Dissipation dynamics and half-lives of cypermethrin, emamectin benzoate, and indoxacarb insecticides in different parts of amaranth (*Amaranthus tricolor* L) and mustard greens (*Brassica juncea*)

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The current study aimed to examine dissipation dynamics of cypermethrin, emamectin benzoate, and indoxacarb insecticides in different parts of amaranth (*Amaranthus tricolor* L) and mustard greens (*Brassica juncea*) and determine their half-livesfor a safe harvest. Two experiments, one in the open-field and one in a net-house were set up to grow the two crops; the three insecticides were applied 20 days after transplanting. Different crop parts (leaves, stem, and root) were collected after 2 hours, 2, 4, 6, 8, and 10 days after the insecticide application for measurements of their residue using GC-MS/MS (cypermethrin and indoxacarb) and LC-MS/MS (emamectin benzoate). Results showed that dissipation dynamics of the pesticides fitted well to the pseudo-first-order kinetics model with three parameters. The pesticide's half-lives were significantly dependent on the crop types and crop parts. The half-lives of cypermethrin varied from 3.0 – 5.3 days; the half-lives of emamectin benzoate were from 0.37 – 0.64 days, and those of indoxacarb were from 12.5 – 13.5 hours. The highest half-lives were in the stem part. The open-field conditions resulted in shorter half-lives than the net-house condition. Based on the Codex Maximum Residue Limit, harvesting the leaves of amaranth and mustard greens in the open-field condition should not be carried out until after 7.7 and 7.1 days respectively from the last application of cypermethrin and after 1.4 and 1.3 days of emamectin benzoate. In the net-house condition, the harvest of the leaves of amaranth and mustard greens could be implemented after 9.4 and 7.8 days for cypermethrin and after 1.8 and 1.4 days for emamectin benzoate, respectively. Meanwhile, it could be safe to harvest the leaf part of the two tested crops after 12 hours from the last application of indoxacarb.

Keywords: Dissipation dynamics, half-lives, cypermethrin, emamectin benzoate, indoxacarb, vegetable crop

The use of pesticides to control various pests, which significantly reduce crop productivities by causing harm to crops (Kazemi et al. 2012; de Bon et al. 2014)is increasing, along with the rapid growth rate of global population (Vela et al. 2007). The use of pesticides reduces crop losses and increases productivity all over the world (Cooper and Dobson 2007; Pimentel et al. 1992), and also improves food quality (Fantke and Juraske 2013). On the other hand, inappropriate use may lead to environmental pollution (Popp et al. 2013; Kumar and Kumar 2019) and public concern on human health after consuming pesticide polluted products (Popp et al. 2013; Wentzell et al. 2014; Kumar et al. 2012). The adverse impacts on human health and the environment depend on the residual levels of the chemicals on/in crop products, which can be determined by the pesticide half-lives and the time interval from the last application to the harvest (Wang et al. 2013).

Consequently, pesticide residues and half-lives in different parts of vegetable crops are the most important characteristics in affecting the crop product quality. Many studies reported the residual dynamics and half-lives of various pesticides on different host crops (Fantke and Juraske 2013; Jacobsen et al. 2015; Lewis and Tzilivakis 2017). In general, the half-lives of pesticides can vary from 1 hour to 918 days, but the majority of half-lives (95%) vary from 0.6 – 29 days, depending on many factors (Fantke and Juraske 2013). These factors can be grouped into three main categories; the chemicals, the crops and the environmental conditions. For example, Fantke and Juraske (2013) documented that imidacloprid pesticide had half-lives varying greatly with crop types, lowest in cucumber

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(around 1 day) and highest in sugar beet (around 15 days), while in tomato abamectin pesticide had half-lives of around 1 day and cadusafos pesticide had around 24 days. Overall, Lewis and Tzilivakis (2017) documented that pesticide dissipation can be dependent on the physical-chemical properties of the active substance, crop properties (skin, leaves, fruit and leaf shape), and environmental conditions (temperature, humidity, sunlight etc.). These indicated that a particular pesticide can have varying half-lives, depending on agricultural crops in different environmental conditions, which need to be determined.

Amaranth (*Amaranthus tricolor* L) and mustard greens (*Brassica juncea*) are two vegetable crops commonly cultivated in Vietnam and in many countries worldwide (Huong et al. 2013). A recent survey conducted by the research group indicated that mustard greens and amaranth were the first and the third most planted vegetables, respectively, in Cu Chi District, Ho Chi Minh City, Vietnam (data not shown). The two crops are regularly affected by various insect pests (Seni 2018; Bustami et al. 2019) and cypermethrin, emamectin benzoate, and indoxacarb pesticides are recommended for use to control the pests (Wang et al. 2012; Chau and Hieu 2017; Shi et al. 2019). Consequently, crop productivity can increase with these pesticides use, but on the other hand, they could be contaminated with the residue of the applied pesticides. It is necessary to study the half-lives and time interval after the application of these pesticides for a safe harvest. Therefore, the current study was conducted to (1) examine dissipation dynamics of cypermethrin, emamectin benzoate and indoxacarb pesticides in different parts of amaranth and mustard greens and (2) determine their half-lives for a safe harvest at different sites.

Materials and methods

Experimental sites and materials

The current study was conducted in the Research and Development Center for High Technology Agriculture (RDCHTA), Cu Chi District, Ho Chi Minh City, Vietnam, located at 11°01'05''N, 106°31'05''E. The area receives an average annual rainfall of 1300 – 1700 mm and has a mean monthly temperature of 24.8 – 28.8°C. The vegetable crops amaranth and mustard greens, used in the current study were provided by the Trang Nong commercial company. The two crops have a life cycle of around 30 – 35 days with yields of 12 – 15t/ha (amaranth) and 25 – 35 t/ha (mustard greens). The three pesticides (cypermethrin, emamectin benzoate, and indoxacarb pesticides) used for the study were bought from retail suppliers in Vietnam.

Experimental setup

Two experiments were conducted for the study, one in the open-field and one in a net-house in the RDCHTA. Both experiments were conducted twice with planting times in September 2018 and June 2019. For both crops, seedlings were transplanted on raised beds (1.0 m x 5 m x 0.25 m, width x length x height) in the open-field and on compost-made beds (1.0 m x 5 m x 0.15 m, width x length x height) in the net-house. A buffer bank of 0.2 m width was established to separate these experimental beds. The plant spacing for the two crops was 20 cm between the rows and 10 cm between two the plants in a row with a plant density of 50 plants/m^2; for amaranth there were three seedlings planted at each point and for mustard greens one seedling was planted at each point (50 points/m^2.) Drip irrigation with five watering lines was applied to maintain water content for the crops during the experiment. After 22 days from transplanting, three insecticides cypermethrin (Sherpa 25EC, Bayer Vietnam), emamectin benzoate (Mikmire 7.9 EC, Nam Bac company), and indoxacarb (Opulent 150SC, NongDuoc 2 company) were sprayed on their corresponding planting beds at the recommended rates of 120, 31.6, and 60 active ingredient per ha (a.i. ha^{-1}), respectively.

Sampling and chemical analysis

Two hours, and 2, 4, 6, 8, and 10 days after spraying, crop parts (root, stem, and leaves) were sampled for measurement of pesticide residues. The sampling was carried out by cutting the aboveground parts of 5 experimental plots, each plot $0.4m^2$ (diagonal rule) on each bed and digging soil within these areas for root collection. The aboveground parts were further separated into the stem and leaf parts for amaranth and soil material was washed with tap and distilled water to collect root mass for measurements. The aboveground parts were not washed with any water before measurement.

The chemical analysis for the residue of the three tested pesticides was started by drying the samples at 70°C until unchanged mass, then they were ground to pass through a 2-mm sieve before analysis. The quantification of the residue of cypermethrin and indoxacarb was implemented following the procedure by Chau and Hieu. (2017) with 3 basic steps. The three-step procedure was:

1. Extraction: after addition of 100 ng surrogate δ-HCH into 5 gram ground biomass samples, 60 mL acetone and 30g $NaSO_2$ were added to this mixture, which was ultrasonically extracted and filtered three times.
2. Drying: the total extract was evaporated to about 10 mL followed by a two-step solid-phase extraction, and further evaporated to near dryness.
3. The nearly dry extract was transferred to a 1-mL amber vial containing 100 ng fluorine-D10 and 100 ng phenanthrene-D10 followed by a toluene fill-up to 1mL and stored until measurement.

The pesticide measurement was carried out using a gas chromatography - triple quadrupole mass spectrometry system (GC-MS/MS TQ model 8040, Shimadzu, Japan). The concentration of pesticide residue was determined, based on an additional standard curve.

The quantification of the residue of emamectin benzoate was implemented following the procedure of AOAC INTERNATIONAL (2007) with some modifications. The procedure was:

1. Ground samples weighing around 15g were placed in a 50 mL Teflon centrifuge tube, to which was added 15 mL of acetonitrile with 1% acetic acid, 6 g of $MgSO_4$ and 1.5 g of anhydrous sodium acetate.
2. Shaking and centrifuging the tubes to get 8-mL extract into 15-mL tubes containing 400 mg of PSA (Primary and Secondary Amine) adsorbent and 1200 mg of magnesium sulfate.
3. Shaking and centrifuging the tubes again to get a 4-mL extract into other tubes.

Evaporating the extract to around 0.5 mL, to which was added 0.5 mL acetonitrile. Ultrasonicating and filtering the extract into a vial for measurement. The emamectin benzoate concentration was determined using a Triple Quadrupole Liquid Chromatograph Mass Spectrometer (LC-MS/MS model 8040, Shimadzu, Japan). The analysis methods used for the current study were described and used with some modifications by other studies on various crops (Kottiappan and Anandh 2012; Chau et al. 2020; Lee et al. 2019; Coello-Villanueva et al. 2018). The quantification was carried out using corresponding standard curves with varying good linearities of coefficients of determination of 0.99 or higher. For the current study, the limits of detection (LOD) and limits of quantification (LOQ) 0.05 and 0.17 (ppm) for cypermethrin, 0.04 and 0.13 (ppm) indoxacarb, and 0.01 and 0.04 (ppm) for emamectin benzoate, respectively.

Statistical analyses

Various mathematical models were used to quantify the dissipation dynamics of pesticide residue in different parts of plants (Fantke and Juraske 2013). For the leaf and root parts the current study used the pseudo-*fi*rst-order kinetics with three parameters because it fitted very well to the measured data (Lewis and Tzilivakis 2017). The model was:

$$y = a + be^{-cx}$$

Where y and x are the residual concentration and time; a, b, and c are constants. For the cypermethrin concentration in the stem parts a linear regression equation fitted very well to the measured data:

$$y = ax + b$$

Where y and x are residual concentration and time; a and b are constants. The half-lives, defined as the time needed for compounds to reduce to 50% of their initial concentrations, of the three pesticides were computed by solving the relevant equation to get the x value (day or hour) when y was set to a half of the pesticide concentration estimated on the application day. The scatter plot method, curve fitting, and statistical analysis were conducted using Sigmaplot 12 (Systat Software Inc.).

Results

Concentration dynamics of three insecticides

Figure 1a shows that the concentrations of cypermethrin in the leaves and root of amaranth collected from the field trial decreased significantly and exponentially with time after application with decrease rates of 0.127 and 0.16 µg kg^{-1}, respectively. The concentration in the leaves changed from 2691 µg kg^{-1}on the spraying day to 0 after 10 days. Concentration of the root was 0 on the spraying day but was the highest on the second day of 1031 µg kg^{-1}, decreasing to 0 on day 10. Unlike in the leaves and root, the cypermethrin concentration in the stem of amaranth decreased linearly and significantly with time. Its concentration was changed from 1566 µg kg^{-1}on the spraying day to 0 after 10 days, with a daily decrease rate of 161 µg kg^{-1}.

Similar to the field trial, the net-house trial showed that the concentration of cypermethrin in the leaves and roots of amaranth was decreased significantly and exponentially with time after application, with a decrease rates of 0.119 and 0.13 µg kg^{-1}, respectively (Figure 1b). The concentration in leaves on the application day was 2696 µg kg^{-1}, which decreased to 0 on day 10. Concentration in the root on the spraying day was 0, but on the second day was 1104 µg kg^{-1} decreasing to 0 on day 10. In the stem, the cypermethrin concentration was negatively and linearly related to the time after its application with a daily decrease rate of 149 µg kg^{-1} for every day after application.

For emamectin benzoate, the two trials showed that the concentration in the two analyzed parts (leaves and stem) of amaranth decreased significantly and exponentially from the application day to day 10 (Figures 2a and 2b). Four days after application, the concentration of emamectin benzoate reached 0 from the initial concentrations on the spraying day of 2540 µg kg^{-1} (leaves - field trial), 1200 µg kg^{-1} (stem - field trial), 2550 µg kg^{-1}(leaves - net-house trial) and 1208 µg kg^{-1} (stem – net-house trial). The emamectin benzoate concentration in the root part of amaranth in the field trial was detected only on the second day 6.4 µg kg^{-1}, and that in the net-house trial was only observed on days 2 and 4 after application (57 and 2.7 µg kg^{-1}respectively).

For mustard greens, the leaves and roots of crop were analyzed for cypermethrin residue and the results are shown in Figures 3a and 3b. The cypermethrin concentration in the leaves of the crop from both trials decreased significantly and exponentially with time after the insecticide application, from 2178 µg kg^{-1}field trial and 2184 µg kg^{-1} (net-house trial) to 0 after 10 days. In the roots, the residue was not detected on the spraying day but was detected on the other days until day 10. The change in the cypermethrin concentration with time after application significantly followed apseudo-first-order kinetics model, varying from 830 µg kg^{-1} (field trial) and 856 µg kg^{-1} (net-house trial) on the second day to 0 on day 10.

The emamecthrin benzoate concentration in the mustard greens leaves from the field trial significantly decreased from 2043 µg kg^{-1} on the application day to 0 on day 10 (Figure 4a). Its concentration in the root from the same trial was detected only on the second day (3.6 µg kg^{-1}). Similarly, Figure 4b showed that the residual dynamics of emamecthrin benzoate in the mustard greens leaves from the net-house trial was apseudo-*fi*rst-order kinetics model with a decrease rate of 1.72 µg kg^{-1}, decreasing from 2067 µg kg^{-1} on the spraying day to 0 on day 6. The root of this crop was detected to contain emamecthrin benzoate residue only on the second day from the application (34.1 µg kg^{-1}).

Similar to cypermethrin and emamecthrin benzoate, the residue of indoxacarb was measured on days 0, 2, 4, 6, 8, and 10, but its presence was detected only on the application day. Therefore, the dynamics of indoxacarb concentration were examined on an hourly basis and the results are shown in Figures 5a and 5b. Apseudo-first-order kinetics model provided a good fit to the dynamics of the indoxacarb concentration over time after the application. The concentration in the amaranth leaves decreased from 1927 µg kg^{-1} right after the insecticide application to 0 in 48 hours afterward, with a reduction rate of 0.0431 µg kg^{-1}. Similarly, its concentration in the stem part of the same crop decreased from 1090 µg kg^{-1} to 0 after 48 hours. In the leaves of the mustard greens, the indoxacarb concentration decreased from 1491 µg kg^{-1} to 0 in 48 hours with a decrease rate of 0.0392 µg kg^{-1}.

The half-lives of three insecticides

Overall, the half-lives of the three insecticides in different parts of the two crops were higher from the field trial than from the net-house trial (Figures 6a, 6b, and 6c). The half-lives of cypermethrin varied from 2.5 – 5.3 days and were the highest in the stem and lowest in the root of two crops. The half-lives of emamectin benzoate varied from 0.37 – 0.64 days and were higher in the stem than in the leaf of amaranth. Indoxacarb had the half-lives varying from 12.5 – 13.2 hours and was higher in the stem than in the leaf of amaranth.

Discussion

Residual dynamics of the examined insecticides

Overall, the residual concentration of the three pesticides was highest in the leaves, followed by the stem (in case of amaranth crop) and then the root of the two tested crops. Directly spraying the chemical solution over the leaves, the crop part directly receiving the sprayed compounds, could be the reason for the difference. Movement of the sprayed insecticides from the leaves to the stem and the root may partially explain the dissipation dynamics in different crop parts. The common dynamics of the three pesticide residues in the current study were apseudo-first-order kinetics model, starting from the spraying day, having the highest pesticide concentration, to around day 10 afterward. These dissipation dynamics were similar to those reported by Song et al.(2019), who showed that the cypermethrin concentration in the aerial parts of six leafy vegetables decreased exponentially to almost 0 within a few weeks. A similar dissipation pattern was also reported from studies by Lu et al. (2014) on chlorpyrifos, Saber et al. (2016) on hexythiazox and Malhat et al. (2013) on emamectin benzoate.

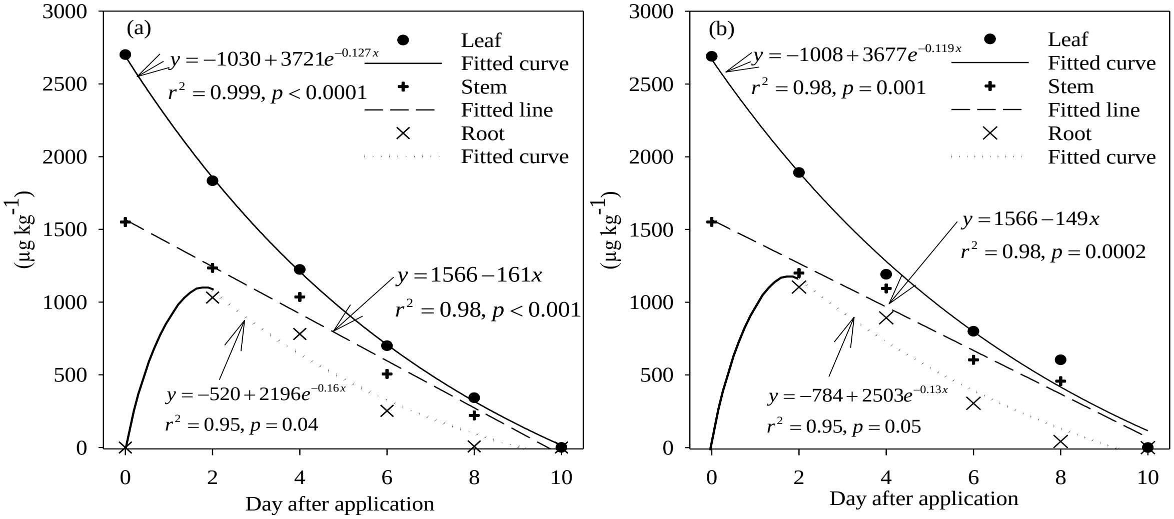

Figure 1: Time-based dissipation dynamics of the cypermethrin concentration in different parts of amaranth from the open-field trial (a) and the net-house trial (b). Data are the means over two growing seasons.

On the leaves of the two tested crops, the dissipation dynamics of the three insecticides could be separated into two phases, the first was characterized with a rapid decay rate for around 2–4 days and the second was with a slow and stable rate. The rapid dissipation in the first phase could be a consequence of two main reasons: (1) movement of the chemicals from the leaves to the stem and down to the root system through a wash-off of chemical solution from the crop surface and/or transport through the vessels/phloem (Mashal and Obeidat 2019; Fantke and Juraske 2013) and (2) dissipation of the chemicals due to photo-degradation on plant surfaces, volatilization, and dilution due to plant growth (Fantke and Juraske 2013; Jacobsen et al. 2015). The first of these could be the most important process, happening typically right after the chemical application (Farha et al. 2016), because the surface wash-off of the sprayed solution may result in (a) a rapid decrease of the pesticide concentration on the leaves and (b) relative slowing down of the dissipation rate of the chemicals in the stem part. The continuous movement of the insecticides from the leaves (the part directly receiving sprayed insecticides) to the stem for the first few days from application may compensate the dissipated portion of the chemicals, lowering the dissipation rate of the compounds in the stem for the first few days. Consequently, the concentration dynamics of cybermethrin in the stem of amaranth could be represented by a straight line (the dissipation rate did not change for the whole test period). Nevertheless, a similar process was not observed with emamectin benzoate in the amaranth stem. This could be the consequence of the rapid dissipation of the compound with short half-lives of 0.39 to 0.46 days.

The dissipation concentration of the three pesticides in the root was relatively low, compared to those in the aboveground parts. For cypermethrin, residue in the root was not found on the application day (2 hours after application), but was found on days 2, 4, 6, and 8, indicating that the compound possibly migrated from the aboveground parts. The migration could occur through the phloem system because the root was washed with tap and distilled water before measurement. The migration from the aboveground parts to the roots could be also inferred for emamectin benzoate, which was not detected on the application day and was only detected in the roots of the two crops on day 2, because the compound had relatively short half-lives so that its migration from the aboveground parts, through plant tissues, could not last longer than 2 days from its application. The diffusion of insecticides to vicinal soil may also be another reason to rapidly reduce the insecticide concentration in the root.

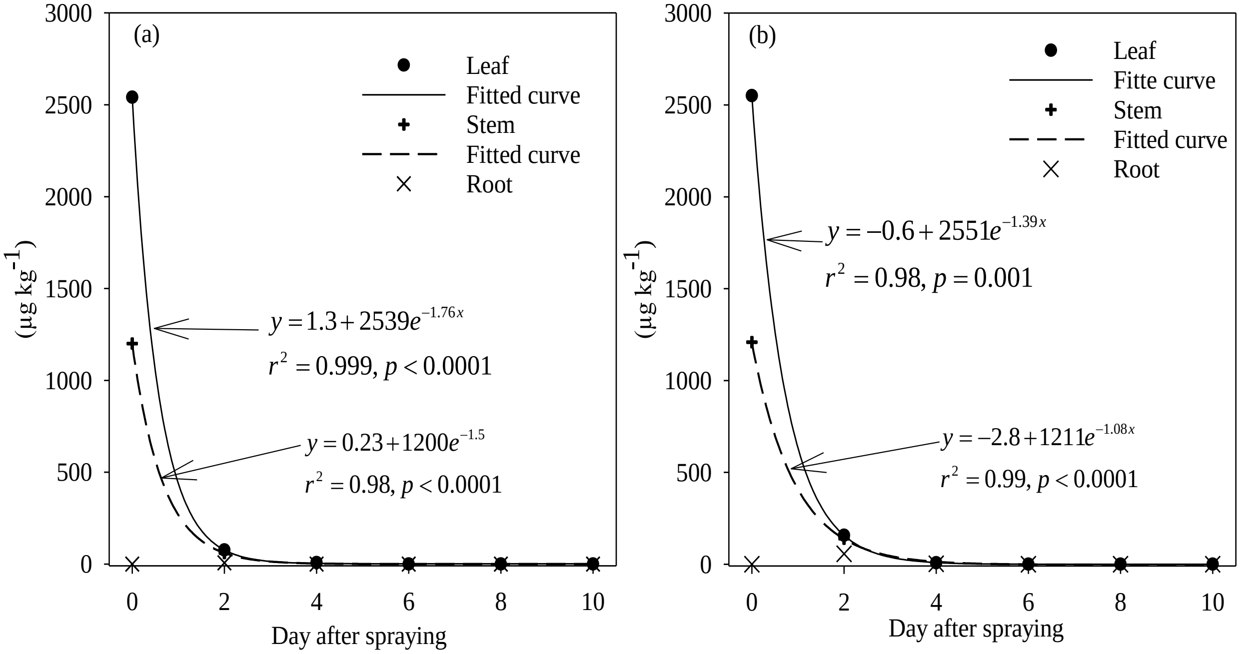

Figure 2: Time-based dissipation dynamics of the emamectin benzoate concentration in different parts of amaranth from the field trial (a) and the net-house trial (b). Data are the means over two growing seasons.

The Codex Maximum Residue Limits (MRL) of cypermethrin (200 µg kg^{-1}) and emamectin benzoate (200 µg kg^{-1}) for mustard greens (FAO/WHO 2020), were reached in the field trial in 7.1 and 1.3 days and in the net-house trial in 7.8 and 1.4 days respectively. Because the MRL for amaranth was not established by FAO/WHO (2020), we assumed that its MRLs could be similar to those for mustard greens. Consequently, cypermethrin and emamectin benzoate in the leaves of amaranth needed 8.7 and 1.4 days in the field trial and 9.4 and 1.8 days in the net-house trial, respectively, to reach the MRL. These indicate that harvesting the vegetable leaves of these crops should be implemented after these corresponding time intervals. The MRL for indoxacarb was established to be 1000 µg kg^{-1} for mustard greens (European Union 2014), predicting 12 and 8-hour periods respectively, for amaranth and mustard greens leaves to be safely harvested.

The half-lives of the three insecticides

Half-lives of cypermethrin of 4.10 – 4.38 days were reported by Fantke et al. (2014). The current study found that the half-lives of this pesticide varied with vegetable parts as well as vegetable crops, from 2.5 to 5.3 days, which covered the period stated by Fantke et al. (2014). The half-lives of this compound were found to be around 2.6 – 7.0 days in various leafy vegetables and were 2.7 days in mustard leaves (Song et al. 2019). Fantke et al. (2014) reported that emamectin benzoate had half-lives of around 1.2 – 2.8 days. In cabbage and apple, Wang et al. (2012) found that the half-lives of emamectin benzoate varied from 1.3 – 3.1 days, dependent on the tested location. The current study found that the half-lives of this compound were less than 1 day, which could be due to location and crop variation.

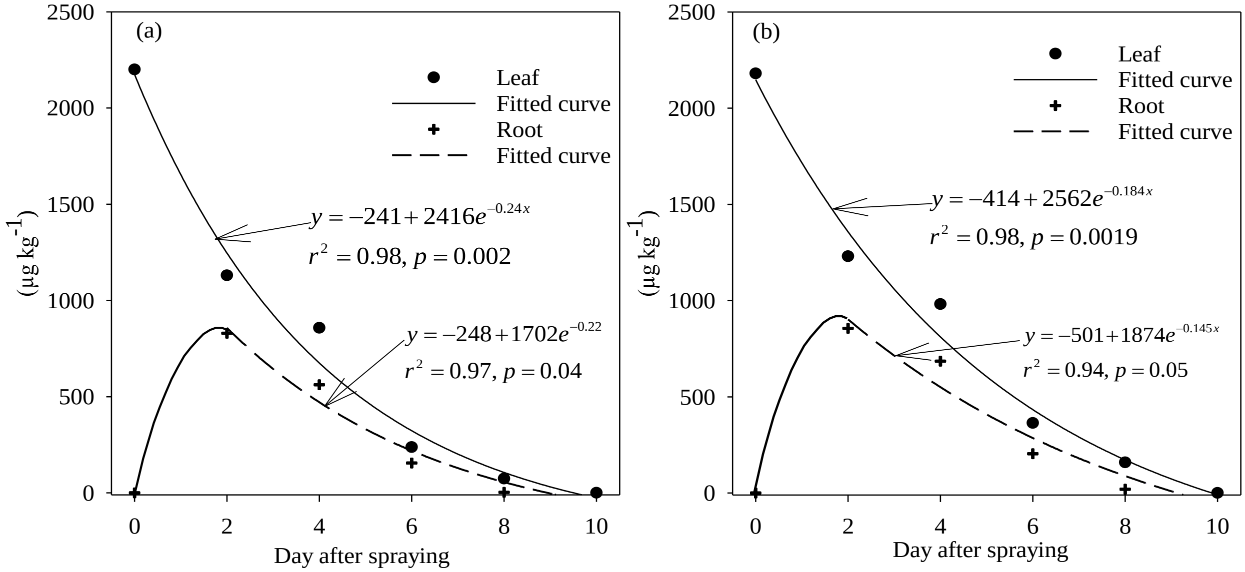

Figure 3: Time-based dissipation dynamics of the cypermethrin concentration in different parts of Mustard greens from the field trial (a) and the net-house trial (b). Data are the means over two growing seasons.

Of the three chemicals tested, cypermethrin had the highest half-lives varying from 2.5 – 5.3 days in the three crop parts of the two trials. A higher spray rate of cypermethrin (120 g a.i. ha^{-1}) compared to the other pesticides (31.6 and 60 g a.i. ha^{-1} for emamectin benzoate and indoxacarb, respectively) could be one of the reasons to explain the difference. Indoxacarb had the lowest half-life of around 12.5 – 13.5 hours in the leaves and stem of amaranth and in the leaves of mustard greens in the net-house trial. This chemical was reported to have half-life of 3.0 – 3.8 days in fruits of eggplant in a field experiment (Saimandir and Gopal 2009). Fantke et al. (2014) reported that indoxacarb had half-lives of 2.3 – 3.8 days. The difference in half-lives could be mainly due to the host crops, as well as the environmental conditions among the studies. Fantke et al. (2014) explained that (1) temperature may affect microbial activities to decompose the pesticides and (2) crop characteristics such as growth, transpiration, transportation, and crop shape may significantly affect the dissipation rate of the pesticides on or in the crop parts.

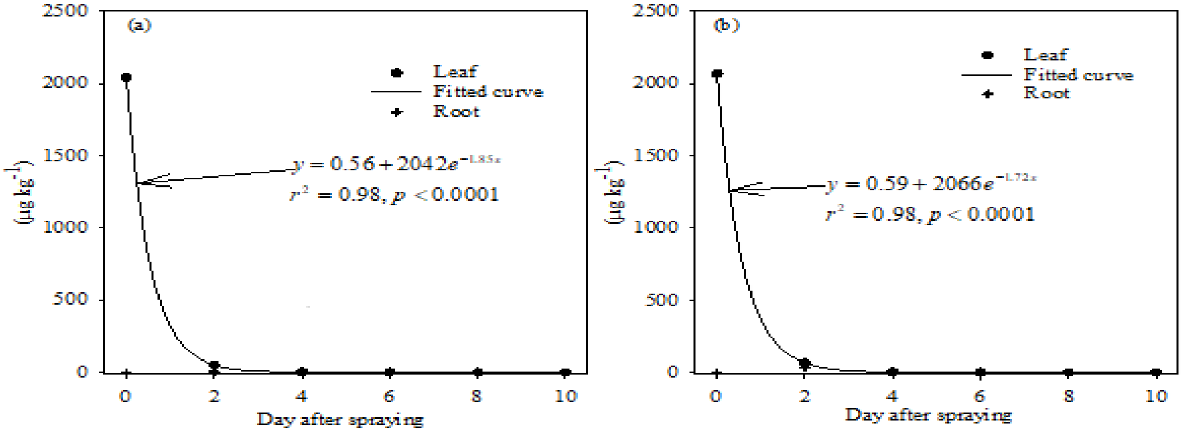

Figure 4: Time-based dissipation dynamics of the Emamectin benzoate concentration in different parts of the mustard greens from the field trial (a) and the net-house trial (b). Data are the means over two growing seasons.

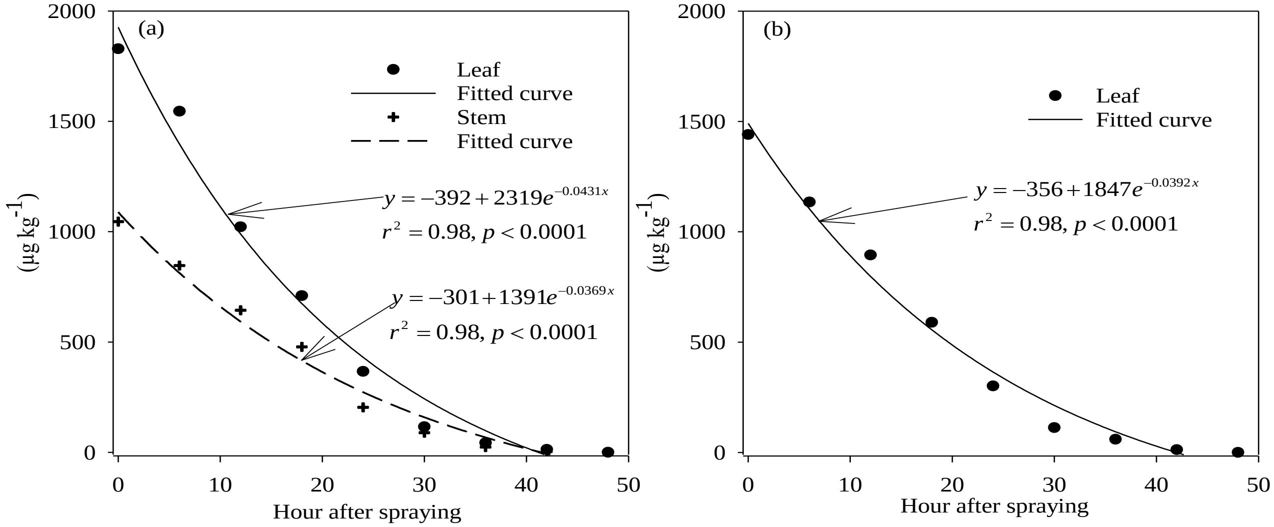

Figure 5: Time-based dissipation dynamics of the Indoxacarb concentration in different parts of the amaranth (a) and the mustard greens (b) from the field trial. Data are the means over two growing seasons.

Effects of crop parts andexperimental locationon the insecticide half-lives

In general, the crop types did not seem to result in much difference in the half-lives of the three tested pesticides, but their parts did show both crop types and crop parts seemed to show differences. The vegetable stems had the half-lives higher than the leaves and root. The stem part is partially enclosed in the leafy structure of crops, resulting in more protection of insecticides from environmental factors, such as sunlight, winddrift, and rainfall. These experimental factors were reported to significantly affect the dissipation rate of insecticides (Cabras et al. 2002; Taylor1986). In addition, the wash-off of the sprayed pesticide solution from the crop leaves may supply the chemicals to the stem from the leaves, prolonging the existence of the tested insecticide in the stem. Meanwhile, the chemicals in the root could be rapidly dissipated, due to their diffusion to vicinal soil.

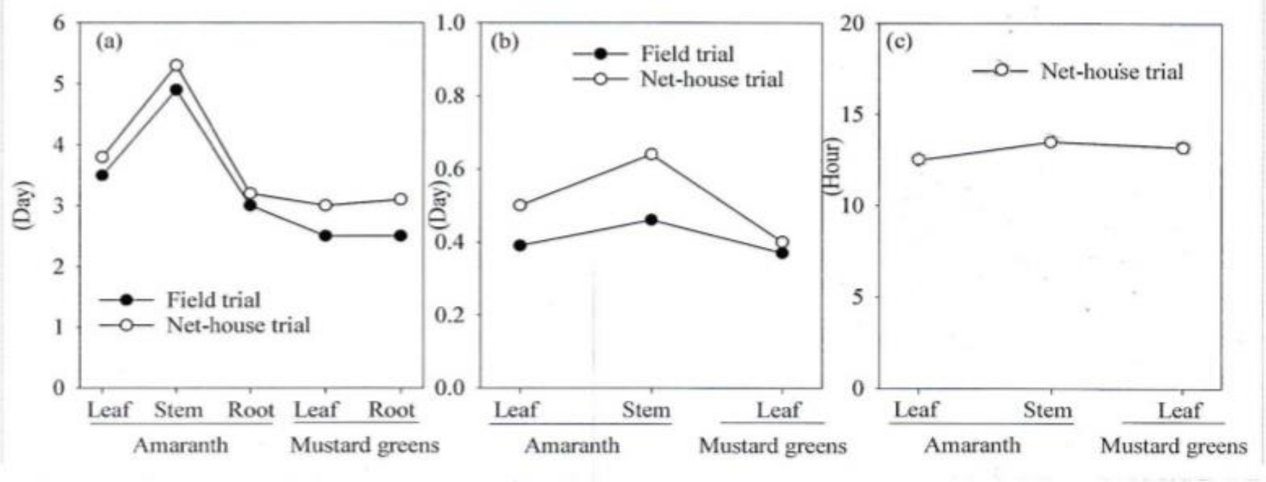

Figure 6: The half-lives of cypermethrin (a), emamectin benzoate (b) and indoxacarb (c) in different parts of the two crops from two trials; indoxacarb concentration was measured only from the net-house trial. Data are the means over two growing seasons.

The half-lives of the three pesticides were greatly affected by the experimental location, the field trial had half-lives of cypermethrin and emamectin benzoate lower than the net-house trial in all crop parts and crop types. This indicated that the dissipation rate of the two pesticides was faster in the field trial than in the net-house trial. Environmental parameters such as temperature, wind velocity and sunlight were all higher in the field trial than in the net-house trial and could be the reason for the difference. Fantke et al. took these parameters into account in estimating the half-lives of various pesticides in different conditions.

Conclusions

The dissipation dynamics and the half-lives of the three tested pesticides (cypermethrin, emamectin benzoate, and indoxacarb) were significantly dependent on the crop types (amaranth and mustard greens) and crop parts (leaves, stem, and root). The pseudo-first-order kinetics model with three parameters provided good fit to the dissipation dynamics of the pesticides. The half-lives of cypermethrin varied 3.0 – 5.3 days and highest in the stem part; those of emamectin benzoate were from 0.37 – 0.64 days, and these of indoxacarb was from 12.5 – 13.5 hours. The three pesticides in different parts of the two crops cultivated in the open-field condition had the half-lives shorter than those in the net-house condition. The safe harvest of the edible crop leaves could be implemented after a certain time period, varying with the pesticides, crop types, and environmental conditions.

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