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The fate of Chlorpyrifos-Ethyl 480 g/l in Horticultural crops in Ghana

Koomson, C. K[1]., Owusu, E.O[2]., Obeng-Ofori, D[3]., and Kingsford-Adaboh, R[4]

[1]Department of Integrated Science Education, University of Education, Winneba, P.O. Box 25, Winneba, Central Region, Ghana.
[2]Department of Animal Biology and Conservation Science, University of Ghana, Legon.
[3]Crop Science Department, University of Ghana, Legon
[4]Chemistry Department, University of Ghana, Legon
Corresponding Author: Dr. Charles Kwesi Koomson, Department of Integrated Science Education, University of Education, Winneba, P.O. Box 25, Winneba, Central Region, Ghana.
E-mail: *charleskoomson@yahoo.co.uk*

Abstract

This study investigated the fate of chlorpyrifos-ethyl 480 g/l in cabbage (*Brassica olerecea* var. capitata L.) using both field experiments and laboratory analysis. GC analysis showed that the insecticide undergoes volatilization and degradation which could reduce the concentration of the active ingredient making it less effective to control insect pests. The results revealed that the dissipation of insecticides occur through volatilization and degradation of the insecticide in the crop which may be influenced by temperature. It is therefore important that the Environmental Protection Agency and the Pesticides Technical Committee demand studies on the rate of volatilization of agrochemical as part of the bioefficacy trials done by local researchers. This will help in the determination of the correct application rates under tropical conditions that can leave a desirable amount of the agrochemical in the crop to control insect pests and diseases. It is believed that when this is done it will reduce incidence of pest resistance and pest resurgence in the country.

Keywords: Chlorpyrifos-ethyl 480g/l, dissipation, volatilization, evapouration, degradation, and bioefficacy trials.

1. Introduction

Insecticides are one of the major groups of chemicals responsible for environmental contamination (Ntow, 2006). Many of these insecticides are highly toxic and considered a potential risk to both human health and the environment (Ntow, 2006). Farmers often spray their crops with hazardous agrochemicals such as organochlorines and organophosphates and at different application rates to protect their crops and investments (Koomson, 2003). Runoff of these agrochemicals may contaminate water bodies which may pose serious health problems to human and animals that drink from them, or may contaminate soils and their residues can concentrate in crops and pose health hazards to consumers if the maximum residue limit (MRL) set by the FAO/WHO is exceeded (Ntow, 2001). Non-target flora and fauna concentrate these residues in their tissues and pass them on along the food chain (Koomson, 2003).

One of such insecticides is chlorpyrifos-ethyl 480g/l which is a broad-spectrum organophosphate insecticide. While originally used primarily to kill mosquitoes, it is now registered to control cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice (He, 1994). It is used as an insecticide on grain, cotton, field, fruit, nut and vegetable crops, and well as on lawns and ornamental plants (EPA, 2010). Chlorpyrifos acts on pests primarily as a contact poison, with some action as a stomach poison (Racke, 1992). It is available as granules, wettable powder, dustable powder and emulsifiable concentrate (Racke, 1992).

Chlorpyrifos-ethyl 480g/l has been found in water bed residue monitoring in Akomadan in the Ashanti Region (Ntow, 2006) and cabbage crops studies in the Greater Accra (Amoah *et al***,** 2006), and it is one of the commonly used insecticide in horticultural production in Ghana. It has also been reported that farmers also spray their crops with agrochemicals well above the recommended application rate in order to effectively control insect pests and diseases (Ntow, 2003).

An important aspect of understanding the health and environmental impact of chlorpyrifos-ethyl on farmers in a typical Ghanaian farming community and also to determine the concentration of insecticides in crops that can effectively control insect pest and diseases is to assess the distribution, persistence and dissipation of the insecticide in tropical crops.

The objectives of this study were:

1. To determine how Chlorpyrifos-ethyl 480g/l is distributed, qualitatively and quantitatively, in the cabbage crop following spraying on plant foliage.
2. To determine the persistence of Chlorpyrifos-ethyl 480g/l in cabbage leaves and stem as total residues to which consumers and/or insects may be exposed
3. To determine the factors that can affect the effectiveness of Chlorpyrifos-ethyl 480 g/l application rate under the tropical conditions of Ghana in controlling insect pests and diseases.

2. Materials and Methods

2.1 Reagents

Analytical standards of chlorpyrifos-ethyl 480 g/l (97.5% purity) were supplied by Water Research Institute (WRI) of the Centre for Scientific and Industrial Research (CSIR). Termex 48EC containing 480 g/l ai of chlorpyrifos-ethyl was obtained from Saro Agro Sciences Ghana Limited. Stock solutions of chlorpyrifos-ethyl (100 µg/ml) were prepared separately in n-hexane. All organic solvents used were of GC, grade (Sigma, Munich, Germany; or BDH, VWR International, Poole, UK).

2.2 Determination of the fate of chlorpyrifos-ethyl 480 g/l in cabbage

2.2.1 Soil analysis

The field study was conducted during December 2009-April 2010 in Accra, behind the Water Research Institute (W.R.I) of the C.S.I.R. Soil samples (about 200 g dry weight) from 0-10, 10-20, 20-30, and 30-40 cm depths of the experimental field were collected with a corer (5.0 cm diameter) at random and analysed for water content, pH, organic matter content, texture (clay, silt, and sand contents), and bulk density. Each of the soil composites was brought to the laboratory to air-dry. The air-dried soil samples were pounded and pulverized; passed through a 2 mm sieve for course and through 0.425 mm for the fine earth samples.

Soil pH

Soil pH was determined using the potentiometric method at a soil-water ratio of 1:2.5 (ISRIC, 1995). About 20 grams of soil was weighed and placed in appropriate plastic cups and added with 50 ml distilled water. The solution was stirred for nearly two minutes and allowed to stand for 30 minutes. Afterwards, pH was read after the pH meter was calibrated using pH 4 and pH 7 buffer solutions.

Soil Organic matter content

Organic matter was analyzed using the modified Walkley-Black method (Jackson, 1958). Exactly 0.5 gram of soil (sieved through a 0.425 mm wire mesh) was weighed and placed in a 500 ml Erlenmeyer flask and added with 10 ml potassium dichromate ($K_2Cr_2O_7$). The flask was swirled gently to disperse the soil in the solution. Under a fume hood, 10 ml concentrated H_2SO_4 was dispensed rapidly and immediately swirling the flask gently then more vigorously for one minute. The flask was allowed to stand under the hood for one hour. Afterwards, 200 ml of distilled water was added. Six drops of phenanthroline indicator was mixed into the solution; stirred, and titrated with 0.5N $FeSO_4$. The endpoint

was reached when a maroon colour observed in the solution was clearly visible.

Soil texture (particulate size)

The soil particulate size was determined using the method described by (Day, 1965). About 50 mL of Clorox was added to 40 g of oven dry soil and allowed to stand for 24hrs after which 100 mL of dispersant (40 g sodium hexametaphosphate liter^{-1}) solution and 100 to 200 mL of distilled water were added. The solution was allowed to stand 10 min with occasional stirring to thoroughly disperse the soil particles. 3-5 drops of amyl alcohol was added to the sample to defoam it. The dispersed sample was transferred into a sedimentation cylinder, mixed and placed upright on a table and timed immediately. A hydrometer was carefully inserted into the suspension and readings taken at the top of the meniscus, 30 seconds after the start of sedimentation and the reading recorded as R after which the hydrometer was removed, rinsed and dried. The readings were taken at 3, 10, 30, 90, and 120 min after the start of sedimentation to obtain R1, R2 etc. The particulate size is calculated as:

$$\text{Sand \%} = \frac{(\text{oven dry soil mass}) - (\text{R sand} - \text{RC1})}{(\text{oven dry soil mass})} \times 100$$

$$\text{Clay \%} = \frac{(\text{R clay} - \text{RC2})}{(\text{oven dry soil mass})} \times 100$$

$$\text{Silt \%} = 100 - (\text{Sand \%} + \text{Clay \%})$$

Three sizes were estimated: <0.002 mm (clay), 0.002-0.02 mm (silt), and >0.02 mm (sand)].

2.2 Planting of cabbage

About 30 m^2 plot was prepared for the planting of the KK-cross variety of cabbage (*Brassica olerecea* var. capitata L.). No chlorpyrifos-ethyl had been sprayed on the field for over 5 years. Nine plots each measuring 15 x 15 m were demarcated in a 2 x 2 randomized complete block design for two treatments (Tl) and a control treatment (TC), leaving a border area of about 2.5 m around the plots. Each treatment was replicated three times. On January 9, 2010, 14-day-old cabbage seedlings were transplanted at 65 cm apart in rows with row to row distance of 60 cm. There were 30 plants per plot. On February 18, 2010 (i.e., 40 days after transplanting), Chlorpyrifos-ethyl 480g/l (Termex 48EC; labeled application rate is 1L/ha) was applied on Tl plots from a height of 20-25 cm above the plant canopy at a rate of 1L/ha (480g of ai/ha) in 215 L of water using a portable [Knapsack CP 15 L sprayer equipped with one conical nozzle operated at 40 psi (275 kPa)]. The reason for spraying at maturity was to prevent the accumulation of residues of chlorpyrifos-ethyl from previous application which could affect the concentration of the insecticide at the time of analysis. Before use, the spraying device was calibrated with respect to homogeneity of the spray beam and pumping volume per time unit. The delivery rate was 1L of the active ingredient per ha. The application of the insecticide to the plots was applied bandwise and in a criss-cross pattern to ensure uniform distribution. Control plots had no insecticides applied on them. Throughout the experiment, the plots were kept free of weeds by hand picking, taking care not to disturb the upper layer of soil. The plots were irrigated twice daily (morning and evening) with tap water. The irrigation water was analyzed for the presence of chlorpyrifos-ethyl residues using the GC. During the experimental period, there were rainfalls on February 4th, February 15th, March 5th, April 10th and 19th 2010. Mean relative humidity was 71%; maximum and minimum temperatures averaged 33 and 25 °C, respectively, with a mean of 29 °C.

2.3 Sampling for chlorpyrifos-ethyl residues

Samples of leaves (10 g of fresh weight each), roots and stems (100 g of fresh weight) were randomly taken from 10 plants from each replicate plot at intervals of 0 (2 hr after spray), 1, 2, 6, and 14 days. The samples of leaf, stem, and root were wrapped in aluminum foil, packed in polythene bags, and transported to the CSIR Water Research Institute Laboratory in Accra in clean ice chests. Upon arrival at the laboratory, leaf, stem, and root samples were washed with cold water to remove soil particles and subsequently kept in a freezer at 4 °C until required for extraction, which was carried out within 24 h.

2.4 Analytical procedures

Samples of cabbage plant parts (roots, stems and leaves) were extracted according to procedures described by FAO/IAEA (1997). Briefly, the frozen samples were thawed, and each plant part (approximately 5 g of fresh weight) was cut into small

pieces and homogenized in a mortar. The plant parts were transferred to a pre-extracted Whatman cellulose extraction thimble. Lipids were extracted for 8 hr with methanol (200 ml) in a Soxhlet apparatus cycling four or five times per hour. The extract was passed through a preconditioned SPE column (Bond Elute C-18 3-cc/500 mg; Varian, Palo Alto, CA, USA) as described in Ntow (2001). Residues trapped in the column were eluted with n-hexane (1.5mL) into a glass vial and brought to volume (2mL) with n-hexane for analysis by gas chromatography.

Analyses were performed with a Perkin-Elmer AutoSystem gas chromatograph equipped with a 63Ni electron capture detector. Separations were on a 30 m x 0.32 mm i.d. capillary column with 0.25µm methyl phenyl phase (Perkin-Elmer Elite-225). The gas flow (helium) was set to 16 mL/min through the column and at 30mL/min makeup (nitrogen) through the detector. Sample volumes (1µL) were injected in a split mode at 250 °C, and the oven temperature was programmed as follows: 100 °C for 1 min, increased to 150 °C (10 °C/min), 250 °C (5 °C/min), then at 30 °C/min to 300 °C (held 10 min). The detector temperature was 350 °C. The retention times (RT) of the chlorpyrifos-ethyl was compared with those of the external standards, and the data was recorded. The RT of was observed as 20.4 min.

2.5 Calculation of residue levels

Residue levels were calculated using the equation (NRI, 1994) below:

$$\text{Residue level} = \frac{\text{Concentration in the final extract x dilution factor}}{\text{Weight of sample analyzed}}$$

2.6 Data Analysis

Differences in concentration of the residue levels from the leaves and stems of the cabbage were analyzed by one-way-analysis of variance followed by a Bonferroni test (equal variances assumed) (SPSS software, version 12.0.1 for Windows, SPSS Inc, Chicago, Illinois, USA). The data were analyzed for normal distribution (Kolmogorov-Smirnov test) and LSD test at ($P<0.001$) was used to separate the means. The dissipation of chlorpyrifos in cabbage foliage, stem and root was determined by a nonlinear regression of the insecticide residue concentration against time (treatment Tl) implemented in Microsoft Excel. The statistical parameters, r2, k, and C0 were determined using an iterative nonlinear regression procedure using SPSS software (SPSS software, version 12.0.1 for Windows, SPSS Inc., Chicago, IL). DT50 and DT90 values chlorpyrifos-ethyl 480g/l were also calculated.

3. Results

The results of the residue levels of chlorpyrifos-ethyl in cabbage leaves, stem and roots analyses are presented in tables 1 below.

Table 1. Distribution of Chlorpyrifos-ethyl residue in cabbage parts

	Time (days)	Sample		
		Mean conc. mg/kg FW of leaves	Mean conc. mg/kg FW of stem	Mean conc. mg/kg FW of roots
Control	0	0	0	0
	0	1.85 ± 0.03^{a}	0.90 ± 0.01^{b}	0.26 ± 0.02^{c}
	1	0.57 ± 0.06^{a}	0.63 ± 0.11^{b}	0.17 ± 0.01^{c}
	2	0.24 ± 0.02^{a}	0.61 ± 0.01^{b}	0.13 ± 0.21^{c}
	6	0.13 ± 0.11^{a}	0.20 ± 0.02^{a}	0.08 ± 0.14^{a}
	14	0.09 ± 0.03^{a}	0.02 ± 0.01^{b}	0.03 ± 0.01^{b}

Number of samples= 30 Means of samples on the same row followed by different letters are significantly different at ($P<0.01$), LSD

In table 1, chlorpyrifos-ethyl residue contents in and their distribution among leaves, stems, and roots are presented. Shortly after treatment, the total chlorpyrifos-ethyl residue contents found in the leaves was significantly higher than that of the stems and the roots. Among the plant parts, leaves had the highest content of total chlorpyrifos-ethyl residues, followed by stems, and roots. For cabbage leaves, a sharp decline in the total chlorpyrifos-ethyl contents was observed within 24 hr, followed by a relatively slow decline to the termination of the experiment as found in figure 1. The decline in the cabbage stems was not very sharp initially as compared to that observed in the leaves followed by slow decline till the end of experiment (Figure 2) whiles the decline of the chlorpyrifos-ethyl in the roots was sharper than that observed in the stem but lower than the decline in the leaves (Figure 3).

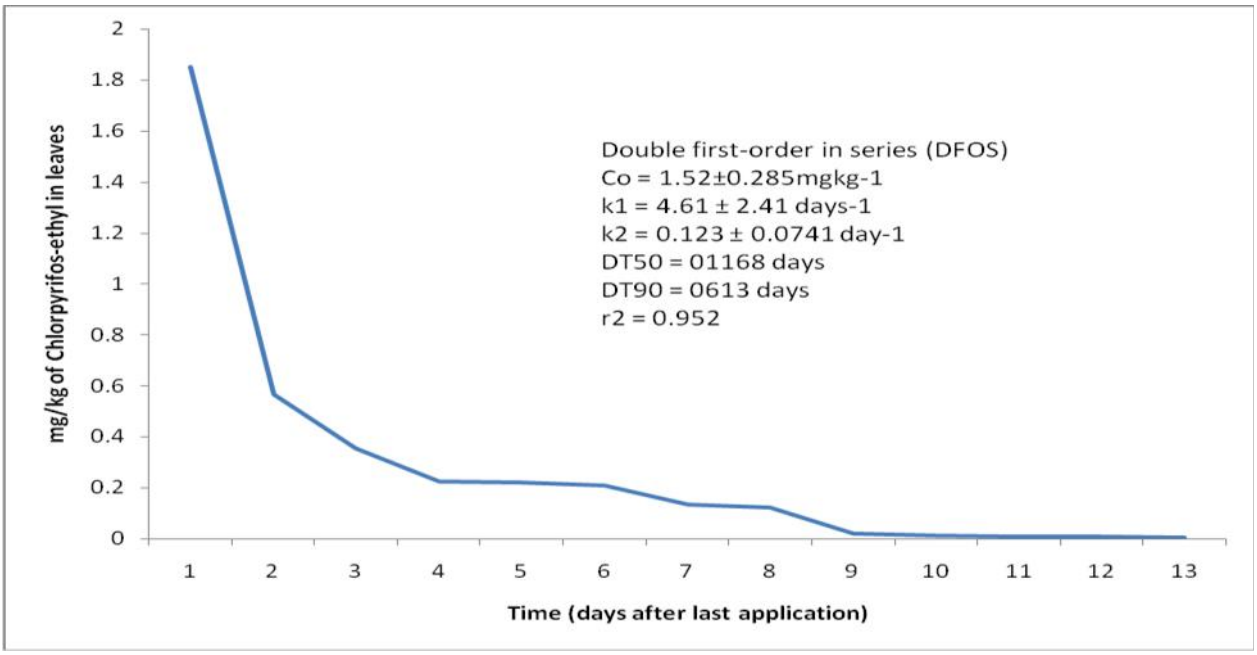

Figure 1 Dissipation of Chlorpyrifos-ethyl in cabbage leaves

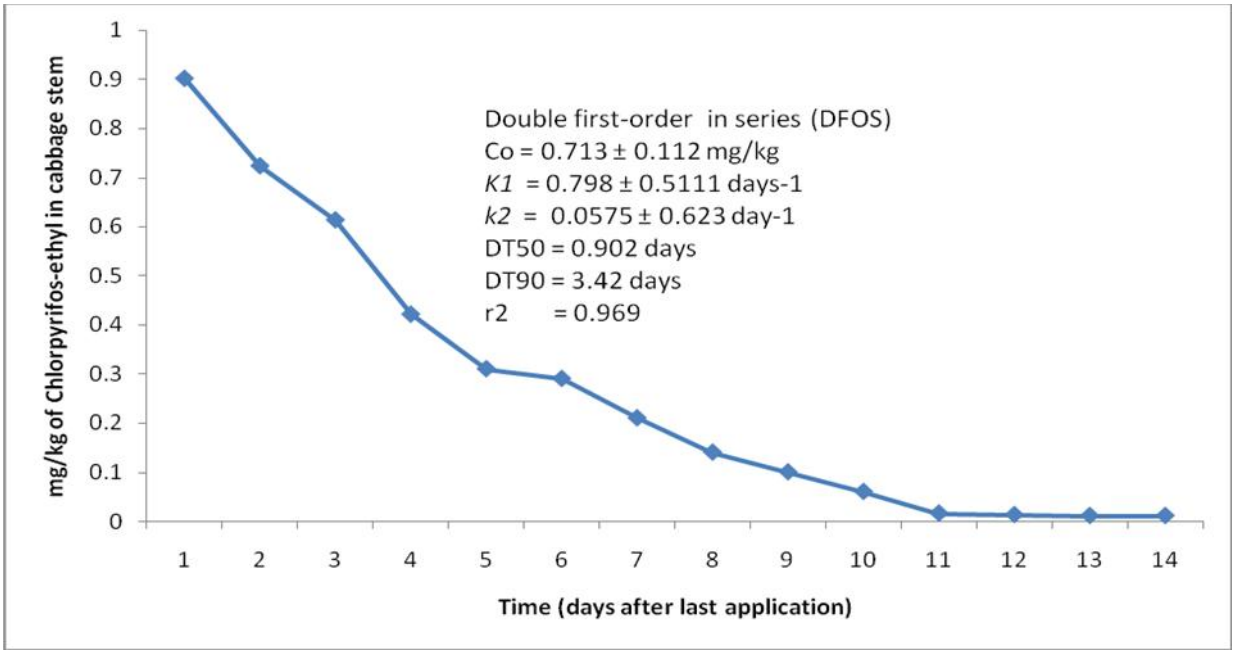

Figure 2 Dissipation of Chlorpyrifos-ethyl in cabbage stems

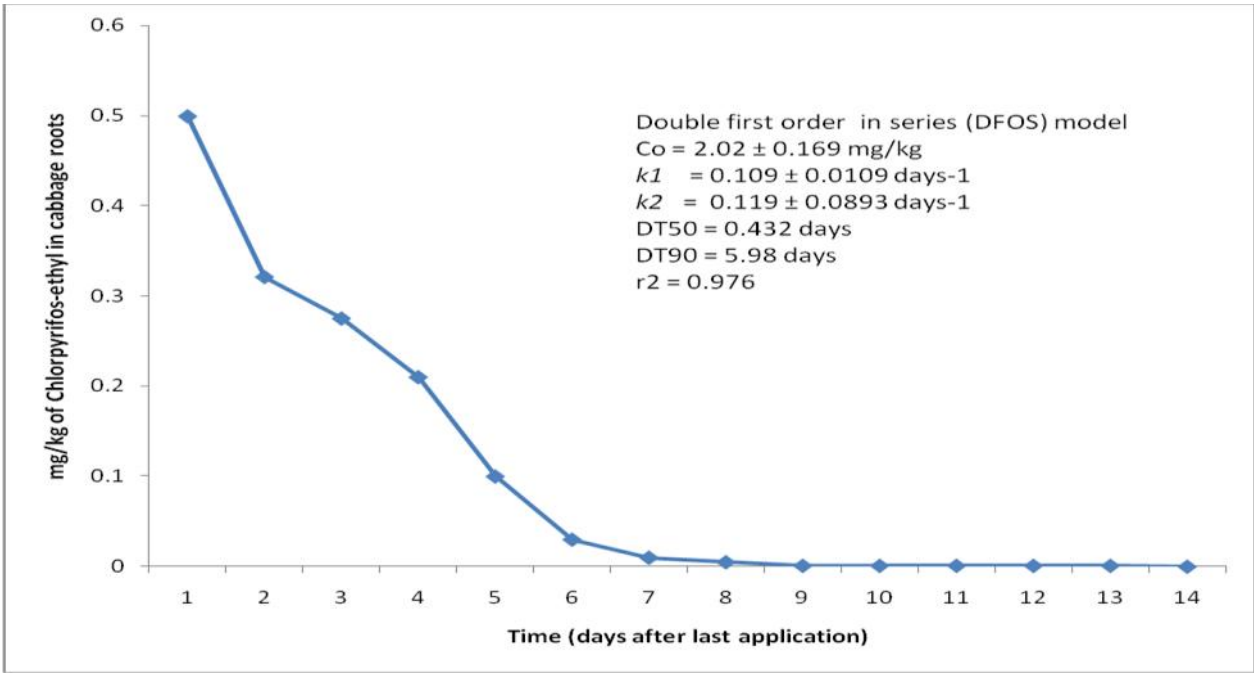

Figure 3 Dissipation of Chlorpyrifos-ethyl in roots of cabbage

In cabbage leaves, an initial total chlorpyrifos-ethyl residue was above 1.85 ± 0.03 mg/kg 2 hr after treatment. The concentration dropped to 0.032 ± 0.03 mg/kg at the end of the 14th day. The stem had an initial concentration of 0.90 ± 0.01 mg/kg of chlorpyrifos-ethyl 2 hrs after application and this dropped to 0.02 ± 0.01 mg/kg at the end of the 14th day after treatment while the roots had 0.26 ± 0.02 mg/kg of the insecticide after 2 hrs of treatment and this dropped to 0.034 ± 0.01mg/kg at the end of the experiment. From table 1, the distribution of total chlorpyrifos-ethyl residues followed the order leaves (58%) > stem (30) > root (12%).

4. Discussion

4.1 The fate of chlorpyrifos-ethyl 480 g/l in cabbage

Chlorpyrifos-ethyl 480 g/l is the most widely used insecticide in horticultural production in Ghana (EPA, 2010). Shortly after treatment of chlorpyrifos-ethyl 480 g/l on field-grown cabbage using Termex 48 EC formulation, the levels of total chlorpyrifos-ethyl residues were about 48% higher in leaves than in stems, or roots due, partly, to the foliar application of the insecticide. The other reasons could be the horizontal position of the lamina of the leaves and the difference in surface area between leaves and other tissues of the plant (Raha, et al, 1993). Miglioranza, et al (1999) also found that high carotenoid levels in the leaves (lipophilic substances) are responsible for retaining pesticides in the body and peel of vegetables.

The measurement of chlorpyrifos-ethyl 480g/l in cabbage is of great importance as its uptake is a major pathway for a toxic substance into the food chain leading to human exposure. The Codex Committee on Pesticide Residues (CCPR) considers the total chlopyrifos-ethyl concentration of 0.20 mg/kg in cabbage to be the maximum residue level (MRL) [CCPR (https://secure.pesticides.gov.uk/MRLs)]. After treatment, the residue level of total chlorpyrifos-ethyl in cabbage leaves was 1.9 mg/kg, and at harvest time, that is, 2 weeks later (according to the pre-harvest interval of the Termex 48EC (chlorpyrifos-ethyl)), it was 0.09 mg/kg, which is lower than the Codex MRL. This clearly indicates that if horticultural farmers employ good agricultural practices such as the use of correct application rates, correct application equipments, correct pre-harvest application interval etc, then the problem with high residues in horticultural crops will be drastically minimized.

To describe the dissipation of residues of chlorpyrifos-ethyl in cabbage foliage, stem and roots, a monophasic dissipation model in first-order kinetics derived from the equation 1 below (Monica *et al*, 2002) was used:

$$C_t = C_0 e^{-kt} \quad \text{.....(1.0)}$$

C_0 is the y-intercept value, C_t the concentration of chlorpyrifos-ethyl residues in matrix at time t (mg/kg), r is the post application time (days), and k is the slope of the dissipation line. DT_{50} and DT_{90} values and the dissipation rate constant (k) were determined from the slope of a nonlinear regression plot of ***C***, versus ***t.***

However, in cabbage crop, chlorpyrifos-ethyl concentration also decreased with time, but more rapidly initially and then slowly (Figures 1, 2 and 3). The initial decrease was more pronounced in the leaves followed by the roots and stem in that order. This could be due to the fact that the leaves are exposed to harsh climatic conditions which increase the volatilization property of the insecticide in the leaves. The roots on the other hand are in contact with the soil and this may lead to diffusion of the insecticide into the soil. This deviation of dissipation kinetic from first-order kinetic, with exhibition of biexponential (two-stage) dissipation kinetic, confirms findings of studies done by several authors (Kennedy *et al,* 2001 and Monica et al, 2002). The biphasic model could be explained by an initial rapid volatilization phase followed by the other phase of a slower rate of dissipation (Miglioranza et al. 1999)**.**

Thus, in cabbage crop, dissipation of chlorpyrifos-ethyl was described by Monica *et al,* **(**2002)'s biphasic model which is:

$$C_t/C_o = ae^{-k_1 t} + (1-a)e^{-k_2 t} \quad \text{.....(2)}$$

where C_0 is the initial concentration of chlorpyrifos-ethyl (mg/kg), **C,** is the concentration at time ***t*** (mg/kg), ***t*** is the post application time (days), k_1 and k_2 are fast and slow dissipation rate constants, and **a**, is a constant.

Figures 1, 2 and 3 show the nonlinear relationships together with the values of the statistical parameters calculated for chlorpyrifos-ethyl using the model. The biphasic shape of chlorpyrifos-ethyl dissipation curves show the two-phase dissipation of insecticides in crops, with an initial period of fast insecticide loss followed by a phase of slower dissipation. The dissipation occurred through volatilization and degradation of the insecticide in the crop. During the experiment, there was no significant off-site movement of in-furrow irrigation water. Therefore, there was little potential for chlorpyrifos-ethyl foliar wash-off which could affect the results of the study.

Several authors (Farmer et al, 1972; Spencer et al, 1973 and Roger and Bhuiyan, 1995) have held the concept that volatilization is a significant route of insecticide loss in the field, particularly when it is applied to the surfaces of soils or plants.

The findings of this experiment could explain why farmers in the tropics find it difficult to control insect pests with the recommended rate of application since volatilization and degradation of the chemical (which may be influenced by tropical temperatures) could reduce the concentration of the active ingredient making it less effective to control insect pests. As a result, farmers in their desperate attempt to protect their investments apply unnecessarily large quantities of insecticides leading to high residues in crops and contamination of the environment.

5. Conclusions and Recommendations

The study is the most recent and comprehensive studies on the fate of organophosphate in the tropics. The results revealed that the dissipation of insecticides occur through volatilization and degradation of the insecticide in the crop which may be influenced by temperature. It is therefore important that the Environmental Protection Agency and the Pesticides Technical Committee demand studies on the rate of volatilization of agrochemical as part of the bioefficacy trials done by local researchers. This will help in the determination of the correct application rates under tropical conditions that can leave a desirable amount of the agrochemical in the crop to control insect pests and diseases. It is believed that when this is done it will reduce incidence of pest resistance and pest resurgence in the country.

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