer.

Conclusion

In Bangladesh context, the Cabbage growers have been using the pesticides frequently to have the higher and insect free yield. But the overdoses of pesticides make the residue problem, which might pollute our food and environment. Appropriate use of pesticides in agriculture needs to be addressed in Bangladesh and other countries. Although the Government is concerned about pesticide residues in the food and the environment, staff and facilities to conduct the necessary monitoring programs are not available. In addition the country is not yet established legal limits for residues and depends upon Codex allowable limit which are not always proposed for all crops and major pesticides used within the country. So in order to remove residual effect of pesticides which are toxic, we should know the exact dose which should be recommended to the farmer and the harvest time of each pesticides so that the amount of residual pesticides in vegetables might be lower than the present time. And an attractive method was provided by this approach with detection limits at parts per million concentrations and could be extended to additional crops and pesticides.

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