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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 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 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=0.0.951) 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 drops 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 Tajgaon 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}, 1.0 ha^{-1}, 2.0 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 sun light 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). Mili-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 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 µL 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=a+bx) 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].

	Calibration		Calibration parameters		
Compound	**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	8.27´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}, 0.883 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}, 2.2.209 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 (r=1.00 for Diazinon and r=0.986 and 0.951 for Dursban (Tables 3a, 3b and 3c; Tables 4a, 4b and 4c) respectively.

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-methoxypyridine and carbon dioxide [17]. Dursban are highly toxic to human and animal. So, the recommended dose of the Dursban in Carrot should be lower.

	Residue (mg kg^{-1})	
Dose	**Diazinon**	**Dursban**
Recommended dose	1.085	1.628
Double of the Recommended dose	1.64	2.243

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

	Treatment	Soil	Plant
Treatment	1	0.980**	0.979**
Soil		1	1.000**
Plant			1

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

Table 3a: Correlations between Diazinon Treatment and Residual and Uptake at 1st sampling.

	Treatment	Soil	Plant
Treatment	1	0.979**	0.980**
Soil		1	1.000**
Plant			1

**Significantly correlated at 0.01 level (2-tailed).

Table 3b: Correlations between Diazinon Treatment and Residual and Uptake at 2nd sampling.

	Treatment	Soil	Plant
Treatment	1	0.979**	0.979**
Soil		1	1.000**
Plant			1

**Significantly correlated at 0.01 level (2-tailed).

Table 3c: Correlations between Diazinon Treatment and Residual and Uptake at 3rd sampling.

	Treatment	Soil	Plant
Treatment	1	0.962**	0.993**
Soil		1	0.986**
Plant			1

**Significantly correlated at 0.01 level (2-tailed).

Table 4a: Correlations between Dursban Treatment and Residual and Uptake at 1st sampling.

	Treatment	Soil	Plant
Treatment	1	0.915*	0.993**
Soil		1	0.951*
Plant			1

*Significantly correlated at 0.05 level (2-tailed); **Significantly correlated at 0.01 level (2-tailed).

Table 4b: Correlations between Dursban Treatment and Residual and Uptake at 2nd sampling.

	Treatment	Soil	Plant
Treatment	1	0.805	0.993**
Soil		1	0.854
Plant			1

**Significantly correlated at 0.01 level (2-tailed).

Table 4c: Correlations between Dursban Treatment and Residual and Uptake at 3rd sampling.

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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