Degradation of Dimethoate and Diazinon Pesticide Residues in *Amaranthus tricolor* L. (Spinach) and Soil under Pesticide-treated Plants

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Abstract

Amaranthus tricolor L. (Spinach) is a very popular fresh salad ingredient in Myanmar. If cooked, it is better to steam it very briefly so as not to destroy the flavor and water soluble nutrients. In this research, dissipation of dimethoate and diazinon pesticide residues in spinach and soil under pesticide-treated plants were studied. The concentrations of these insecticide residues were detected by GC-PFPD chromatography using internal standard of fenitrothion. Mean recovery values of dimethoate and diazinon pesticides at three different standard concentration levels were in the range of between 87.25-87.40% and 85.60-85.90% in spinach. The residues of dimethoate and diazinon in spinach declined from 1.3310 ppm to 0.0157 ppm after 216 hr (9 days) applications and 0.8134 ppm to 0.0104 ppm after 168 hr (7days) applications, respectively. After 9 days and 7 days of applications initial deposit dissipated to 98.82% for dimethoate and 98.72% for diazinon. Complete degradation (i.e 100%) of these insecticides was observed after 264 hr (11 days) and 216 hr (9 days) of their application in spinach. The preharvest intervals (PHI) for dimethoate and diazinon in spinach were found to be 24 hr (i.e 1 day) in this investigation. Soil type was found to be loamy soil in this study. The degradation of dimethoate and diazinon in soil under pesticide-treated spinach plots were studied in parallel. All two pesticides degraded rapidly and were not detected on 168 hr (7 days) for dimethoate and 216 hr (9 days) for diazinon after treatment. The decay rate constants and half-lives of pesticide residues were determined by using double exponential decay model and pseudo-first order equations.

Keywords: *Amarantus tricolor* L., dimethoate, diazinon, GC-PFPD, PHI, double exponential decay model

Introduction

Pesticides are chemicals used to manage pest organisms in both agricultural and nonagricultural situations. By definition, a pesticide is a "substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood, wood products or animal feedstuffs, or which may be administered to animals for the control of insects, mites/spider mites or other pests in or on their bodies (Racke, 2003).

The term pesticides includes all of the following: herbicides, insecticides (which may include insect growth regulators, termicides, etc.), nematicide, molluscicide, piscicide, avicide, rotenticide, bactericide, insect repellent, animal repellant, antimicrobial, fungicide, disinfectant (antimicrobial), and sanitizer (Lee and Seenevassen, 2000). Pesticide residues in air, water, crops, vegetables, animals and man can also be found in the environment. Residual pesticides are known to be decomposed by various natural conditions in the environment by hydrolysis in water, photodegradation, metabolism in plants, and animals, and bacteriological degradation in soil and thus pesticide contributed to the problems.

Organophosphate insecticides are a class of organophosphorus compound with the general structure O=P $(OR)_3$. Organophosphates are good of human-made chemicals that poison insects and mammals. Organophosphates are the most widely used in agriculture, the home, gardens, and veterinary practice. Most organophosphorus compounds have a short residual activity e.g., diazinon, dimethoate, malathion, chlorpyrifos, fenitrothion, etc. (Ware and Whitacre, 2004).

Dimethoate and diazinon are systemic and nonsystemic organophosphate insecticides which are widely used in vegetable farms for plant protection. They act by interfering with the activities of cholinesterase (WHO, 1992).

Spinah (Hin-nu-nwe) is a very popular leafy vegetable in Myanmar. The leaves and stems may be eaten as a salad vegetable. It is usually cooked as a leafy vegetable. It is usually stir fried or steamed as a side dish. It has an earthy and nutty flavor (Larsen *et al*., 2007).

The main aim and objectives of this study were to assess: degradation of dimethoate and diazinon residues in spinach after application and then to determine the preharvest interval (PHI) for these two insecticides in spinach and also in the soil under pesticide-treated spinach plants.

Materials and Methods

Plant Materials

Amaranthus tricolor L. (Spinach) was cultivated on plot (35 x 25 ft) of vegetable farm at Alantapo Village, Hlegu Township, in Yangon. Crops were grown in four 30 x 3 ft rows (on control and three treated plots).

Spraying of Insecticides

A single application of dimethoate (40 EC) and diazinon (40 EC) insecticides, applied in the trials, was performed when the spinach had reached the harvesting stage. These insecticides were diluted with water to obtain the recommended dose by hand spray from a spray tank on each plot. The control plot was sprayed with water only (Sagar, 1985).

Sample Collection, Preparation and Storage

The whole plants were collected symmetrically (i.e., z shape) on day 0, day 1, day 3, day 5, day 7 and day 9 after application of insecticides. All collected samples (1000 g) were chopped and mixed thoroughly by homogenizer and stored in polyethylene plastic sealed bags at -4°C until extraction (Ambrus, 1998). Before analysis, thaw samples to room temperature and out of them, 50 g subsamples were taken and further analyses were carried out (Hernandez *et al*., 2002). Soil samples under spinach plant were also collected according to the sampling schedule of spinach plant. Soil sample was taken down to a depth of 20 cm with auger by turning the auger once anti-clockwise before pulling it, back to surface. The soil portion was cut off using clean steel scissors and placed into a plastic sealed bag at -4°C until extraction. A 50 g of each soil sample was obtained by using coning and quartering method (Black, 1965).

Extraction, Clean-up and Determination of Pesticide Residues

Pesticides were extracted from chopped spinach samples with ethyl acetate and anhydrous sodium sulphate. The ethyl acetate extracts were first cleaned up by activated charcoal and anhydrous sodium sulphate and then followed by deactivated silica gel column chromatography.

Pesticides were extracted from soil samples with a mixture of acetone and normal hexane by soxhlet extraction apparatus. The extracted were cleaned up by anhydrous sodium sulphate and neutral alumina column chromatography. Finally, the extracts were analyzed for dimethoate and diazinon residues by gas chromatographic analysis.

Results and Discussion

Physicochemical Analysis of Spinach

Some physicochemical properties of the spinach were water content (93.68%), pH (5.02), ash (0.88%), fat (0.65%) and protein (1.80%), respectively (Table 1).

Table 1 Physicochemical Analysis of Spinach Plants

Water content (%)	pH	Ash (%)	Fat (%)	Protein (%)
93.68 ± 0.41	5.02 ± 0.01	0.88 ± 0.03	0.65 ± 0.04	1.80 ± 0.03

Preliminary Investigation of Soil Type and Soil Properties of the Experimental Plots

The soil properties and soil type of experimental plots were shown in Table 2. The moisture content, pH, total nitrogen content, organic carbon content and humus of soil were 0.90%, 5.30, 0.19 %, 0.76%, 1.52%, respectively. This soil was slightly acidic. The soil contained 46.55% sand, 36.64% slit, and 14.36% clay, respectively. The texture of soil was found to be loamy soil according to textural triangle (Gee and Bouder,1986).

Table 2 Preliminary Investigation of Soil Type and Soil Properties of the Experimental Plots

Parameters	Properties of soil in experimental plots
Moisture (%)	0.90 ± 0.01
pH	5.30 ± 0.14
Sand (%)	46.55 ± 1.35
Silt (%)	36.64 ± 1.03
Clay (%)	14.36 ± 2.48
Total nitrogen (%)	0.19 ± 0.17
Organic carbon (%)	0.76 ± 0.03
Humus (%)	1.52 ± 0.12

Extraction Efficiency

The "completeness of extracting pesticide residues from a substrate" (extraction efficiency) is an important procedural step (Hamilton *et al*., 1997). For three different standard concentration levels (7.6764 μg/ml, 15.3528 μg/ml and 30.7056 μg/ml), good mean recovery percentages were 87.38±0.52 and 88.32±1.94% for dimethoate and 85.78±0.69 and 92.01±0.69% for diazinon in spinach at 95% confidence level, respectively. These results fall in the accepted range of 70 -120% (Jimenez *et al*., 2001).

Degradation of Pesticide Residues as a Function of Time

The degradation of dimethoate and diazinon residues in spinach was studied as a function of time after application and the results were shown in Figures 1 and 2. The results revealed that the residue concentrations in spinach were found to be 1.3310 ppm and 0.8134 ppm for dimethoate and diazinon insecticides after application of 2 hr. The residue concentrations of both pesticides decreased gradually. On 264 hr (11 days), the dimethoate residues were not detected and diazinon residues were not detected beyond 168 hr (7 days). The maximum residue limit (MRL) of dimethoate and diazinon in spinach were 1.0 and 0.5 ppm and acceptable daily intake (ADI) of dimethoate was 0.002 mg/kg body weight by Codex Alimentarius Commission (2002). Thus, the preharvest interval (PHI) for dimethoate and diazinon in spinach plants was found to be 24 hr (i.e.1 day) in this investigation.

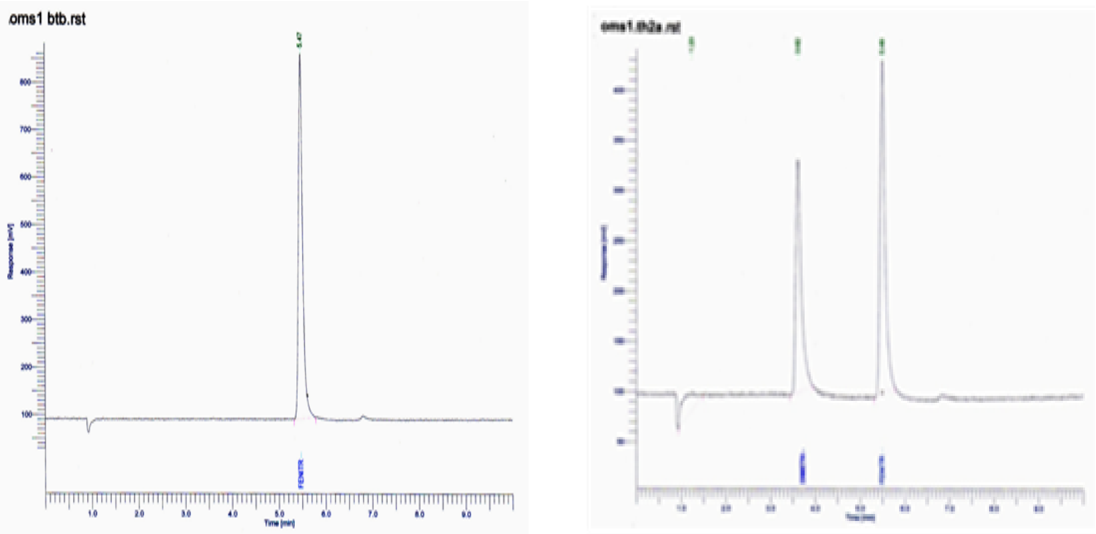


Figure 1 Gas chromatograms of (a) reagent blank and (b) dimethoate residues in spinach

Percent degradation of dimethoate and diazinon in spinach plants are presented in Table 3 and Figure 3. The results obtained showed that 37.84% of dimethoate residue and 37.92% of diazinon in spinach degraded after application of 24 hr (1 day). After 168 hr (7 days), dimethoate degraded 96.78% and diazinon degraded 98.72% in spinach. Complete degradation (i.e., 100%) of dimethoate insecticide was observed on 264 hr (11 days) of its application while diazinon insecticide was degraded on 216 hr (9 days) in spinach. These results revealed that the degradation rates of diazinon were faster than those of dimethoate in spinach.

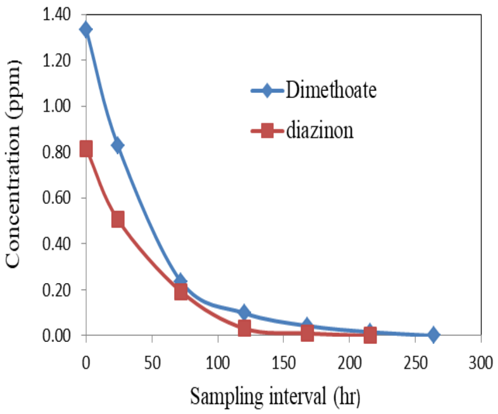


Figure 2 Degradation of dimethoate and diazinon pesticide residues in spinach as a function of time

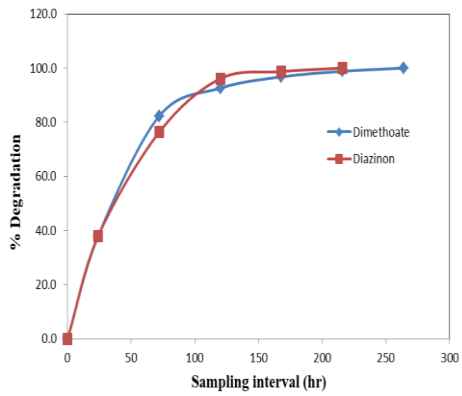


Figure 3 Percent degradation of dimethoate and diazinon pesticide residues in spinach

Table 3 Percent Degradation of Dimethoate and Diazinon Pesticide Residues in Spinach during a Sampling Period

Sampling interval (hr)	(%) Dgradation	
	Dimathoate	Diazinon
24	37.84	37.92
72	82.32	76.37
120	92.61	96.12
168	96.78	98.72
216	98.82	100
264	100	-

Water Contents and pH in Spinach During Study Period

Water contents and pH in spinach were determined as a function of sampling interval. In this study, the water content of spinach (control sample) was found to be about 93.18±0.32% and it remained nearly constant, i.e., 92.18 - 92.22% and 94.09 - 94.21% after spraying with dimethoate and diazinon, respectively, during study period. From this study, it was observed that water content did not change throughout the picking intervals. Similarly, no pronounced change of pH in spinach (control sample) was 5.60±0.04 and the results were in the range of 5.50 to 5.60 and 5.05 to 5.11, respectively throughout the study period. Thus, pH values of spinach plants
remained nearly constant during the treatment period.

Diminution of Pesticide Residues in Soil under Pesticide-Treated Plants

Throughout the sampling period, dimethoate and diazinon were detected in the range of <0.01-0.2347 ppm and <0.01-0.5999 ppm, respectively (Figure 4). Dimethoate degraded rapidly and were not detected on 168 hr (7 days) after treatment while diazinon degreaded slowly and were not detected on 216 hr (9 days) after treatment. All two pesticides in soil samples showed a decrease in concentration with increasing sampling intervals. This is probably due to the combined effects of volatilization, chemical conversion and degradation (hydrolysis, oxidation, reduction or isomerization) and photodegradation (Carter and Stringer, 1970).

Percent degradation of dimethoate and diazinon in soil under pesticide-treated spinach plants was found that after 120 hr (5 days) and 168 hr (7 days) of application, dimethoate and dizinon residues degraded almost completely (i.e., 100%) (Figure 5). The data showed that the degradation of dimethoate proceeded at a much faster rate than that of diazinon in the soil. Because dimethoate is rapidly broken down by soil microorganisms,it will be broken down faster in moist soil.

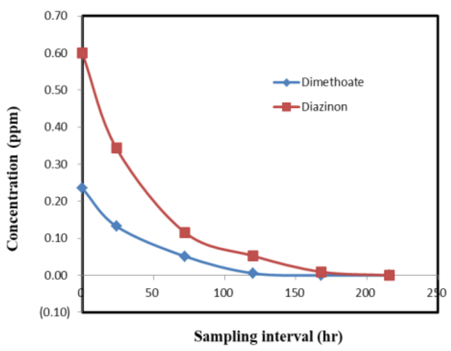
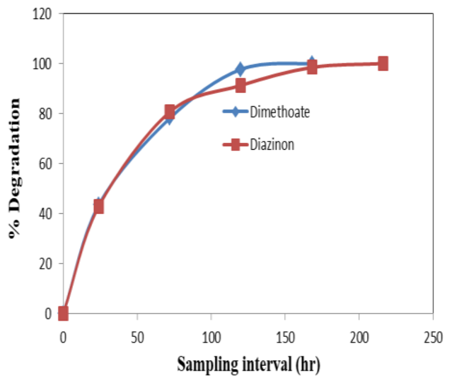


Figure 4 Degradation of dimethoate and diazinon pesticide residues in soil as a function of time



Figuer 5 Percent degradation of Dimethoate and diazinon residues in soil as a function

Rate Constants and Half-Lives of Dimethoate and Diazinon Pesticides in Spinach and Soil under Pesticide-Treated Plants

Rate constants and half-lives of dimethoate and diazinon pesticides in spinach and soil under plants were used to fit double exponential decay model and pseudo-first order equations (Table 4) . The dissipation curves were fitted for the individual pesticides in soil by nonlinear regression with the Sigma Plot for Windows 4.01 curve fitting package ($y = a\ e^{-bx} + c\ e^{-dx}$) (Laab *et al.*, 2002). The results obtained show that the degradation rate constants (0.0221 and 0.0205 hr^{-1} for spinach and 0.0217 and 0.0232 hr^{-1} for soil) and half-lives (33.25 and 33.81 hr for spinach and 30.65 and 29.87 hr for soil) for dimethoate in both cases were found to be not quite differen. In the case of diazinon, the degradation rate constants (0.0212 and 0.0227 hr^{-1} for spinach and 0.0210 and 0.0229 hr^{-1} for soil) and half-lives (32.67 and 30.53 hr for spinach and 31.75 and 30.26 hr for soil) were not quite different. Generally, half-life is rapid in leafy vegetables and forage crops.

Table 4 Comparison of the Rate Constants and Half-Lives of Dimethoate and Diazinon in Spinach and Soil under Pesticide-Treated Plants using Double Exponential Decay and Pseudo-First Order Equations

Samples	Double exponential decay				Pseudo-firstorder			
	Dimethoate		Diazinon		Dimethoate		Diazinon	
	k_1 (hr^{-1})	$t_{½}$ (hr)	k_1 (hr^{-1})	$t_{½}$ (hr)	k'_1 (hr^{-1})	$t'_{½}$ (hr)	k'_1 (hr^{-1})	$t'_{½}$ (hr)
Spinach	0.0221	33.25	0.0212	32.67	0.0205	33.81	0.0227	30.53
Soil	0.0217	30.65	0.0210	31.75	0.0232	29.87	0.0229	30.26

Conclusion

Monitoring pesticide residues is a priority objective in pesticide research to obtain an extensive evaluation of food quality. People are unaware of negative impacts of pesticide. Pesticides are an important but often controversial component of today's integrated pest management system. Myanmar needs sound environmental management regarding persistence of organic pollutants (POPs) chemicals. In this work, physicochemical analysis of spinach, preliminary investigation of soil type and soil properties of the experimental plots, water contents and pH values for pesticide-treated spinach and dimethoate and diazinon residues in spinach and soil under pesticide-treated plots were studied. Comparison of the rate constants and half-lives of double exponential decay model and pseudo-first order equations in spinach and soil under pesticide-treated plots were studied. Physicochemicals analyses of spinach were determined by FAO and AOAC method. In this study, the type of soil was loamy soil according to the textural classes. PHI for dimethoate and diazinion in spinach plants were 24 hr (i.e.1 day) in this investigation. The water percentages and pH value of pesticide-treated spinach remained nearly constant throughout the study period. Comparison of the degradation rate constants and half-lives of spinach and soil by using double exponential decay and pseudo-first order equations indicated that there were no significant differences among the data.

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