



Chlorothalonil Dissipation as Influenced by Growing Season and Cultivation Systems on Green Mustard and Soil

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Received: 10 February 2022 / Accepted: 18 May 2022 / Published online: 13 June 2022
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Abstract

In this study, the dissipation pattern of the fungicide in green mustard and soil was studied through field trials experiments conducted under the net house and open field in wet and dry seasons. Field trial results were then compared with data collected from laboratory trials conducted under controlled conditions. Chlorothalonil residue showed 99.5–99.7% loss in the dry season while in the wet season the residue loss was 94.37–99.8% under the net house and open field, respectively. The half-life of chlorothalonil in the green mustard planted in the net house and open field were 1.03 and 0.86 days in the dry season, while in the wet season, it was 2.21 and 2.02 days. The terminal residue of chlorothalonil exceeded the Maximum Residue Limit of 0.01 mg kg⁻¹, which violates the National Food Act 1983 and food safety regulation. This work is significant in the agriculture sector to provide a better insight into pesticides fate and establish the correct pre-harvest intervals for chlorothalonil on green mustard, thus reducing the pesticide toxicity effect among consumers.

Keywords Pre-harvest Interval · Environmental sustainability · Fungicide · Humid tropics · Photodegradation

1 Introduction

Numerous fungal diseases and pathogens infecting vegetables, fruits, turf and ornamentals are common in many countries including Malaysia. The warm and humid climate favour the resurgence of pests and plant diseases, which led farmers to rely on pesticides for crop protection [1, 2]. Chlorothalonil (2, 4, 5, 6-tetrachloroisophthalonitrile) is a foliar, non-systemic and widely applied chlorinated fungicide [3].

It has low solubility in water (0.81 mg L⁻¹) and vapor pressure of 7.62×10⁻⁵ mPa at 25 °C [4].

Chlorothalonil can disturb the nitrogen cycle, cropping process and crop growth by affecting bacterial growth in soil and producing unwanted residues [5]. Several studies have been conducted to understand the dissipation fate and behaviour of chlorothalonil in crops under both tropical and temperate climates (Table 1).

The dissipation fate and behaviour of chlorothalonil in crops were reported to be diverse and temperature-dependent [6, 7]. A field study conducted on the dissipation fate and behaviour of chlorothalonil for tropical vegetables such as the leafy brassicas cultivated under humid tropical climates is scantily available. The dissipation pathways of chlorothalonil in crops such as enzymatic degradation [8], photodegradation, volatilisation, plant growth dilution, bound residue formation (penetration) and washed-off by rainfall could have taken place simultaneously [9].

Computer simulation results for chlorothalonil suggested penetration into the plants is by far the most important process (total of 43.0% of the dosage applied), followed by wash-off by rains (computed to be 26.3% of the dosage applied) and photodegradation (15.9% of the dosage applied). The volatilisation in the initial period is slightly higher than that derived from the measurements.

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Table 1 Dissipation of chlorothalonil effects study in plants

Plants	Conc. (mg kg ⁻¹)/dose (kg ha ⁻¹)	LOQ (mg kg ⁻¹)	LOD (mg kg ⁻¹)	Recovery (%)	RSD (%)	Half-life (t _{1/2})	MLR (mg kg ⁻¹)	PHI (days)	References
Button mushroom	0.01–5 ^a	0.0063700	0.0019100	–	–	1.05	–	–	[16]
Tomato (O.F)	0.84 ^b	–	–	–	–	–	2.0	20 (14)	[17]
Tomato (greenhouse)	–	–	–	–	–	5.00–5.32	–	49.72–74.73%	[18]
Prickly pear	–	–	0.00005	86.5	7.35	–	–	–	[19]
Shallot	–	0.04	0.012	104.9	2.7	2.8	–	–	[20]
Cucumbers	–	0.02	0.0025	86.2–103.3	4.6	1.6	–	–	[21]
Cabbage	–	0.05	0.005	96	3	1.7	–	–	[22]
Pepper fruit	–	0.05	0.01	88.27–108.01	4–12	1.83	0.05	10	[23]
Apple	–	2.0	0.025	90–97	–	–	84.5–91.1	–	[24]

LOQ limit of quantification, LOD limit of detection, MLR maximum residue limit MLR, RSD relative standard deviation, PHI pre-harvest intervals

^aConcentration

^bDose

Computed cumulative volatilization however gave only 5.2% of the dosage applied [10]. Photodegradation is another predominant way of chlorothalonil dissipation from the plant [11]. The efficiency of the dissipation pathways of chlorothalonil however appeared to be governed by several factors such as climate condition and growing system, as these would affect surrounding temperature, humidity, rainfall intensity and sunlight radiation intensity [9, 12].

Based on Codex Alimentarius Commission (FAO/WHO), the maximum residue limit (MRL) of chlorothalonil in green mustard is 0.01 mg kg⁻¹. Green mustard, *Brassica juncea* (L.) *Coss.* is a widely cultivated crop [13] in Malaysia, both for domestic consumption and exports to neighboring countries such as Singapore and Brunei. Green mustard has a very short cycle (est. 1 month) and is grown throughout the year either in a net house and open field [14]. There are reported cases of MRL violations in chlorothalonil usage on vegetables [15]. The existing application dosage and pre-harvest interval data provided on the pesticide's label are believed to be extrapolated based on the studies from temperate countries. Although good agriculture practice has been strictly followed, violation cases involving chlorothalonil residues in vegetables are still commonly discovered.

Herein, we report on the dissipation rate, half-life (t_{1/2}) and pre-harvest intervals (PHI) of chlorothalonil in green mustard and soil-based under humid tropical climate. These parameters are significant to establishing the correct PHI for chlorothalonil used on green mustard, thus reducing the pesticide toxicity effect among consumers due to prolonged consumption of vegetables containing high pesticides residue.

2 Materials and Methods

2.1 Preparation of Organic Amendments

A commercial formulation of DICONIL 50 EC (Chlorothalonil 50% w/w) was purchased from the local market. Analytical grades of acetonitrile, dichloromethane, *n*-hexane, acetic acid glacial, sodium chloride, and anhydrous magnesium sulfate were purchased from J.T. Baker, USA. The primary-secondary amine (PSA) was purchased from Varian, USA. Chlorothalonil reference standard (98.2%) was obtained from Sigma Aldrich, Steinheim, Germany. Chlorothalonil stock solution of 100 mg L⁻¹ was prepared in *n*-hexane. Working solutions of 10 mg L⁻¹ and 1 mg L⁻¹ were obtained by dilution of an appropriate amount of stock solutions in *n*-hexane. All standard solutions were stored in the dark at 4 °C.

Robot Coupe chopper (Vincennes Cedex, France) was used to chop the vegetable samples, while a Thermo Jouan model B41 centrifuge (Gontier, France) was used to centrifuge the sample. An Agilent Model 6890 gas chromatography (GC) equipped with an electron capture detector (ECD) was used for the analysis of chlorothalonil. This instrument was configured with a non-polar fused silica capillary column ultra 1 (25 m × 32 mm and 0.5 μm) obtained from J & W Scientific. A more polar capillary column, SPB 608, 30 m × 0.53 mm × 0.5 μm obtained from J&W Scientific was used for the confirmation of chlorothalonil. The column temperature was maintained at 120 °C for 0.5 min (10 °C min⁻¹) to 180 °C, followed by another temperature ramp to 240 °C

(6 °C min⁻¹), 280 °C (10 °C min⁻¹) and held constant at 280 °C for 12 min. The injector (in splitless mode) and detector temperatures were maintained at 260 °C and 300 °C, respectively. Nitrogen with a flow rate of 1.2 mL min⁻¹ was used as carrier gas.

2.2 Field Trials and Sampling for Green Mustard and Soil

The field trials were conducted at Agriculture Research Centre, Semongok, Kuching Malaysia (N01°23'05.9", E110°19'44.7') in N.H and O.F. The first experiment held in July 2013 was for the dry season followed by another batch in November 2013 for the wet season. N.H was constructed using 40 mesh polyethylene netting which provides 25–30% shading. The size of each bed was approximately 5.2 m × 1.2 m. Vegetable beds were cleared, tilled, and chicken manure was incorporated into the soil at 0.5 kg m⁻². Three beds were treated with chlorothalonil and three beds were kept aside as a control. Chlorothalonil was applied three times at 2 g L⁻¹ using a knapsack sprayer. The first application was carried out one week after seeds were broadcasted and the subsequent spray was performed at weekly intervals. The final spray was carried out on day 21 and the first green mustard sample was collected 2 h after the final spray (day 0). For each bed, 1 kg of green mustard was sampled randomly on days 0, 1, 3, 5, 7, 9, 11, 15, and 22 for chlorothalonil residue analysis. The roots were removed from the green mustard samples. The three replicates were combined, chopped and mixed homogeneously. Similarly, 0.5 kg of the surface soil sample at 2 cm depth was collected between the green mustard plants using a clean spade. The soil samples from the three plots with similar pesticide formulations were combined and mixed homogeneously before pesticide residue analysis. Debris and stones were also removed from the soil. The plant and soil samples were weighed (10 g for each replicate), packed and stored in a freezer at - 20 °C for further analysis.

The chemical analysis of chlorothalonil on green mustard was validated following the SANCO guidelines (2011) [25]. Green mustard samples which were free from pesticide contamination were homogenized and fortified with an appropriate amount of chlorothalonil standard solutions to obtain fortification levels of 0.01 mg kg⁻¹, 0.05 mg kg⁻¹, 0.1 mg kg⁻¹, 0.5 mg kg⁻¹ and 1.0 mg kg⁻¹. These fortification levels are selected to determine the suitability of the method to detect and quantify chlorothalonil in green mustard at maximum residue limits (MRLs). Each concentration level was prepared in three replicates and blank samples were used as control.

2.3 Parameter for Soil Physicochemical Properties Examined

This study was conducted at Agriculture Research Centre, Semongok (N01°23'05.9", E110°19'44.7') in August 2013 for the dry season and October 2013 for the wet season. The soil is classified as clayey red-yellow podzolic soil (typic paleudults, very fine, mixed and isohyperthermic) formed from sedimentary rock). The topsoil physicochemical properties at 0–20 cm depth adapted from existing data [26] are shown in Table 2.

The study was conducted in the net house (N.H) and open field (O.F). The size of each plot was approximately 15 m × 15 m with sixteen beds measuring about 5.2 m × 1.2 m each (in N.H) and 5.2 m × 1.2 m (in O.F). The vegetable beds were cleared, tilled, and chicken manure was incorporated into the soil at 0.5 kg m⁻². The green mustard was sprayed three times. The first spraying was carried out one week after seeds were broadcasted. The second spray was performed 1 week later and the final spray was on the 21st day after seeds were broadcasted. The pesticide formulation used for this study was chlorothalonil, Daconil 2787 (chlorothalonil 50% w/w). Pesticide application was carried out in three replicates (three beds) and the three remaining beds were kept aside as a control. Climate data were obtained from the local meteorological department throughout the experimental period.

2.4 Extraction of Green Mustard, Soil and Clean-Up Analysis

Chlorothalonil in green mustard and soil was extracted using an established method [27]. Ten grams of the homogenized sample was weighed into a 50 mL Teflon centrifuge tube. Acetonitrile (15–20 mL) containing 1% acetic acid was

Table 2 Physicochemical properties of Semongok topsoil at 0–20 cm depth [26]

Parameter	Semongok topsoil physicochemical properties
pH ^a	4.8
% carbon ^b	2.20
% clay ^b	23.1
% silt ^b	29.6
% fine sand ^b	9.8
% coarse sand ^b	37.6
Moisture content ^c	30%

^apH was determined in 0.01 M CaCl₂ in a 1:1 soil:water suspension

^bMass percentage of carbon determined by dry combustion

^cMoisture content was determined by oven drying and gravimetric analysis

added to the sample and the sample extract was shaken vigorously by hand for 1 min. Anhydrous magnesium sulfate (5–6 g) and sodium chloride (1.5 g) were then added to the sample. The sample extract was vortexed and centrifuged at 2500–3000 g for 1 min each. The supernatant was transferred into another Teflon centrifuge tube, shaken with anhydrous magnesium sulfate (3 g), vortexed and centrifuged at 3000 g for 1 min. The extract (2 mL) was taken and eluted through activated silica gel (0.2 g packed in a 2 mL glass Pasteur pipette). The eluate was left in the fume hood to dry, makeup with 2 mL n-hexane and injected into a gas chromatograph-electron captured detector (GC-ECD) for qualification and quantification of chlorothalonil. The experimental work plan and analysis of chlorothalonil in green mustard and soil are shown in Fig. S1.

2.5 Lab Incubation Study

An amount of 1.5 mL of 50 mg L⁻¹ individual pesticide standard was spiked onto 10 g soil samples prepared in 330 individual vials. The pesticide was prepared in triplicates with 1 sample blank for each batch of samples. Spiked samples were vortexed for 1 min and left in a fume hood for 1 h. Samples were covered with aluminium foil, prick on top to allow aeration and incubated under 25 °C in the dark. Water content was monitored weekly and added accordingly to the sample weight lost. Samples were taken for analysis on days 1, 3, 9, 15, 25, 30, 50, 70, 85 and 100 using the soil extraction method as described earlier.

2.6 Method Development and Validation

Homogenized green mustard and soil were fortified with an appropriate amount of chlorothalonil standard solution to

obtain 0.01 mg L⁻¹, 0.05 mg L⁻¹, 0.1 mg L⁻¹, 0.5 mg L⁻¹ and 1.0 mg L⁻¹, respectively. Each concentration level was prepared in triplicates. Blank samples were also analysed in three replicates as control. After spiking, the samples were left in the fume hood (1 h) to allow solvent evaporation and pesticide interaction with the sample.

3 Results and Discussion

3.1 Method Validation of Pesticides in Green Mustard and Soil

The recoveries obtained for chlorothalonil fortified on green mustard samples at concentration levels of 0.01 mg L⁻¹, 0.05 mg L⁻¹, 0.1 mg L⁻¹, 0.5 mg L⁻¹ and 1.0 mg L⁻¹ were 89.45%, 82.62%, 88.39%, 91.04% and 82.62%, respectively (Table 3). While in soil, the recoveries obtained were 76.51%, 77.15%, 83.97%, 80.97% and 82.62% at 0.01 mg L⁻¹, 0.05 mg L⁻¹, 0.1 mg L⁻¹, 0.5 mg L⁻¹ and 1.0 mg L⁻¹ concentration level, respectively. All recoveries were within the acceptable range of 70–120% following the analytical quality control and validation procedure for pesticide residue analysis in food and feed [25] (Fig. 1). The relative standard deviations (RSD) obtained in this study were ≤ 20%. Linearity was accomplished by using a standard curve, which was prepared by serial dilution of chlorothalonil working stock solution to 5 concentration levels ranging from 0.01 to 1.0 mg L⁻¹. Good linearity was achieved with a correlation coefficient of 0.996. Method selectivity was assessed by comparing the chromatogram of the blank green mustard sample with the corresponding fortified samples (Fig. S2). No interference of matrices with chlorothalonil

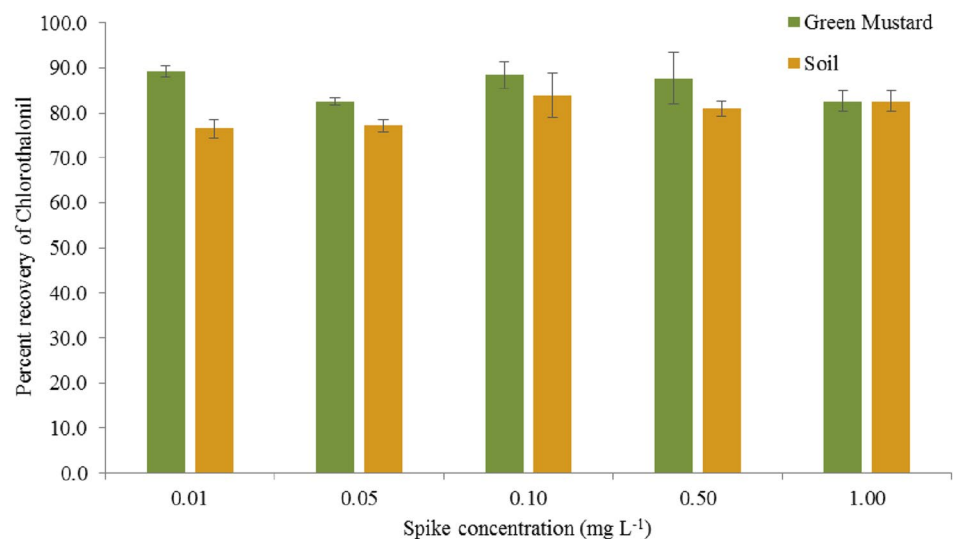
Table 3 Recoveries of chlorothalonil in Semongok mineral green mustard and soil

Chlorothalonil	Spiking level (mg L ⁻¹)	% Recovery (n=3) ^a	SD	RSD (%)	LOD (mg kg ⁻¹)	LOQ (mg kg ⁻¹)	Linearity	MRL (mg kg ⁻¹)	MDL (mg kg ⁻¹)
Green mustard	1.0	82.62	2.26	2.74	0.0077	0.001	0.996	0.01	0.0076
	0.5	91.04	11.42	12.54					
	0.1	88.39	3.02	3.42					
	0.05	82.62	0.82	0.99					
	0.01	89.45	0.73	0.81					
Soil	1.0	82.62	2.26	2.74	0.0077	0.001	0.996	N.A	0.0076
	0.5	80.97	1.80	2.22					
	0.1	83.97	4.86	5.79					
	0.05	77.15	1.39	1.79					
	0.01	76.51	2.05	2.68					

SD standard deviation, RSD relative standard deviation, MRL maximum residue limits, MDL, method detection limit, LOQ limit of quantification, LOD Limit of detection, N.A not available

^aEach value is a mean of three replicates

Fig. 1 Recovery study of chlorothalonil in green mustard and soil. Vertical lines represent standard deviation of replicates ($n = 3$)



was observed. The method detection limit (MDL) was $0.0076 \text{ mg kg}^{-1}$.

Recoveries of chlorothalonil in soil fortified at concentration levels of 0.01, 0.5, 0.1, 0.5, and 1.0 mg kg^{-1} were shown in (Table 3). Recovery obtained for soil sample spiked at 0.01 mg kg^{-1} was 76.51%, 77.9% for 0.05 mg kg^{-1} , 85.5% for 0.1 mg kg^{-1} , 80.6% for 0.5 mg kg^{-1} , and 83% for 1.0 mg kg^{-1} , respectively. The recoveries of chlorothalonil were in the acceptable range of 70–120% with RSD < 15% [25]. Blank soil samples were analysed to assess selectivity and matrix effect. Results obtained showed no interference with the targeted compound.

Injudicious use of pesticides by farmers led to the presence of unwanted residue in food crops produced which may pose risks to human health [28]. Besides, extensive violation of pesticide usage may also cause deleterious effects on the environment [29]. Pesticide's fate and behaviour vary

greatly on the rate and frequency of application, pesticide formulation, crop morphology and weather conditions [30]. Therefore, pesticide fate and behaviour need to be evaluated individually on specific crops and under specific environmental conditions predicted to show a similar pattern of dissipation on green mustard during the two seasons. However, the dissipation rate of chlorothalonil may be different due to its physicochemical properties.

3.2 Field Result of Green Mustard and Soil

The dissipation curves of chlorothalonil in N.H and O.F during dry and wet seasons in green mustard and soil are shown in Fig. 2a, b and Fig. 3a, b, respectively. Chlorothalonil dissipated exponentially against time in both N.H and O.F during both seasons (Tables S1–4).

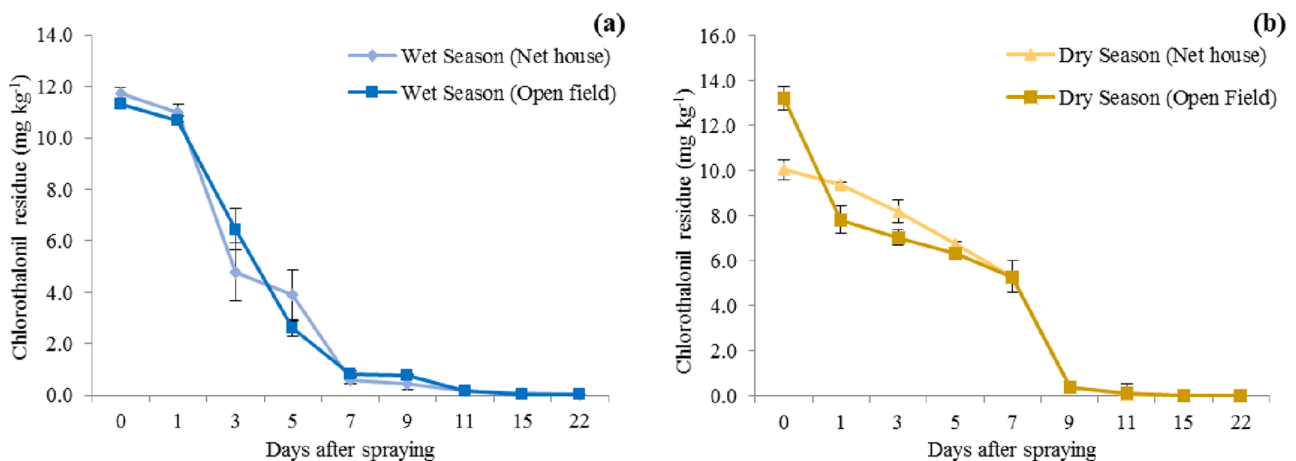


Fig. 2 The dissipation curves of chlorothalonil residue in green mustard cultivated in net house and open field during dry (a) and wet (b) season. Error bars represent standard errors for values of three replicates

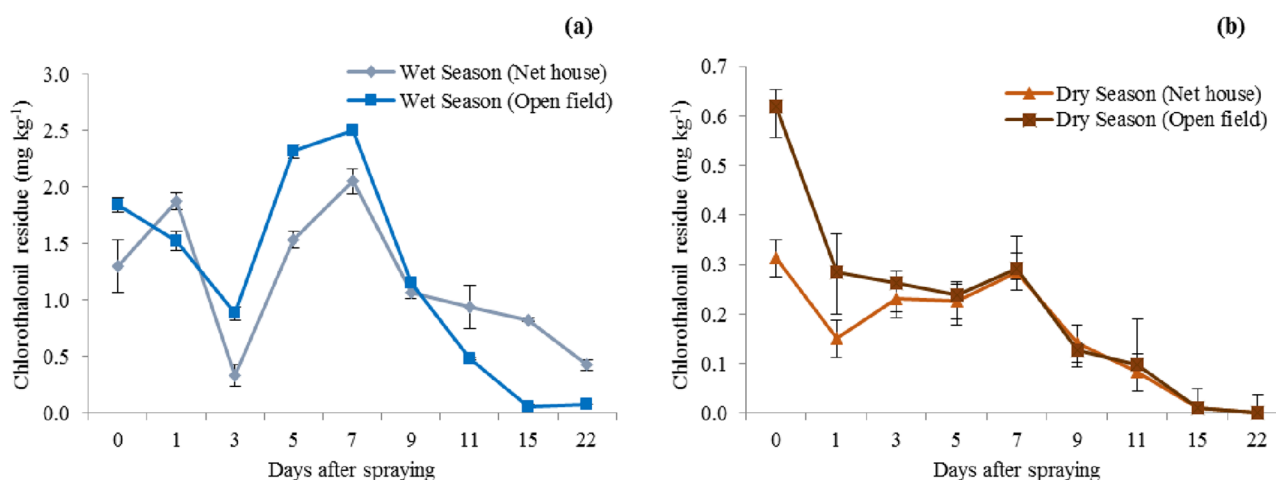


Fig. 3 The dissipation curves of chlorothalonil in cropped topsoil under the net house and open field cultivation system during the dry season (a) and wet season (b). Error bars represent standard errors for values of three replicates

3.2.1 Dissipation Behaviour in Green Mustard During Dry Season and Wet Season

During the dry season, initial concentrations of chlorothalonil detected in green mustard were 10.03 mg kg^{-1} (N.H) and 13.22 mg kg^{-1} (O.F). While during the wet season, chlorothalonil concentrations of 11.72 mg kg^{-1} and 11.33 mg kg^{-1} were detected in N.H and O.F, respectively. Numerous factors lead to the toxicity i.e., concentrations, duration and dosage of chlorothalonil to discharge and frequency of exposure [31].

After 7 days, chlorothalonil concentration declined to 5.29 mg kg^{-1} and 5.24 mg kg^{-1} both in N.H and O.F. This accounts for 47.3% and 60.4% reduction in concentration for N.H and O.F. Meanwhile, during the wet season, chlorothalonil residue declined to 0.59 mg kg^{-1} in N.H resulting in 95% of residue loss. In the O.F, residue declined to 0.82 mg kg^{-1} , resulting in a 92.8% loss. The pronounced loss observed could be due to greater rainfall frequency throughout the wet season. During the dry season, rainfall averaged 46.3 mm per day (excluding day 7) and doubled to 84.9 mm during the rainy season. This observation suggests that rainfall could be the main factor that contributed to rapid chlorothalonil dissipation in the wet season [10, 32]. Another factor that contributed to the dissipation of chlorothalonil could be the growth dilution from the rapid growth of the green mustard plant.

On day 22, almost all chlorothalonil residues dissipated in both seasons and cultivation systems (Figs. 4, 5). A total of 99.2–99.7% residues loss was observed during the dry season and 99.8–99.9% during the wet season in both N.H and O.F, respectively. Terminal residue concentration of chlorothalonil was found to be higher during the dry season where the remaining residue concentrations

detected were 0.08 mg kg^{-1} and 0.04 mg kg^{-1} for N.H and O.F. During the wet season, lower chlorothalonil residue concentrations of 0.02 mg kg^{-1} and 0.01 mg kg^{-1} were detected on green mustard in the N.H and O.F, respectively. The dry season has less frequent rainfall compared to the wet season. The continuous rainfall during the wet season may cause more bound residues to be washed off from the plant (Table S5).

Although rainfall intensity plays an important role in dislodging the residues from leaf surfaces, longer rainfall duration seemed to cause a more pronounced loss of chlorothalonil [33]. Despite the frequent rainfall from day 12–19 (average 197.8 mm) during both dry and wet seasons, the chlorothalonil residues nevertheless were detected above the permissive level (MRL) of 0.01 mg kg^{-1} (Table S1). Chlorothalonil has low water solubility [34] and a tendency to diffuse into the inner part of the plant, which would therefore inhibit its removal via rainfall wash-off [28]. It was reported that penetration of chlorothalonil and formation of bound residues led to a slower rate of dissipation hence volatilisation, wash-off and photodegradation were no longer effective [33].

The dissipation of chlorothalonil in green mustard was fitted into the first-order kinetic equation. Calculated half-lives of chlorothalonil in green mustard during the dry season were 1.03 and 0.86 days in the N.H and O.F, respectively. While, during the wet season the calculated half-lives of chlorothalonil were 2.21 days and 2.02 days in the N.H and O.F, respectively (Table 4). In other words, the half-lives of chlorothalonil in green mustard planted during the wet season were longer than in the dry season. Despite a faster dissipation rate due to rainfall events during the wet season, higher surrounding temperature and longer sunshine during the dry season might have accelerated the whole dissipation

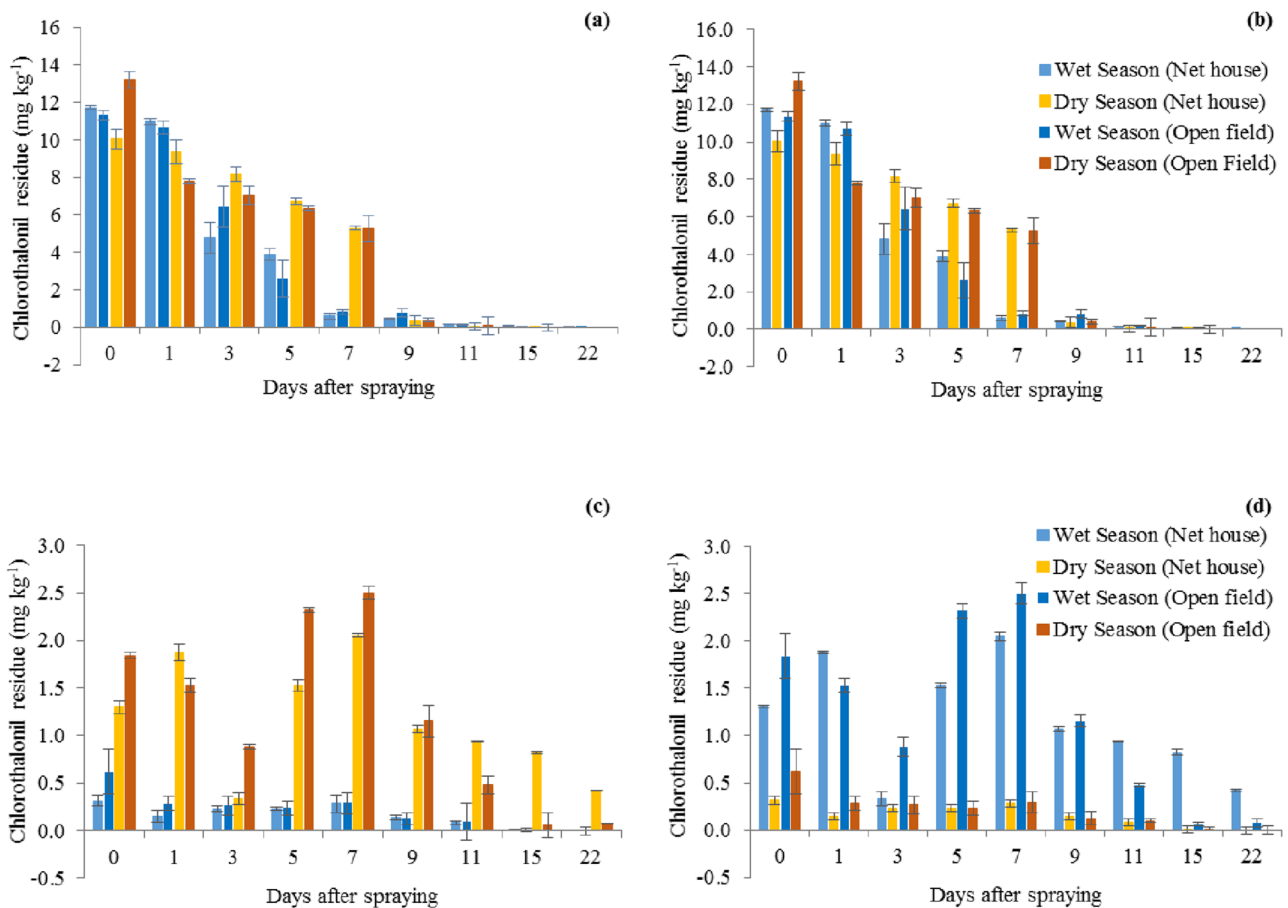


Fig. 4 Seasonal and cultivation system effect on chlorothalonil dissipation in green mustard (a, b) and soil (c, d). Error bars represent standard errors for values of three replicates

process, which resulted in a shorter half-life of chlorothalonil compared to the wet season.

Both seasons showed slightly shorter half-lives of chlorothalonil (0.86 and 2.02 days) in the O.F compared to N.H (1.03 and 2.21 days). It was envisaged that the shading effect in N.H reduced the intensity of both sunlight radiation and rainfall, which is believed to be some of the important factors required to facilitate the dissipation of chlorothalonil from green mustard. These findings were similar to the earlier studies on the effect of polyethylene cover which decreased solar radiation intensity and photodegradation of pesticide residue on crops [16]. It is suggested that chlorothalonil dissipation pathways from plants mainly occurred via photodegradation, washed off by rains and penetration. Other dissipation pathways such as volatilization and chemical degradation might also have taken place at a much slower rate [11]. Chlorothalonil half-lives on green mustard obtained from this study were found to be shorter compared to other previously studied on other crops such as cucumber [12], tomatoes [21] and cabbage [35] under tropical and temperate climates. In other words, the dissipation fate of

chlorothalonil varies greatly under certain conditions as well as between certain types of plants. A higher temperature climate seemed to increase the dissipation rate and thus shorten the PHI required.

3.2.2 Dissipation Behaviour in Soil During Dry Season and Wet Season

Rainfall and cultivation systems used were likely to affect Chlorothalonil dissipation from topsoil via different physicochemical properties. Chlorothalonil degradation in the soil has been widely studied because of the interest in its metabolites formation in soil, which is more stable and toxic than the parent compound itself [9]. Chlorothalonil metabolite of 4-hydroxychlorothalonil, for instance is 30 times more toxic in acute toxicity and mobile in soil and water than the parent compound [36].

The trend of chlorothalonil dissipation in Semongok cropped topsoil is depicted in Fig. 3. Chlorothalonil showed a non-linear dissipation pattern which could be due to the uneven amount of deposit distribution on soil

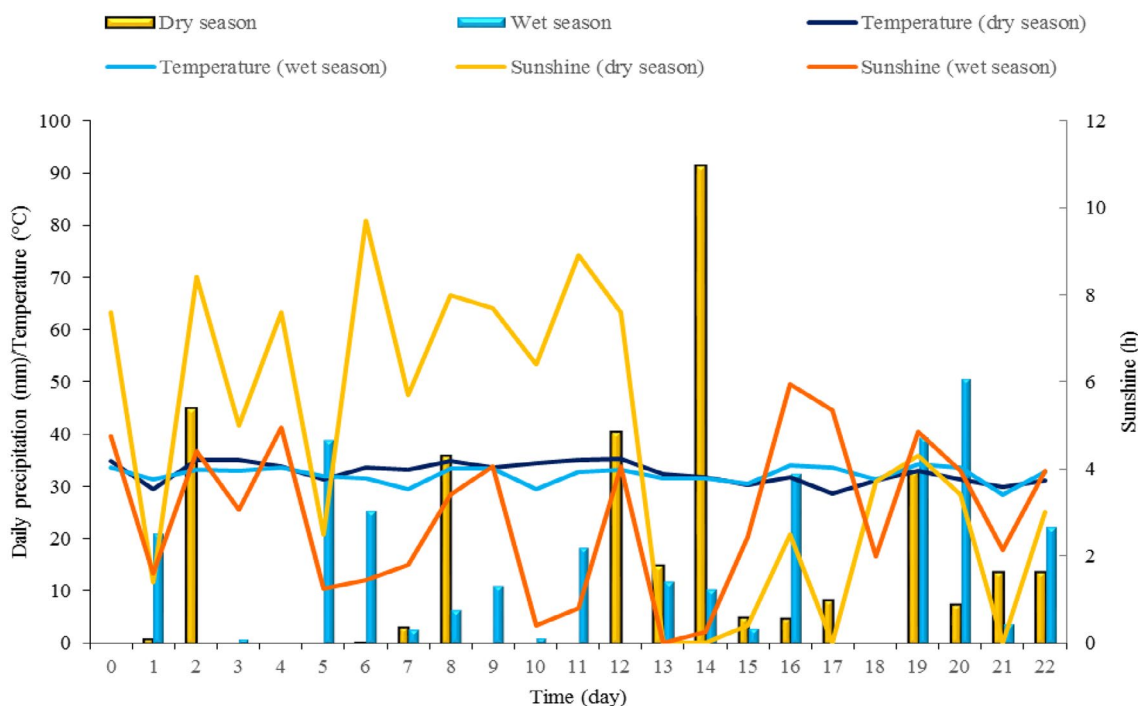


Fig. 5 Metrological data collected throughout the experiment period

Table 4 Linear regression equation and half-life of chlorothalonil in green mustard and soil in N.H and O.F during dry and wet season

Study	Season	Cultivating system	Linear regression equation	R ²	Half-life, t _{1/2} (day)
Green mustard	Dry season	N.H	$C = 10.03 e^{-0.6760t}$	0.8787	1.03
		O.F	$C = 13.22 e^{-0.8038t}$	0.8887	0.86
	Wet season	N.H	$C = 11.72 e^{-0.3133t}$	0.9525	2.21
		O.F	$C = 11.33 e^{-0.3448t}$	0.9782	2.02
Soil	Dry season	N.H	$C = 1.30 e^{-0.0411t}$	0.2176	16.9
		O.F	$C = 1.84 e^{-0.1673t}$	0.7145	4.14
	Wet season	N.H	$C = 0.39 e^{-0.1790t}$	0.6737	3.87
		O.F	$C = 0.62 e^{-0.2144t}$	0.8097	3.23

Net house (N.H) and open field (O.F)

due to the green mustard canopy enclosure. The difficulty of accurately and evenly applying pesticides through a growing crop onto the soil variation may cause uneven deposits in different sampling spots on the soil [37].

The initial concentration of chlorothalonil detected in topsoil was 1.30 mg kg⁻¹ in N.H and 1.84 mg kg⁻¹ in O.F, respectively. After 24 h, analysis of topsoil sampled from N.H showed a slight increment in concentration, while O.F showed decreasing concentration after 24 h (Fig. 3). The humid condition under N.H is believed to enhance the chlorothalonil degradation. This phenomenon could be due to the dislodged of chlorothalonil residue from green mustard which led to chlorothalonil accumulation in the

topsoil. Besides, the hermetic condition of N.H may limit the chlorothalonil volatilization [18].

There was a steep drop in residue concentration from soil collected on day 1 and day 3. This was presumed to be due to heavy rainfall on day 2. Similar studies reported a huge drop in pesticide residue concentration in topsoil, especially on polar pesticides due to the rainfall that drew the pesticides downward into a deeper soil layer leading to dissipation from the topsoil layer [38]. As the rainfall continues, the concentration continued to decline. Therefore, during the wet season, volatilization of chlorothalonil from the soil is not likely to occur as it has leached into a deeper layer of soil.

On day 5, the concentration of chlorothalonil residue increased in both N.H and O.F. The residue continues increasing on day 7 in which 2.05 mg kg^{-1} was detected under N.H and 2.50 mg kg^{-1} in O.F. The increasing concentration of chlorothalonil in topsoil on day 5–7 was attributed to chlorothalonil washed off from the green mustard by a stint rainfall event on day 6. As dewfall was reported to be sufficient enough to dislodge the pesticides from the leaf surface, a small amount of rain should be able to wash off the chlorothalonil from green mustard into the soil. Besides, the possible occurrence of the ‘wick effect’ phenomenon where chlorothalonil residues are drawn upward toward the soil surface led to residue accumulation in topsoil [38].

From day 9–22, chlorothalonil declined continuously in topsoil. Heavy downpour on day 8 (35.9 mm) was suspected to leach down the chlorothalonil residue into a deeper layer of soil and reduced its concentration in topsoil. Apart from leaching, the preferential flow may cause rapid dissipation of chlorothalonil from topsoil as the chlorothalonil tend to form bound residues in soil [16]. Topsoils with high moisture contents may induce macropore flow which facilitates pesticide migration in solution or as sorbed particles [15]. Slow and continuous dissipation of chlorothalonil on day 9–22 could be partially attributed to biodegradation. This is because biodegradation is always correlated to the slower dissipation process of pesticides in soil [39]. Soil moisture, soil organic matter content and soil temperature are the key parameters for the activity of pesticides degrading microbes in the soil. Linear correlation between soil temperature and mineralization rate of pesticides in soil reaffirmed the crucial role of temperature in pesticides biodegradation [40]. Overall, faster dissipation trend was observed in O.F compared to N.H during the dry season (Fig. 3a).

During the wet season, initial chlorothalonil residue detected in topsoil was much lower compared to the dry season. Under N.H, chlorothalonil residue was 0.39 mg kg^{-1} while in O.F chlorothalonil residue was 0.62 mg kg^{-1} (Fig. 3b). Chlorothalonil residue showed steep drop on day 1, which accounted for 61% of the initial concentration loss under N.H from 0.3933 to $0.1500 \text{ mg kg}^{-1}$ and 55% loss in O.F, from 0.6200 to $0.2850 \text{ mg kg}^{-1}$. The high surrounding temperature ($28.8 \text{ }^\circ\text{C}$) recorded within this period may have caused rapid chlorothalonil losses via volatilization [41]. Besides, the formation of bound residue may have started within 24 h after the last application as higher temperature accelerates pesticide binding to soil particles [42].

Chlorothalonil concentration rose slightly on day 3 under N.H condition, but continuously declined in O.F. Heavy downpour on day 2 (20.9 mm) was suspected to wash chlorothalonil residue from green mustard into the topsoil. More intense rainfall in O.F however might have caused some amount of chlorothalonil to leach into a deeper layer of soil [38]. On day 7, chlorothalonil concentration in topsoil

increased under both cultivation systems. This trend was due to residue wash-off from the plant and the ‘wick effect’ following continuous rainfall on day 5 and day 6. Chlorothalonil residue declined from day 9 onwards, whereas the terminal residue was no longer detectable on day 22.

During the dry season, chlorothalonil residue was still detectable on day 22, while during the wet season, the residue completely dissipated on day 22. Chlorothalonil was reported to dissipate mainly by abiotic processes, volatilization and biodegradation [36]. Moreover, the formation of bound residue is also frequently correlated with the fast dissipation of chlorothalonil in soil [43]. Bound residue formed caused pesticides to be less accessible to degradation, and therefore the residue in this study was still detectable on day 22.

3.2.3 Degradation of Chlorothalonil in Tropical Soil Under Laboratory Condition

The dissipation of pesticides in soil under field conditions involved several processes such as degradation, leaching, volatilization and surface run-off. These processes are dependent on environmental factors such as soil type, climate, and cultivation system [38]. The complicated overlapping processes and factors make further interpretation of dissipation dynamics rather difficult. By conducting the study under standardized laboratory conditions, some factors can be isolated to understand some specific processes and conditions that affect the rate of chlorothalonil dissipation in soil [39].

Several studies reported a shorter pesticide dissipation rate under field conditions compared to laboratory conditions due to several processes such as surface run-off, leaching, and volatilization [38]. Variation and fluctuation of soil moisture and temperature in the field may also lead to enhanced microbial and chemical degradation. Besides, sunlight radiation promotes soil surface photolysis, which is absent under laboratory conditions [39]. Chlorothalonil showed an exponential dissipation curve in Semongok soil (Fig. 6) with an initial chlorothalonil concentration of 4.05 mg kg^{-1} . The lower concentration recovered for chlorothalonil was due to the possible formation of non-extractable bound residue in soil which may require a rigorous acids extraction [43].

In the first 9 days, half of the initial concentration had dissipated which accounted for a 46% loss in concentration. The fast initial dissipation of chlorothalonil in incubated soil was due to the possible volatilization losses [39]. A pesticide with high vapour pressure tends to be volatile under $25 \text{ }^\circ\text{C}$. Besides, the chlorothalonil losses could also be attributed to the formation of bound residues. Bound residue formation may hinder microbial degradation as the pesticides became less accessible to the soil

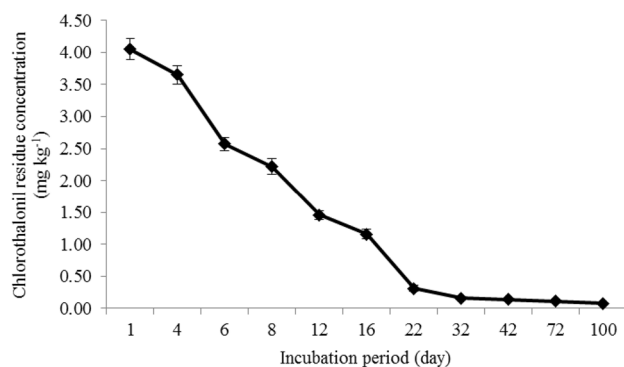


Fig. 6 Chlorothalonil degradation trend in fresh Semongok field soil incubated in the darkness at 25 °C with a relative humidity of 80% and soil moisture 30%. Error bars represent standard errors for values of three replicates

microbes thus impeding further degradation [23]. After the first week, the chlorothalonil dissipation rate was observed to be reducing. As the pesticide started to bind to soil particles, it became less available for microbial degradation. Although mineralization might occur, the rate was rather slow due to the formation of bound residues [43]. Limited microbial community in incubated soil might have also led to a slower dissipation of chlorothalonil in the incubation study.

On day 32, chlorothalonil exhibited a higher rate of dissipation with a 93% loss of the initial concentration. Chlorothalonil is more susceptible to binding with the soil particles, thus less available for degradation by the soil microbes [23]. On day 100, chlorothalonil showed the highest loss with 98% of the initial concentration. The rapid chlorothalonil dissipation could be due to the formation of bound residue in the soil that is responsible for fast dissipation [18]. Other than photodegradation, the volatilization rate under field conditions is higher compared to the laboratory conditions. The losses of chlorothalonil in soil under field conditions were observed to be higher than in the incubated soil. Some pesticides with a low vapour pressure can volatilize under field conditions [39]. A higher dissipation rate under field conditions can be correlated with the presence of sunlight and wind which favour photodegradation and volatilization.

The half-life calculated for chlorothalonil in Semongok incubated soil was 15.2 days. Hypothesis testing suggests that the dissipation rate of chlorothalonil in incubated Semongok soil and field study was significantly different. Variation in the degradation rate of chlorothalonil in soil under controlled conditions and field study suggested the significant influence of climate conditions on the degradation rate of chlorothalonil in soil. Besides, climate conditions have also proven to be essential in dictating chlorothalonil dissipation rate from green mustard.

4 Conclusion

The dissipation mechanism of chlorothalonil from green mustard was successfully investigated. Faster dissipation and shorter half-life of chlorothalonil were observed in the tropical and hotter region compared to the colder climate. Chlorothalonil has a shorter half-life in crops with higher water contents. The dosage and the frequency of pesticides should be based on the types of crops and climate conditions to avoid residue violations that may cause adverse health effects after prolonged consumption of vegetables containing high pesticide residue. This is imperative to ensure food safety as well as optimize the effect of chlorothalonil for crop protection purposes.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42250-022-00384-7>.

Acknowledgements The authors would like to thank The Ministry of Higher Education Malaysia for financial support.

Funding This work was supported by The Ministry of Higher Education through FRGS/ST01(02)/968/2013(09).

Declarations

Conflict of interest The authors declare that they have no competing interests.

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