Residual Concentrations of Diazinon and Dursban and its Impacts on Soil and Carrot

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Abstract
At early growth stage after application of two pesticides, the growth of Carrot (*Daucus carota* L.) was enhanced but at middle and mature stages the growth rate was slowed down. At initial stage the response of uptake Diazinon ranged from 0.302 µg l⁻¹ to 3.064 µg l⁻¹ (Diazinon doses were 0.50 l ha⁻¹, 1.00 l ha⁻¹, 2.00 l ha⁻¹ and 4.00 l ha⁻¹); in second stage and at maturity stages the uptake ranged from 3.064 µg l⁻¹ to 3.757 µg l⁻¹ and 0.400 µg l⁻¹ to 4.089 µg l⁻¹ respectively. On the other hand, the residual values of Diazinon at different doses at different times ranged from 0.270 µg l⁻¹ to 3.426 µg l⁻¹. The residual effect and responses between soil and carrot were positively correlated at 0.01 level (r=1.00). In case of Dursban application at different doses were responded of uptake by Carrot ranged from 0.205 µg l⁻¹ to 2.580 µg l⁻¹ at rate of 0.50 l ha⁻¹, 1.00 l ha⁻¹, 2.00 l ha⁻¹ and 4.00 l ha⁻¹ throughout the growth stages. On the other hand, the residual values of Dursban at different doses ranged from 0.443 µg l⁻¹ to 0.329 µg l⁻¹. The significant level of residual effect and responses between soil and carrot were correlated positively at 0.01 level (r=0.986) and at 1st sampling correlated at 0.05 level (r=0.951) and at 2nd and 3rd sampling there were no significant relation were found (r=0.854) with respective sampling time. The experiment concluded that there was a positive response of plant uptake and residual effect in soil was occurred in both pesticides.

Keywords: Pesticide; Diazinon; Dursban; Carrot; Residual effect; Plant uptake

Introduction

Carrot (*Daucus carota* L.) is an important and nutritious winter root vegetable and is widely grown in Bangladesh mainly in Robi season. It contains a wide range of essential β-carotene, which is metabolized into vitamin A in humans when bile salts are present in the intestines. Massive over consumption of carrots can cause carotenosis, a benign condition in which the skin turns orange. Carrots are also rich in dietary fiber, antioxidants, ascorbic acid and minerals as well as small amount of protein and its nutritional value per 100 g (3.5 oz) [1].

In recent years, vegetable consumption has been increased in our country. However, the productivity of Carrot per unit area is quite low as compared to developed countries of the world [2]. The response of Carrot is high to nitrogen application and moderate to phosphorus application [3,4] For the higher productivity, pesticides are also other major agro-chemicals that controlling pest to destroy or decaying the vegetable growth but unfortunately, the application of pesticides are heavily sprayed on Carrot 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 Carrot [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. 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 organic and inorganic compounds are frequently used for vegetable growing; accumulation of these trace organic and inorganic compounds in the edible portion of these crops can occurred and poses significant health risks once entered into the human food chain [6,7]. 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; soil and climatic factors as well as crop management [8,9]. Thus, inorganic and organic pesticide accumulation in soils and subsequently plant uptake of those elements under natural open field conditions is a great interest of green house or container studies may not be truly representative of field conditions [8,10].

The present inquisition was carried out to see the residual effects of Diazinon and Dursban and its response between Carrot and soil at different growth stages.

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 Taigaon Series, and there was no pesticide concentration found before after analysis. In this experiment *Daucus carota* L. variety of Carrot was used.

Experimental design
The total plot size was 12 m × 24 m which required 45 small unit plots. The per unit plot size was 2 m × 2 m which accommodated 30 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 0.50 l ha⁻¹, 1.00 l ha⁻¹, 2.00 l ha⁻¹.

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and 4.0 l ha\(^{-1}\) for Diazinon and for Dursban in 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 45 days after first sampling and third or last sampling was done 60 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 sunlight protected non-polythene bag with well labeled to prevent exposing and contamination or alteration of organic properties. Soil samples were collected at the depth of 15 cm from surface.

**Reagents for estimation of compounds presence in two pesticides**

The organic solvents, acetonitrile, ethyl acetate used were HPLC grade were obtained from Center for Advanced Research 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 \(\mu\)m pore size) before injection on the column. Anhydrous sodium sulphate for residue analysis, 12-60 mesh, was maintained at 30°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.

**Extraction**

25 g of Triturate with dry, powdery mixture of Sodium Sulphate with the aid of an extraction thimble; extract the mixture exhaustively with Petroleum Ether in Soxhlet apparatus. The extract solution was concentrated to dryness by a concentrator and dilute to 25 ml with Petroleum Ether saturated with Dimethylformamide [11]. 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 Carrot sample (75 g) was mixed with anhydrous sodium sulphate (50 g) and extracted with ethyl acetate [12] 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 [13,14].

**HPLC systems**

A Shimadzu SCL-10AVP, Version 5.22 High performance liquid chromatography having UV/visible detector was used for identification and quantification of compounds present in pesticides. Separation was performed on reversed phase C-18 column (Nova pack). Samples were injected manually through a Rheodyne injector. Detector was connected to the computer for data processing. The working condition of HPLC was binary gradient, mobile phase was acetonitrile: water; (70:30), flow rate was 1 mL min\(^{-1}\). Injection volume was 20 \(\mu\)L and the wavelength of the UV/visible detector was fixed at 254 nm for the residual analysis of Diazinon and 230 nm for the analysis of Dursban [13].

**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 [13].

**Recovery**

Recovery studies were performed to examine the efficiency of extraction and clean up. Untreated Carrots 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 [13]. 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 (signal-to-noise ratio) >4 so that it is in the linear range of the standard calibration [15]. The LOD of Diazinon and Dursban was 0.02 mg kg\(^{-1}\). Recoveries which were obtained by triplicate analysis of Carrots sample spiked with each type of pesticide at one fortification level were satisfactory for response and residue analysis. 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 (\(y=ax+b\)) parameters for method calibration are shown in Table 1. The determination coefficients (\(R^2\)) 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 [16].

| Compound     | Calibration Range RT (Min) (mg kg\(^{-1}\)) | Slope | Intercept | \(R^2\) |
|--------------|---------------------------------------------|-------|-----------|--------|
| Diazinon     | 8.1                                         | 0.066-1.46 | 1.3’105 | 65000 | 0.998 |
| Malathion    | 5.7                                         | 0.080-1.66 | 4.5’104 | 43000 | 0.992 |
| Chlorpyrifos | 12.9                                        | 0.076-1.15 | 3.9’104 | 54000 | 0.991 |
| Cypermethrin | 9.8                                         | 0.064-0.99 | 8.2’104 | 18400 | 0.988 |

*Table 1: Retention Times Windows (RTWs) and typical calibration parameters of the method in Carrot matrix.*
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.

Results and Discussion

Diazinon and Dursban in all samples were detected (Table 2). According to MRL Status Report, 2009, it is found that Diazinon was detected above Maximum Residue Limit (0.01 µg kg\(^{-1}\) of sample) and value was found above Maximum Residue Limit (0.05 mg kg\(^{-1}\) of sample) in the samples where Diazinon and Dursban were 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 0.335 µg l\(^{-1}\), 1.083 µg l\(^{-1}\), 2.342 µg l\(^{-1}\) and 3.405 µg l\(^{-1}\) respectively at four different doses of application, whereas the amount of the residues of Dursban detected were 0.443 µg l\(^{-1}\), 1.325 µg l\(^{-1}\), 2.591 µg l\(^{-1}\) and 3.426 µg l\(^{-1}\) respectively. At 2nd sampling time Diazinon values were 0.313 µg l\(^{-1}\), 0.835 µg l\(^{-1}\), 2.209 µg l\(^{-1}\) and 3.205 µg l\(^{-1}\) respectively at four different doses of application, where Dursban value obtained were 0.182 µg l\(^{-1}\), 0.609 µg l\(^{-1}\), 0.881 µg l\(^{-1}\) and 1.062 µg l\(^{-1}\) respectively. The amount of the residues of Diazinon detected at 3rd sampling time were 0.270 µg l\(^{-1}\), 0.720 µg l\(^{-1}\), 1.905 µg l\(^{-1}\) and 2.763 µg l\(^{-1}\) respectively at four different doses of application and on the other hand, Dursban detected values were 0.074 µg l\(^{-1}\), 0.280 µg l\(^{-1}\), 0.299 µg l\(^{-1}\) and 0.329 µg l\(^{-1}\) respectively.

The amount of the uptake response to Diazinon detected at 1st sampling time were 0.302 µg l\(^{-1}\), 0.804 µg l\(^{-1}\), 2.114 µg l\(^{-1}\) and 3.064 µg l\(^{-1}\) respectively at four different doses of application whereas Dursban values were found 0.205 µg l\(^{-1}\), 0.691 µg l\(^{-1}\), 1.372 µg l\(^{-1}\) and 2.321 µg l\(^{-1}\) respectively. The amount of the uptake response to Diazinon detected at 2nd sampling time were 0.367 µg l\(^{-1}\), 0.979 µg l\(^{-1}\), 2.571 µg l\(^{-1}\) and 3.757 µg l\(^{-1}\) respectively at different doses of application and on the other hand Dursban values obtained were 0.209 µg l\(^{-1}\), 0.705 µg l\(^{-1}\), 1.399 µg l\(^{-1}\) and 2.367 µg l\(^{-1}\) respectively. The amount of the uptake response to Diazinon values found at 3rd sampling time were 0.400 µg l\(^{-1}\), 1.026 µg l\(^{-1}\), 2.819 µg l\(^{-1}\) and 4.089 µg l\(^{-1}\) respectively at different doses of application and whereas Dursban showed the values at same sampling time were 0.228 µg l\(^{-1}\), 0.768 µg l\(^{-1}\), 1.525 µg l\(^{-1}\) and 2.580 µg l\(^{-1}\) respectively.

Correlations of Diazinon and Dursban Treatment against Residual and Uptake in all cases were statistically significant at 1% and 5% level (*=0.05 level (2-tailed); **Significantly correlated at 0.01 levels (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-oxypyrimidine and the another 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 Carrots 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-methoxyppyridine and carbon dioxide [17]. Dursban are highly toxic to human and animal. So, the recommended dose of the Dursban in Carrot should be lower.

\[\text{Table 2: Amounts of residues detected in Carrots samples treated with the respective pesticide.}\]

| Treatment | Soil   | Plant   |
|-----------|--------|---------|
| Diazinon  | 1.085  | 1.628   |
| Dursban   | 2.243  |         |

\[\text{Significantly correlated at 0.01 levels (2-tailed).}\]

**Significantly correlated at 0.01 levels (2-tailed).**
Conclusion

In Bangladesh context, the Carrot 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, which causes different types of diseases and damaged the natural ecosystem. So, it is necessary to monitoring and establish a legal limits of pesticide uses 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 crops. So that the amount of residual pesticides in vegetables might be lower and after harvest, some processing might remove the remaining residual concentration. Since the organophosphorus and pyrethroid pesticides residues are not degraded into nontoxic 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 Carrots, should be lower or pre-harvest interval should be longer.

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