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Research Article

Uptake and Dissipation of Carbofuran and Its Metabolite in Chinese Kale and Brinjal Cultivated under Humid Tropic Climate

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Carbofuran is an insecticide with a broad spectrum of activity and is relatively cheap. It is banned in many countries in the world; however, it remains widely used in Asia, Australia, and South America. Carbofuran is commonly used in vegetable farming in Malaysia and it is a legally registered pesticide. This study reports the uptake and dissipation of carbofuran and 3-ketocarbofuran in Chinese kale and brinjal under humid tropic field conditions. The residue profile in plants demonstrated an increase to a maximum, followed by a consistent reduction to a level below the limit of determination (<0.01 mg/kg) over the experimental period. The maximum residue concentration was attained on Day 3 for kale (1.16 mg/kg fresh weight) and Day 7 for brinjal (0.06 mg/kg fresh weight) after carbofuran application. In order to comply with the maximum residue level (MRL) of 0.01 mg/kg, the preharvest interval for kale and brinjal were suggested at 23 and 28 days, respectively. The preharvest interval indicates that carbofuran is not recommended for Chinese kale but it is acceptable for brinjal. The average half-life of carbofuran in soil is 1.24 days, shorter than the literature values reported based on temperate condition, indicating accelerated dissipation under tropical climate. The estimated half-life of carbofuran in leaves was shorter than that in fruits with kale leaves reported at 2.54 days whilst brinjal leaves and fruits recorded at 3.22 and 10.33 days, respectively.

1. Introduction

The use of pesticides is an almost indispensable practice in vegetable farming in order to meet the continuous increase in demand and expectation. In 2016, Malaysia exported a total of 1,504,918 tonnes of vegetables, compared to 281,391 tonnes in 2011 [1, 2]. The consumer expectation of unblemished vegetables further intensifies the reliance of farmers on pesticides.

Carbofuran is an insecticide, acaricide, and nematicide that is widely used in vegetable farming in Malaysia. It has a broad spectrum of activity and is relatively cheap. In paddy and oil palm plantations, carbofuran is used for control of rhinoceros beetle and rodent [3]. The use of carbofuran has become an issue after several incidents of carbofuran detection in vegetables, higher than the Maximum Residue Level (MRL). These include a news report of 900 kg of vegetables tainted with carbofuran from Malaysia and Thailand [4] and The European Commission has also reported a residue concentration of 0.33 mg/kg in fresh Chinese kale imported to Finland from Thailand [5]. The concern is further escalated with more claims of excessive use of carbofuran in durian and watermelon from Thailand, as pointed out by Wanwimolruk et al. [6].

As a matter of fact, carbofuran has been banned in Canada, the European Union, and United States due to numerous cases of bird poisoning [7]. According to the Royal Society for Protection of Birds [8], a total of 316 cases of bird poisoning involving carbofuran were reported in the UK in year 2002–2011. The secondary metabolites of carbofuran, specifically 3-ketocarbofuran and 3-hydroxycarbofuran, are also found lethal. In 2004, a total of 187 vultures and hyenas were killed after scavenging the bird carcasses containing carbofuran residue and its metabolites [9]. Despite that, carbofuran continues to be widely used in Asia, Australia, and South America; it is a legally registered pesticide in Malaysia. The next question to pose is whether carbofuran can be potentially accumulated in agricultural crops and whether the crops are safe to consume.

After numerous allegations of excessive carbofuran in watermelon and durian from Thailand, Wanwimolruk et al. [10] examined these products in the market across eight provinces declaring that the residue was lower than the recommended MRL value, posing no risk of contamination. The coworkers further investigated the presence of carbofuran and other pesticides in the commonly consumed vegetables; carbofuran was occasionally detected at a level higher than the MRL [10, 11]. In another study by Chowdhury et al. [12], carbofuran residue was reported above the MRL value in some eggplants sampled from Bangladesh markets. Although carbofuran is widely used in Malaysia, its dissipation behavior under the tropical climate is scarcely studied. So far, only the dissipation of carbofuran in soil [13] and water [3] was studied. The concern over excessive use of pesticides in vegetables is continuously highlighted, of which carbofuran is often questioned. Carbofuran is in fact not recommended for all vegetables; however, its efficiency and cost have rendered this product to be favorably used by farmers. It is very toxic and numerous poisoning cases have been reported. In 2011, 408 cases were recorded by the Columbia National System for Public Health Surveillance, whilst in Thailand, 2342 cases of poisoning among farmers were identified [14]. Hence in this study, we attempt to evaluate the uptake and dissipation of carbofuran and its metabolite in two vegetables namely brinjal (often referred to as eggplants) and Chinese kale under field conditions. The finding is imperative for the decision-making authorities in assessing and reviewing the status of carbofuran in the country.

2. Materials and Methods

2.1. Reagents and Chemicals. Carbofuran standard was purchased from Dr. Ehrenstorfer GmbH. Carbofuran granules (AGRITOX 3G) were purchased from Hextar Chemicals Sdn. Bhd. Analytical and residue grades of acetonitrile, sodium chloride, acetone, glacial acetic acid, and anhydrous magnesium sulphate were purchased from J.T. Baker, Phillipsburg, USA. Florisil (2% deactivated) was used as a sorbent for extraction.

2.2. Apparatus and Instrumentations. A Robot Coupe CL50E food chopper was used to homogenise the vegetable samples, whilst centrifugation of the sample extracts was performed using Thermo Jouan Model B4i multifunctional centrifuge. An Agilent Model 7000 gas chromatography equipped with mass spectrometry (GC–MS/MS) was used to determine the pesticides present in the samples.

2.3. Preparation of Planting Beds. The uptake and dissipation of carbofuran were assessed based on field experiments with brinjal (*Solanum melongena*) and Chinese kale (*Brassica oleraceae var alboglabra)* as the models. The field experiments were conducted between September 2017 and January 2018. The seeds of brinjal and Chinese kale were sown in nursery for two weeks. Whilst waiting for the seeds to grow, six concrete raised beds (3×1.5 m each) filled with soil were cleared of weed and tilled with 1.0–2.0 kg/m^2 of chicken manure, 100 g/m^2 of dolomite and 30 g/m^2 of NPK fertilizer. The soil was clayey red-yellow podzolic soil at pH 8 with electrical conductivity of 78.6 μmhos/cm and organic matter content of 13%.

2.4. Field Experiments. A total of 210 healthy Chinese kale seedlings were transplanted into six beds with 35 plants/bed (5×7) at a distance of 50 cm between seedlings. The seedlings were left for two days to adjust to the field environments. Following that, the seedlings of three beds were treated with commercially available granulated carbofuran (Agritox 3G) at a recommended dosage of 3 kg/ha, whilst three other beds served as the control. When carbonfuran was applied, the seedlings were at the growth stage of 14 (4th true leaf unfolded) according to BBCH-scale (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) for leafy vegetable not forming heads. The granulated carbofuran was embedded in soil at a distance of 5 cm around each seedling. After two days, a seedling was uprooted from each plot, with and without carbofuran, randomly (Day 0). The growth performance in terms of plant weight, plant height, root length, and number of leaves was recorded. The top soil around the seedling at 0–5 cm depth was also tilled and collected. The soil and plant samples were subjected to analysis of carbofuran and 3-ketocarbofuran. Subsequent to Day 0, samplings were carried out on Days 1, 3, 5, 7, 9, 12, 14, 16, 18, 20, and 23. The field experiment took place between 28 September and 16 November 2017. Note that on normal practice, carbofuran is applied at the early stage of growth and during fruiting for fruit-bearing plants.

For brinjal, a total of 162 seedlings were transplanted into six beds with 27 plants/bed in one single row at an inter-plant distance of 50 cm. The seedlings were similarly left for acclimatization and carbofuran granules were only applied (the recommended dosage: 30 kg/ha) when the plants began to bear fruits (three weeks after transplanting)—at the stage of flowering (second flower open, code 602 according to BBCH-scale). From each bed, one brinjal fruit was harvested and weighed (Day 0). The plant was then uprooted and the soil was similarly collected. The fruits, the leaves and the soil samples were subjected to analysis of carbofuran and its metabolite. Subsequent to Day 0, samplings were carried out at an interval of three days for the first four weeks and at a seven-day interval for the succeeding three weeks. Note that carbofuran is normally applied at the early stage of the growth, but in this study, it was delayed until fruiting, to examine the uptake of carbofuran in leaves and fruits under the effect of single application. The field study was carried out between 19 November 2017 and 19 January 2018.

The plants were watered daily, except rainy days. The temperature, humidity, and precipitation data were provided by the Meteorology Department at Semenggok Agriculture Research Centre. Figure 1 shows the photographs of the field experiments for Chinese kale and brinjal.

2.5. Sample Preparation. Carbofuran and its metabolite (3-ketocarbofuran) were extracted from the plants and

(a) (b)

Figure 1: Photographs of field experiments for (a) Chinese kale and (b) brinjal.

soils according to the modified QuEChERS method [15]. The fruit and leave samples were cut into small pieces and homogenized. For soil, the samples were air-dried, ground, and sieved through 2 mm sieve to remove roots and other debris. Ten grams of samples was weighed into a Teflon centrifuge tube with addition of 20 mL acetic acid (1%) in acetonitrile. The mixture was shaken vigorously for 1 min and added with 1.5 g of NaCl and 6.0 g of anhydrous $MgSO_4$. The mixture was then vortexed and centrifuged (12000 rpm) for 1 min, respectively. Two milliliters of the supernatant was leached through a Pasteur pipette packed with 0.2 g of deactivated Florisil for clean-up. The eluent was left to dry naturally, added with 2 mL acetone, and transferred to a vial. The sample was analyzed in triplicates for carbofuran and its metabolite using a Gas Chromatography Mass Spectrometer with tandem mass spectrometry (GC–MS/MS Agilent Model 7000). The concentration is reported in mg/kg fresh weight based on the calibration and the residue concentrations in plants are reported after subtraction the control.

2.6. Analysis of Carbofuran and Its Metabolite. A stock standard solution of carbofuran and 3-ketocarbofuran at 500 mg/L was prepared in acetone. The stock solution was diluted to standard solutions of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 50 mg/L and stored at 4°C in the dark. The standard solutions were analyzed using GC–MS/MS with a column of HP-5MS 5% phenyl methyl Silox (30 m $\times$ 0.25 μm $\times$ 0.25 μm). The carrier gases were helium and nitrogen set at flow rates of 2.250 mL/min and 1.500 mL/min, respectively. The column temperature was maintained at 70°C for 2 mins and subsequently increased to 150°C at 25°C/min. The temperature was then increased to 200°C at 25°C/min and further to 280°C at 65°C/min. The temperature was held constant for 2 mins.

2.7. Method Validation. Chinese kale, brinjal, and soil samples were spiked with standard carbofuran and ketocarbofuran to attain final concentrations of 0.01, 0.1, and 1 mg/kg, respectively. The samples were mixed well and left for 15 mins before extraction and analysis with GC–MS/MS. The recovery performance was calculated as the percentage of experimental concentration over the expected concentration. The blank samples were also analyzed. The limit of determination was evaluated based on the lowest recoverable concentration.

2.8. Estimation of Half-Life. The half-life was estimated based on the first order kinetic reaction of $Y = Ae^{-kt}$, where Y is the residue concentration at time, t (day), A is the initial residue concentration, and k is the dissipation rate constant (day^{-1}). From the experimental results, the natural log of the residue concentration, Y at sampling time, t was determined. The linear regression of $\ln(Y)$ versus t was modelled yielding $\ln(Y) = \ln(A) - kt$. The half-life ($t_{1/2}$) was then estimated from the equation of $t_{1/2} = \ln(2)/k$. Note that the half-life is calculated only if there is a significant regression between the residue concentration and time ($p < 0.05$).

2.9. Statistical Analysis. One-Way Analysis of Variance (ANOVA) was employed to compare the growth performance at 95% confidence interval, with Tukey's test applied for multiple comparisons. All statistical analyses were performed using Matlab R2013b.

3. Results

Table 1 summarizes the recovery of carbofuran and 3-ketocarbofuran from different matrices. The recovery performance of carbofuran is consistently more than 90% in all matrices with the minimum quantifiable concentration established at 0.01 mg/kg (concentrations less than 0.01 mg/kg is unrecoverable). For 3-ketocarbofuran, the recovery remains promising for soil and Chinese kale, but appears impeded in brinjal at lower concentrations, likely due to matrix effects.

Figure 2 illustrates the growth performance of Chinese kale treated with and without carbofuran (plant weight, height, root length, and number of leaves). The treated Chinese kale observably exhibits better growth than the control; nonetheless, no statistical difference is deduced ($p > 0.05$). Figure 3 shows the carbofuran and 3-ketocarbofuran residues detected in Chinese kale over 23 experimental days, under the climatic conditions with an average temperature and rainfall of 28.9°C (min 22.8°C and max 36.2°C) and 13.2 mm (min 0 mm and max 85.7 mm), respectively. The profiles of both residues demonstrate a similar pattern with carbofuran attaining its maximum concentration of 1.16 mg/kg on Day 3, whilst 3-ketocarbofuran is present at 0.22 mg/kg. After reaching the maximum concentration, the residues begin to reduce

Table 1: The recovery performance of carbofuran and 3-ketocarbofuran in different matrices ($n = 3$).

Matrix	Expected concentration (mg/kg)	Measured concentration (mg/kg)		Percentage of recovery	
		Carbofuran	3-Ketocarbofuran	Carbofuran	3-Ketocarbofuran
Soil	0.01	0.009 ± 0.000	0.010 ± 0.000	92.7 ± 4.4	100.0 ± 2.7
	0.1	0.092 ± 0.002	0.105 ± 0.002	91.6 ± 2.7	105.8 ± 2.1
	1	0.939 ± 0.007	1.064 ± 0.034	93.9 ± 0.8	106.4 ± 3.4
Chinese kale	0.01	0.010 ± 0.000	0.009 ± 0.000	99.0 ± 4.4	99.7 ± 1.5
	0.1	0.100 ± 0.002	0.097 ± 0.002	100.5 ± 2.4	96.4 ± 2.6
	1	1.053 ± 0.005	0.950 ± 0.023	105.3 ± 0.5	94.8 ± 2.4
Brinjal	0.01	0.010 ± 0.000	0.007 ± 0.000	100.0 ± 4.5	74.0 ± 2.7
	0.1	0.103 ± 0.003	0.066 ± 0.002	102.8 ± 3.0	65.5 ± 3.4
	1	0.126 ± 0.053	0.942 ± 0.151	103.0 ± 5.2	94.2 ± 1.6

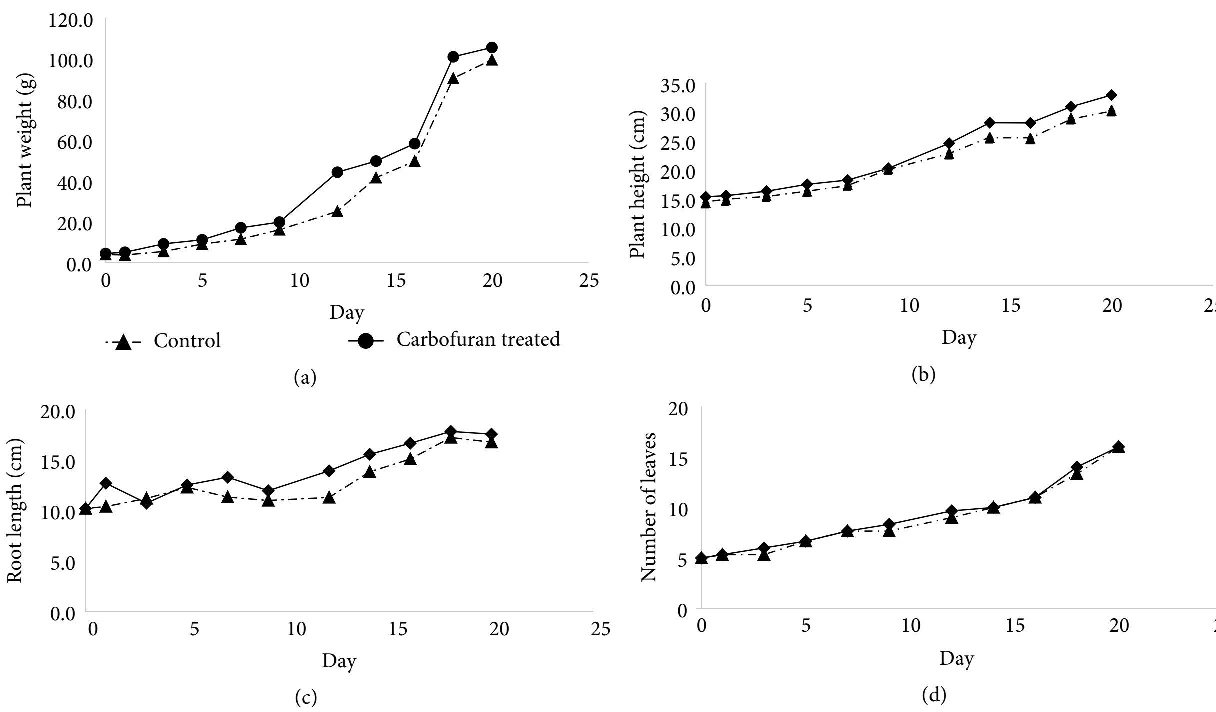

Figure 2: The growth performance of Chinese kale, treated with and without carbofuran. (a) Weight, (b) height, (c) root length, and (d) number of leaves.

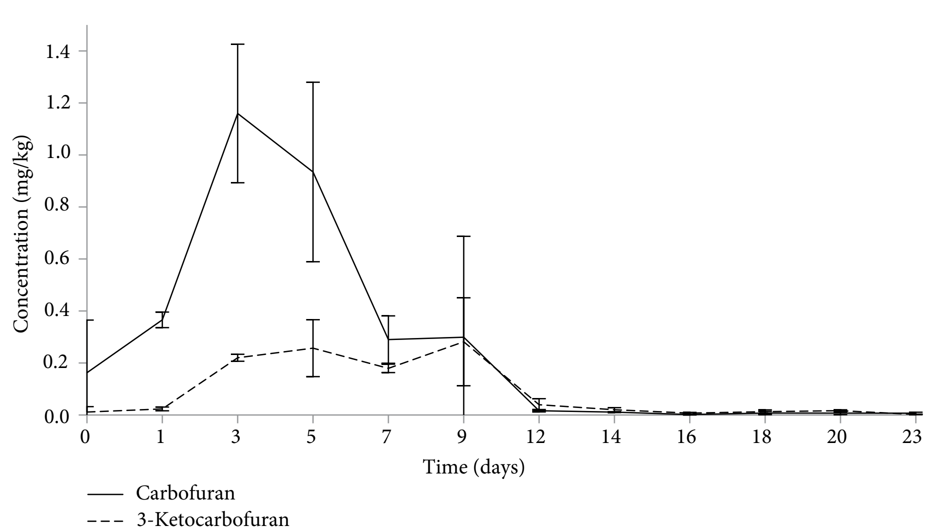

Figure 3: Carbofuran and 3-ketocarbofuran residues in Chinese kale over the experimental period.

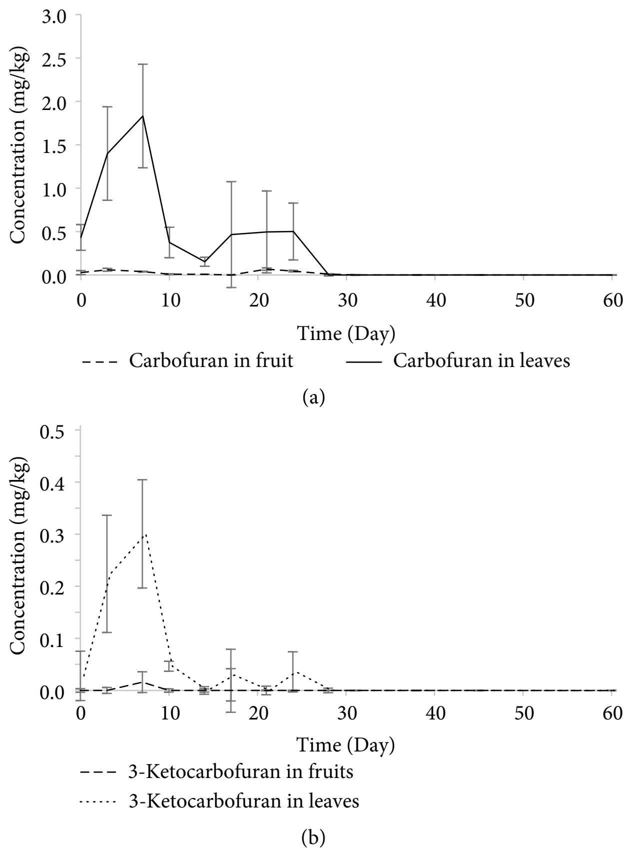

Figure 4: (a) Carbofuran and (b) 3-ketcarbofuran residues in fruits and leaves of brinjal plants.

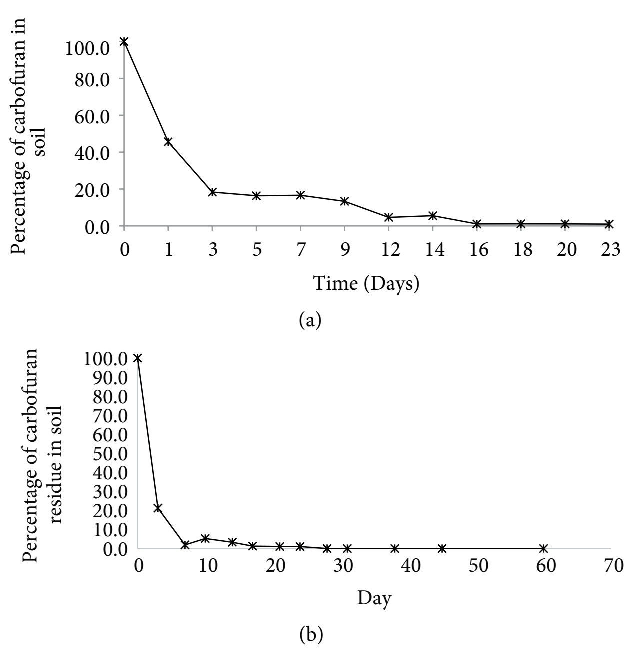

Figure 5: The percentage of carbofuran residue in soil of (a) Chinese kale and (b) brinjal over the experimental period.

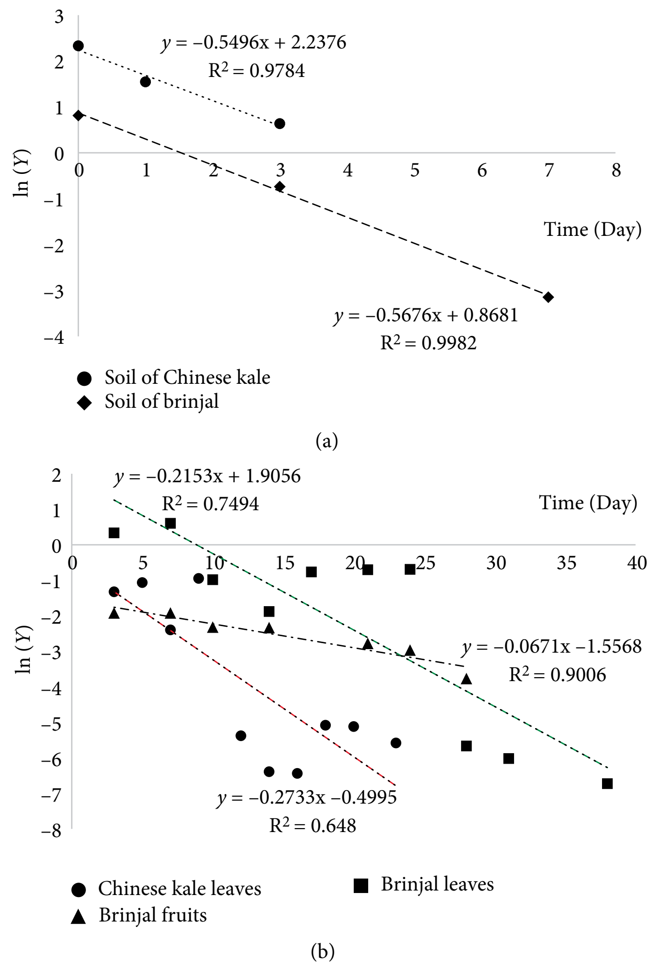

Figure 6: The regression models for the estimation of carbofuran half-life in (a) soils and (b) plants.

consistently meeting the level below MRL (<0.01 mg/kg) on Day 16 and Day 23 for carbofuran and 3-ketocarbofuran in turn. Both compounds are undetected in the control samples.

For brinjal, the growth was monitored over 60 days. The weight of fruit, root length, and plant height were measured (results not shown); statistically, no significant difference is found between plants treated with and without carbofuran ($p > 0.05$). Figure 4 illustrates the carbofuran and ketocarbofuran residues in leaves and fruits of brinjal plants. The carbofuran residue reaches its height at 1.83 mg/kg in leaves and 0.06 mg/kg in fruits on Day 7 and Day 3, respectively, with the former demonstrating higher affinity for accumulation, which is in good agreement with the findings of Zheng et al. [16] and Farha et al. [17]. Like Chinese kale, the residue concentration continues to reduce steadily after its peak, converging the level below MRL on Day 28. The metabolite of 3-ketocarbofuran demonstrates a similar profile with the maximum concentration recorded on Day 7 at 0.30 mg/kg in leaves and at a trace amount of 0.02 mg/kg in fruits (which is then rapidly dissipated).

In soil of experiment with Chinese kale, the concentration of carbofuran reduces exponentially from 10.30 mg/kg on Day 0 to a constant of 0.11 mg/kg on Day 16. The metabolite of 3-ketocarbofuran, however, was undetected. As observed, almost 83% of carbofuran was dissipated after one week. The degradation continued into the second week, reaching a constant on the third week. For the field experiment with brinjal, carbofuran residue was found at 2.25 mg/kg on Day 0 and the level rapidly declines to 0.48 mg/kg on Day 3 (loss of 78.7%). The residue concentration fell below the limit of detection (<0.01 mg/kg) on Day 28. Figure 5 illustrates the loss of carbofuran in soil (in percentage) of Chinese kale and brinjal.

Figure 6 shows the regression models for estimation of half-life of carbofuran in soil and plants. The models are statistically significant with the regression coefficients computed between 0.65 and 0.99. The half-life of carbofuran in soil was deduced with the concentrations attained for the first 5 days for Chinese kale and 7 days for brinjal. The half-life determined from the soil of kale (1.26 days) and brinjal (1.22 days) are comparable with a mean of 1.24 days. For the half-life in leaves and fruits, the maximum concentration from Day-3 and onward was used to express the regression fit. The half-life of carbofuran in the leaves of kale and brinjal was predicted at 2.54 and 3.22 days, whilst in brinjal fruits, it was somewhat longer at 10.33 days.

4. Discussion

The detection of carbofuran was benchmarked with the stipulated maximum residue level (MRL) of various guidelines. The MRL recommended by the European Commission in 2015 was 0.002 mg/kg (specifically for kale and brinjal). In 2008 and 2011, the level stipulated was 0.02 mg/kg, but was tightened to 0.01 mg/kg in 2012 and further to 0.002 mg/kg in 2015 [18]. In Malaysia, the Sixteenth Schedule of Food Act [19] specifies the MRL of carbofuran in brinjal at 0.1 mg/kg, whilst the National Bureau of Agricultural Commodity and Food Standards [20] in Thailand sets the limit at 0.03 mg/kg for *brassica* vegetables and 0.1 mg/kg for brinjal. In this study, the uptake and dissipation of carbofuran in Chinese kale and brinjal is assessed based on the MRL of European Union at 0.01 mg/kg.

The residue profiles of both Chinese kale and brinjal illustrate a gradual increase over time. The concentrations soon peaked and were followed by a consistent reduction, suggesting uptake and degradation of carbofuran. According to a study, the lipophilicity of carbofuran is optimum for transpiration stream of plants [21]. Upon degradation, carbofuran is converted into 3-hydroxycarbofuran, and thereafter into 3-ketocarbofuran. Robert and Hutson [21] assert that both metabolites are rather unstable and are unlikely to be accumulated. In Chinese kale, it took 23 days for carbofuran residue to dissipate below MRL of 0.01 mg/kg, where evaporation, photodegradation, and growth dilution are anticipated as the main pathways [22]. Typically, Chinese kale can grow to maturity in 28–35 days inclusive of 14 days in nursery. The experimental results suggest that Chinese kale treated with carbofuran, upon transplanting from the nursery, will only be safe to consume after 35 days. By then, the kale will be over matured for harvesting; hence the application of carbofuran is not recommended. For brinjal, the experimental results suggest a preharvest interval of 28 days. It usually takes 50–60 days for brinjal fruits to be harvested. On application of carbofuran upon fruiting, the mature fruits will be safe for consumption.

In soil, carbofuran was reported to remain in the field for two to six weeks before it began to dissipate [23]. This initial lag phase, however, was not evidenced in this study likely due to the climatic conditions of greater rainfall and higher temperature compared to that carried out under the temperate environments [15]. With the tropical climatic conditions, the loss of carbofuran *via* volatilization, photodegradation, and runoff is expected to accelerate. According to Sanchez–Bayo and Hyne [24], carbofuran is a relatively persistent pesticide; its half-life in soil was recorded between 23 and 46 days, falling within the range of 28–110 days reported by Toxnet [25]. The literature half-life is noticeably longer than that predicted in this study (1.24 days), likely associated with the tropical climate of greater solar intensity, higher temperature, and rainfall. A higher temperature will foster faster degradation with increased volatilization, whilst higher precipitation encourages losses through leaching and runoff [24]. The findings by Chai et al. [15] likewise reported a shorter half-life for chlorpyrifos and acephate under tropical climatic conditions. The foliar half-life of kale (2.49 days) is comparable to that of acephate, chlorpyrifos, and cypermethrin in green mustard, reported by Chai et al. [26] (1.1–3.1 days). For brinjal leaves and fruits, their fitted dissipation half-lives are also comparable to a database of carbofuran half-lives in plants with an average of 5.03 days in leaves (variability 1.10–13.00 days) and 11.62 days in fruits (variability 11.00–13.10 days) [27].

5. Conclusion

The dissipation profile of carbofuran and its metabolite in Chinese kale and brinjal postulated a preharvest interval of 23 and 28 days, respectively, in order to comply with the MRL of 0.01 mg/kg under tropical conditions with a single application at the recommended dosage. Application of carbofuran was found unsuitable for quick-growing plants such as Chinese kale as the plants may not be safe to consume on maturity, but it is acceptable for fruiting plants such as brinjal. The average half-life of carbofuran in soil was 1.24 days, shorter than the literature half-life reported primarily based on temperate conditions, suggesting accelerated photodegradation, volatilization, and leaching under the hot and humid climate. The half-life in kale and brinjal leaves as well as brinjal fruit was 2.54, 3.22, and 10.33 days, respectively.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Chung Ling Yan and Jocephine Jonip have equal contribution to this paper.

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