Pertanika J. Trop. Agric. Sci. 47 (1): 77 - 89 (2024)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Accumulation and Phytotoxicity of Cypermethrin and Deltamethrin to Aquatic Plants

Wilailuck Khompun[1*], Chonlada Dechakiatkrai Theerakarunwong[2] and Waraporn Chouychai[1]

[1]Biology Program, Department of Science, Faculty of Science and Technology, Nakhonsawan Rajabhat University, 60000 Nakhonsawan, Thailand
[2]Chemistry Program, Department of Science, Faculty of Science and Technology, Nakhonsawan Rajabhat University, 60000 Nakhonsawan, Thailand

ABSTRACT

Synthetic pyrethroid contamination in water is a serious environmental concern as this pesticide is highly toxic to aquatic animals. Phytoremediation using aquatic plants that can tolerate and accumulate pyrethroid pesticides is an interesting alternative. In this study, the phytotoxicity of cypermethrin and deltamethrin, alone or in combination, to three aquatic plants, *Azolla microphylla*, *Salvinia cucullata*, and *Spirodela polyrrhiza* were tested. The results show that *S. cucullata* was the most sensitive species because the pigment content in the fronds significantly decreased when exposed to pyrethroid in water. *Azolla microphylla* was the most tolerant species because the pigment content in their fronds significantly increased when exposed to pyrethroid and cypermethrin, which could also significantly increase the plant fresh weight of *A. microphylla*. Both species could accumulate synthetic pyrethroid pesticides in their tissue. The bioconcentration factors of cypermethrin and deltamethrin in *A. microphylla* were 3,508.8 and 2,323.5, respectively, while the bioconcentration factors of cypermethrin and deltamethrin in *S. cucullata* were 453.0 and 381.7, respectively. *Azolla microphylla* is appropriate for use in pyrethroid phytoremediation in water.

Keywords: Aquatic fern, *Azolla*, pyrethroid pesticide, *Salvinia*, *Spirodela*

ARTICLE INFO

Article history:
Received: 02 June 2023
Accepted: 21 July 2023
Published: 19 February 2024

DOI: https://doi.org/10.47836/pjtas.47.1.06

E-mail addresses:
wililuck.k@nsru.ac.th (Wilailuck Khompun)
chonlada.d@nsru.ac.th (Chonlada Dechakiatkrai Theerakarunwong)
waraporn.c@nsru.ac.th (Waraporn Chouychai)
* Corresponding author

INTRODUCTION

The increasing use of insecticides worldwide has increased the contamination and toxicity in the ecosystem. A synthetic pyrethroid is one group of popular insecticides used

ISSN: 1511-3701
e-ISSN: 2231-8542

for agricultural purposes in rice farms in Thailand and soybean farms in Argentina (Maneepitak & Cochard, 2014; Mugni et al., 2011). Contamination with synthetic pyrethroid in water and sediment is a serious environmental problem due to its high toxicity to fish and aquatic animals. For example, the 50% lethal concentration (LC_{50}) of cypermethrin in fish is 2.8 µg/L (Sangchan et al., 2014). Cypermethrin has been reported to be toxic to the valve activity of marine mussels (*Mytilus galloprovincialis*), with the lowest effect concentration at 100 µg/L (Ayad et al., 2011). Contamination with synthetic pyrethroid pesticides in water and sediment has been reported. Pyrethroid concentrations in water from California's San Joaquin River, Orestimba and Del Puerto Creeks ranged from 0.005 to 0.021 µg/L (Ensminger et al., 2011). Cypermethrin has been found in surface water, riverbed sediment, and suspended sediment in the Mae Sa River, northern Thailand, at 0.01 µg/L, 10.5, and 82.8 µg/kg, respectively (Sangchan et al., 2014). The removal of synthetic pyrethroid contamination in water should be done immediately.

Phytoremediation is an environmentally friendly method to remove synthetic pyrethroid contamination in water. Rhizofiltration, using hyperaccumulating aquatic plants to adsorb and absorb pollutants from aquatic environments (Rahman & Hasegawa, 2011), is a phytoremediation process appropriate for pollutant removal in water. Many aquatic plants have been reported to remove pollutants via this process. For example, *Spirodela polyrrhiza* has been reported to accumulate cadmium (Cd) at 54.45 mg/kg fresh weight when cultured in 10 µM Cd contaminated water for four days with 55% of the Cd accumulated in the cell wall (Su et al., 2017). *Spirodela polyrrhiza* also accumulates fluoride when grown in water contaminated with fluoride at 3–20 mg/L (Karmakar et al., 2016). Plants in the genus *Salvinia* were reported to accumulate arsenic (Rahman & Hasegawa, 2011), chromium (Prado et al., 2020), cadmium, nickel, lead, and zinc (Iha & Bianchini Jr., 2015). In addition, plants in the genus *Azolla* have been reported to accumulate arsenic (Rahman & Hasegawa, 2011), mercury, cadmium (Rai, 2008), and methyl violet 2B dye (Kooh et al., 2018). These plant species are naturally found in the aquatic environment in central Thailand, which is the main rice growing area and pyrethroid pesticides are used normally (Maneepitak & Cochard, 2014). However, reports on the phytotoxicity and phytoaccumulation of pyrethroid pesticides are rarely found. This study selected *Azolla microphylla*, *Salvinia cucullate*, and *Spirodela polyrrhiza* to test their tolerance to synthetic pyrethroids, cypermethrin and deltamethrin, alone or in combination. The most tolerant and sensitive species were selected to assess the accumulation capacity for cypermethrin and deltamethrin co-contaminated water. If these aquatic plants could tolerate and accumulate synthetic pyrethroids in water, it would be useful for phytoremediation in agricultural aquatic sites in central Thailand.

MATERIALS AND METHODS

Plant and Water Preparation

Two aquatic ferns, *A. microphylla* and *S. cucullate*, and one species of aquatic angiosperm plant, *S. polyrrhiza*, were used in this study. *Azolla microphylla* and *S. polyrrhiza* were collected from Thung Pueng Sub-district, Nnong Kha Yang District, Uthai Thani Province, Thailand, and *S. cucullata* was collected from Khwae Yai Sub-district, Mueang Nakhon Sawan District, Nakhon Sawan Province, Thailand. The water used in this experiment was tap water with chlorinevolatiled for 3–5 days before use. This water was contaminated with commercial pyrethroid pesticides (Good Knock©, Thailand) that contained 10% (w/v) cypermethrin and 3% (w/v) deltamethrin (Delta 3%©, Thailand) to final concentrations of cypermethrin and deltamethrin in water at 2.5, 5, 7.5, and 10 mg/L.

Experimental Design

For each plant species, the experimental design for each compound, cypermethrin, deltamethrin, and cypermethrin and deltamethrin (1:1), was a completely randomized design (CRD) with one factor, five treatments per experiment and three replicates per treatment. The pots used for *A. microphylla* and *S. polyrrhiza* were 10.61 cm in diameter and each contained 300 ml of water. For *S. cucullata*, the pots were 16.24 cm in diameter and 600 ml of water. There were 6, 6, and 10 g of *A. microphylla*, *S. polyrrhiza*, and *S. cucullata* per pot. All plants were cultured in a nursery that received natural sunlight and was maintained at room temperature for seven days.

Plant Growth Analysis

Plants from each treatment were collected on day seven after planting to determine the plant's fresh weight and dry weight. The moisture in each plant was calculated by (Fresh weight – Dry weight)/Fresh weight (Aveek et al., 2019). The chlorophyll and carotenoid contents in the leaves or fronds were determined according to the method described in Arnon (1949). Briefly, 200 mg of fresh leaves or fronds were crushed with 80% acetone (Merck, Germany), and the volume was adjusted to 10 ml. The absorbances were measured at 663, 645, and 470 nm, and the concentrations of each pigment (mg per g tissue) were calculated as below:

$$\text{Chlorophyll } a \text{ content} = [12.7(A_{663}) - 2.69(A_{645})] * V/(1000*W)$$

$$\text{Chlorophyll } b \text{ content} = [22.9(A_{645}) - 4.68(A_{663})] * V/(1000*W)$$

$$\text{Total chlorophyll content} = \text{Chl.}a + \text{Chl.}b$$

$$\text{Carotenoid content} = [1{,}000(A_{470}) + 3.27(\text{chlorophyll } a - \text{chlorophyll } b)] * V/(W * 229)$$

where A = absorbance at the wavelength mentioned, V = final volume of chlorophyll extract in 80% acetone, and W = fresh weight of tissue extracted.

In addition, the proline contents in the leaves were analyzed by spectrophotometry by measuring the absorbance of the leaf solution at 520 nm. The leaf or frond solutions were extracted by sulphosalicylic, which was then reacted with acid ninhydrin and extracted with toluene before being measured (John et al., 2008).

Phytoaccumulation Experiment and Pyrethroid Analysis

Based on the dried weight of each plant, *A. microphylla* and *S. cucullata* were selected to study the phytoaccumulation capacity. For the phytoaccumulation experiment, *A. microphylla* and *S. cucullata* were cultured in cypermethrin and deltamethrin co-contaminated water for seven days in the same environment described above. The pots used for *A. microphylla* and *S. cucullata* were 44.5 cm in diameter, each containing 6 L of water. There were 500 and 250 g of *A. microphylla* and *S. cucullata* per pot, respectively. For each replicate, 2 L of pyrethroid-contaminated water was collected and sent for analysis of the pyrethroid pesticide concentration at the Central Laboratory Thailand, Ltd., Bangkok branch, using an in-house method based on the TE-CH-207 method using a gas chromatograph with µ-electron capture detector (GC-µECD) with a limit of detection at 0.50 mg/L. The starting concentrations of cypermethrin and deltamethrin were 9.6 and 8.4 mg/L, respectively. In addition, dried plant tissue of *A. microphylla* and *S. cucullata* was collected and sent for analysis of the pyrethroid pesticide accumulation at the Central Laboratory Thailand, Ltd., Bangkok branch, using an in-house method with TE-CH-030 based on Steinwandter (1985) with the limit of detection at 0.01 mg/kg. Each synthetic pyrethroid's bioconcentration factor (BCF) was calculated from each synthetic pyrethroid concentration in the plant tissue/each synthetic pyrethroid concentration in the water (Somtrakoon & Chouychai, 2023).

Statistical Analysis

One-way analysis of variance (ANOVA) and Duncan tests were used for variance analysis and pairwise comparison. The *t*-test was used to compare the bioconcentration factors of the two plant species.

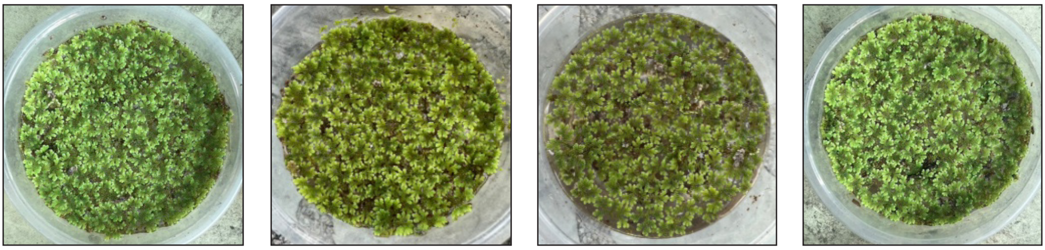
RESULTS AND DISCUSSION

Toxicity of Synthetic Pyrethroid on Weight and Moisture of Aquatic Plants

The toxicity of synthetic pyrethroid on plant weight differed depending on the type of pyrethroid compound and plant species. Cypermethrin or deltamethrin, alone or in combination, did not affect the moisture content of *A. microphylla* and *S. cucullata*. In addition, cypermethrin significantly increased the plant fresh weight of *A. microphylla* but did not affect the fresh weight of *S. cucullata* and the dry weight of both species. Deltamethrin decreased the plant fresh weight of both *A. microphylla* and *S. cucullata* significantly, at 10.0 and 2.5 mg/L, respectively (Table 1). Deltamethrin did not affect the plant dry weight of *S. cucullata* but decreased the plant dry weight of *A. microphylla* at 10.0 mg/L. The combination of cypermethrin and deltamethrin (1:1) only decreased the plant fresh weight of *S. cucullata* significantly at 5.0-10.0 mg/L (Table 1).

The characteristics in fronds of *A. microphylla* were exposed to different concentrations of deltamethrin, cypermethrin, or the combination of deltamethrin and cypermethrin (1:1) were not different when compared with control (Figure 1), but *S. cucullata* sensitive as the color of fronds were slightly different from control (Figure 2). Even though the combination of cypermethrin and deltamethrin (1:1) did not affect the weight and moisture of *S. polyrrhiza*, just cypermethrin decreased the plant dry weight and increased the moisture of *S. polyrrhiza* significantly. Deltamethrin also decreased the fresh and dry weight of *S. polyrrhiza* at 7.5 and 2.5 mg/L, respectively, but it did not affect the plant's moisture (Table 1). The characteristics in leaves of *S. polyrrhiza* were exposed to different concentrations of deltamethrin, cypermethrin, or the combination of deltamethrin and cypermethrin (1:1) not different from the control (Figure 3).

The concentration of synthetic pyrethroid pesticides in this experiment (2.5–10.0 mg/L) was not toxic to the weight and moisture of aquatic plants. Cypermethrin tended to increase the fresh weight of *A. microphylla*. It was the same with the fresh weights of *Eichornia crassipes*, *Pista stratiotes*, and algae grown in 1 mg/L pyrethroid pesticide for seven days that increased dramatically from 15.70, 15.09, and 15.08 mg on day 0 to 17.35,



(a) (b) (c) (d)

Figure 1. Azolla microphylla grew in cypermethrin, deltamethrin, cypermethrin, and deltamethrin (1:1) for 7 days. (a) Non-contaminated water, (b) cypermethrin 10 mg/L, (c) deltamethrin 10 mg/L, and (d) cypermethrin and deltamethrin (1:1) 10 mg/L

(a) (b) (c) (d)

Figure 2. Salvinia cucullata grew in cypermethrin, deltamethrin, cypermethrin, and deltamethrin (1:1) for 7 days. (a) Non-contaminated water, (b) cypermethrin 10 mg/L, (c) deltamethrin 10 mg/L, and (d) cypermethrin and deltamethrin (1:1) 10 mg/L

Table 1
Weight and moisture of three aquatic plants growing in pyrethroid-contaminated water for 7 days

Pyrethroid concentration (mg/L)	*Azolla microphylla*			*Salvinia cucullata*			*Spirodela polyrrhiza*		
	Plant fresh weight (g)	Plant dry weight (g)	% Moisture	Plant fresh weight (g)	Plant dry weight (g)	% Moisture	Plant fresh weight (g)	Plant dry weight (g)	% Moisture
Deltamethrin									
0	3.8±0.11a	0.15±0.011a	96.0±0.24a	18.6±0.71a	0.58±0.03a	96.9±0.28a	8.5±0.15a	0.45±0.01a	94.8±0.20a
2.5	4.1±0.32a	0.17±0.008a	95.1±0.76a	14.0±1.07b	0.59±0.02a	95.8±0.32a	8.1±0.22a	0.36±0.02b	95.3±0.30a
5.0	3.6±0.32a	0.13±0.008b	96.4±0.29a	13.4±0.31b	0.67±0.07a	95.0±0.45a	7.9±0.30a	0.36±0.01b	96.4±1.25a
7.5	3.6±0.26a	0.15±0.007ab	95.8±0.23a	14.4±1.37b	0.64±0.03a	95.4±0.54a	7.0±0.12b	0.35±0.01b	95.0±0.14a
10.0	2.8±0.03b	0.10±0.002c	96.3±0.05a	15.0±0.36b	0.72±0.04a	95.2±0.36a	7.0±0.26b	0.35±0.01b	94.9±0.09a
Cypermethrin									
0	2.9±0.09b	0.13±0.013a	95.6±0.56a	18.6±0.71a	0.58±0.03a	96.9±0.28a	7.9±0.80a	0.49±0.04a	93.8±0.17c
2.5	4.5±0.29a	0.16±0.012a	96.4±0.06a	20.2±0.59a	0.70±0.06a	96.5±0.40a	6.1±0.68a	0.35±0.02b	94.2±0.24b
5.0	4.8±0.23a	0.16±0.021a	96.8±0.35a	19.8±2.05a	0.61±0.07a	96.9±0.12a	6.2±0.30a	0.35±0.01b	94.4±0.13b
7.5	4.7±0.22a	0.14±0.005a	96.9±0.03a	16.7±0.32a	0.73±0.06a	95.6±0.37a	5.4±0.14a	0.29±0.01b	94.6±0.20ab
10.0	4.4±0.30a	0.15±0.009a	96.6±0.05a	16.6±0.97a	0.66±0.08a	96.0±0.37a	6.5±0.31a	0.32±0.01b	95.0±0.13a
Deltamethrin + Cypermethrin (1:1)									
0	3.7±0.41a	0.12±0.016a	96.8±0.12a	18.6±0.71ab	0.58±0.03a	96.9±0.28a	6.4±0.18a	2.27±0.09a	64.5±1.52a
2.5	4.7±0.77a	0.23±0.085a	95.5±0.85a	22.0±2.20a	0.66±0.04a	97.0±0.27a	6.3±0.32a	1.61±0.15a	74.2±2.45a
5.0	3.8±0.40a	0.14±0.018a	96.1±0.23a	16.6±1.31b	0.59±0.04a	96.4±0.06a	6.8±0.30a	1.67±0.27a	75.3±4.13a
7.5	3.2±0.16a	0.12±0.004a	96.3±0.24a	14.9±1.01b	0.61±0.07a	95.8±0.68a	5.8±0.18a	1.65±0.15a	71.6±2.57a
10.0	2.8±0.25a	0.12±0.008a	95.8±0.08a	14.7±0.14b	0.72±0.08a	95.1±0.52a	6.3±0.34a	1.62±0.12a	74.1±2.39a

Note. Different small case letters showed a significant difference ($P<0.05$) at different concentrations of each compound

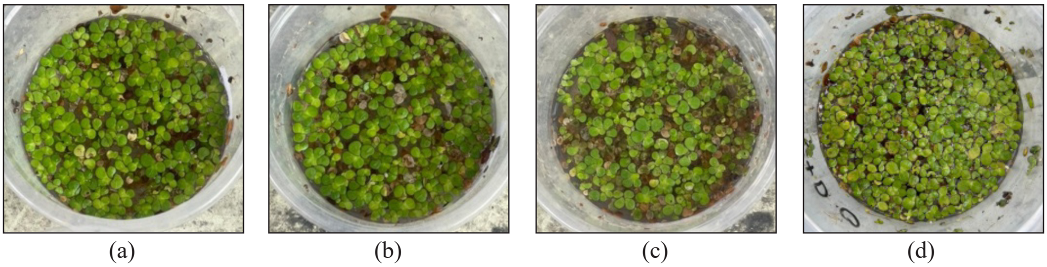


Figure 3. *Spirodela polyrrhiza* grew in cypermethrin, deltamethrin, and cypermethrin and deltamethrin (1:1) for 7 days. (a) Non-contaminated water, (b) cypermethrin 10 mg/L, (c) deltamethrin 10 mg/L, and (d) cypermethrin and deltamethrin (1:1) 10 mg/L

17.51, and 17.56 mg on day seven (Riaz et al., 2017). However, higher concentrations of cypermethrin have been reported to be more toxic. The biomass of *Azolla pinnata* significantly decreased when grown in 30 mg/L cypermethrin for 96 hr (Prasad et al., 2015). Increases in the dry weight and decreases in the moisture content were found in three plant seedlings, *Zea mays*, *Allium cepa*, and *Lathyrus sativus*, exposed to 0.2–0.8 g/L cypermethrin before germination (Aveek et al., 2019).

Toxicity of Synthetic Pyrethroid on Pigment and Proline Content in Leaves/Fronds of Aquatic Plants

The pigment response to pyrethroid-contaminated water in each aquatic plant's leaves differed. The chlorophyll content in the fronds of *A. microphylla* exposed to 7.5–10.0 mg/L deltamethrin increased compared to that grown in non-contaminated water. The chlorophyll *a* content in the fronds of *A. microphylla* in non-contaminated water was 0.020 mg/g FW, while the chlorophyll *a* content in the fronds of *A. microphylla* exposed to 7.5–10.0 mg/L deltamethrin was 0.024 mg/g FW (Table 2). However, increases in the total chlorophyll and carotenoid in the fronds of *A. microphylla* were seen when exposed to 2.5 mg/L deltamethrin. Surprisingly, the chlorophyll *a* and total chlorophyll contents in the fronds of *A. microphylla* were highest when exposed to 5.0 mg/L cypermethrin, and they dramatically decreased when the cypermethrin concentration was increased. The chlorophyll *b* content in the fronds of *A. microphylla* was not affected by any pyrethroid pesticide, and the combination of deltamethrin and cypermethrin did not affect any pigment content in the fronds of *A. microphylla* (Table 2). The proline content in the fronds of *A. microphylla* increased significantly from the control when exposed to 7.5 mg/L deltamethrin, while the proline content decreased significantly from the control when exposed to 10.0 mg/L. The combination of deltamethrin and cypermethrin (1:1) at 2.5–10.0 mg/L decreased the proline content in the fronds significantly (Table 2).

The all-pigment content in the leaves of *S. polyrrhiza* was not significantly different when exposed to different concentrations of deltamethrin, cypermethrin, or the

Table 2
Chlorophyll, carotenoid, and proline content in fronds of Azolla microphylla *growing in pyrethroid-contaminated water for 7 days*

Pyrethroid concentration (mg/L)	Chlorophyll *a* (mg/g FW)	Chlorophyll *b* (mg/g FW)	Total Chlorophyll (mg/g FW)	Carotenoid (mg/g FW)	Proline (mg/g FW)
Deltamethrin					
0	0.020±0.001b	0.012±0.002a	0.032±0.002b	8.02±0.27b	0.65±0.04ab
2.5	0.021±0.001b	0.015±0.001a	0.036±0.001a	10.69±0.91a	0.53±0.06bc
5.0	0.022±0.001b	0.017±0.001a	0.038±0.000a	11.94±0.48a	0.51±0.04c
7.5	0.024±0.000a	0.013±0.002a	0.037±0.002a	12.26±0.63a	0.77±0.02a
10.0	0.024±0.000a	0.015±0.000a	0.039±0.000a	11.68±0.61a	0.73±0.03a
Cypermethrin					
0	0.017±0.000b	0.016±0.001a	0.033±0.001b	11.57±0.70a	0.75±0.02ab
2.5	0.018±0.001b	0.013±0.001a	0.031±0.001b	10.63±0.42a	0.67±0.02b
5.0	0.028±0.001a	0.016±0.003a	0.044±0.003a	13.68±1.18a	0.70±0.04ab
7.5	0.018±0.000b	0.017±0.004a	0.036±0.004ab	12.45±1.44a	0.79±0.03a
10.0	0.018±0.001b	0.014±0.003a	0.033±0.003b	11.69±1.55a	0.57±0.02c
Deltamethrin + Cypermethrin (1:1)					
0	0.021±0.000a	0.015±0.001a	0.036±0.000a	9.58±0.54a	1.00±0.02a
2.5	0.024±0.002a	0.013±0.001a	0.036±0.001a	9.82±0.54a	0.55±0.10b
5.0	0.024±0.001a	0.012±0.000a	0.036±0.001a	10.61±0.81a	0.49±0.19b
7.5	0.026±0.004a	0.031±0.020a	0.058±0.017a	9.62±0.48a	0.41±0.08b
10.0	0.022±0.001a	0.012±0.000a	0.035±0.000a	10.54±0.61a	0.55±0.08b

Note. Different small case letters showed a significant difference ($P<0.05$) at different concentrations of each compound

combination of deltamethrin and cypermethrin (1:1). The proline content in the leaves of *S. polyrrhiza* increased from the control (1.0 mg/g FW) to be 1.8–3.0 mg/g FW when exposed to 2.5–10.0 mg/L deltamethrin while cypermethrin and the combination of deltamethrin and cypermethrin (1:1) did not affect the proline content in the leaves of this plant (Table 3).

The response of the pigment contents in the fronds of *S. cucullata* was sensitive when exposed to pyrethroid pesticides. The chlorophyll *a*, *b*, and total chlorophyll contents in the fronds of *S. cucullata* exposed to 5.0–10.0 mg/L deltamethrin (0.10–0.12 mg/g FW) significantly decreased when compared to the chlorophyll content in the fronds of *S. cucullata* grown in non-contaminated water (0.19 mg/g FW). However, 7.5–10.0 mg/L cypermethrin only significantly decreased the chlorophyll *a* and total chlorophyll contents but did not affect the chlorophyll *b* content in the fronds of *S. cucullate*. The combination of deltamethrin and cypermethrin (1:1) decreased the chlorophyll *a*, *b,* and total chlorophyll contents in the fronds of *S. cucullata* significantly by 10.0, 2.5, and 5.0

Table 3
Chlorophyll, carotenoid, and proline content in leaves of Spirodela polyrrhiza *growing in pyrethroid-contaminated water for 7 days*

Pyrethroid concentration (mg/L)	Chlorophyll *a* (mg/g FW)	Chlorophyll *b* (mg/g FW)	Total chlorophyll (mg/g FW)	Carotenoid (mg/g FW)	Proline (mg/g FW)
Deltamethrin					
0	0.10±0.003a	0.05±0.002a	0.15±0.01a	46.6±0.33a	1.0±0.02c
2.5	0.10±0.005a	0.05±0.002a	0.15±0.01a	47.8±1.23a	3.0±0.26a
5.0	0.10±0.006a	0.06±0.005a	0.16±0.01a	50.0±2.72a	2.1±0.19b
7.5	0.10±0.012a	0.06±0.005a	0.15±0.02a	47.7±4.34a	1.8±0.15b
10.0	0.08±0.008a	0.05±0.004a	0.13±0.01a	42.6±3.33a	2.0±0.27b
Cypermethrin					
0	0.10±0.003a	0.05±0.002a	0.15±0.01a	46.6±0.33a	1.0±0.02a
2.5	0.11±0.002a	0.06±0.004a	0.17±0.01a	54.0±2.36a	1.0±0.22a
5.0	0.11±0.007a	0.06±0.003a	0.17±0.01a	54.0±1.70a	0.9±0.05a
7.5	0.11±0.005a	0.06±0.001a	0.17±0.01a	55.2±2.10a	1.0±0.13a
10.0	0.11±0.011a	0.06±0.007a	0.18±0.02a	54.5±4.00a	0.8±0.08a
Deltamethrin + Cypermethrin (1:1)					
0	0.10±0.003a	0.05±0.002a	0.15±0.01a	46.6±0.33a	1.0±0.02a
2.5	0.10±0.003a	0.04±0.002a	0.14±0.01a	43.8±1.43a	0.7±0.11a
5.0	0.09±0.004a	0.04±0.003a	0.14±0.01a	43.4±1.71a	0.8±0.26a
7.5	0.10±0.010a	0.04±0.006a	0.14±0.02a	43.8±3.95a	1.1±0.13a
10.0	0.10±0.009a	0.04±0.005a	0.14±0.01a	44.9±4.47a	0.9±0.30a

Note. Different small case letters showed a significant difference ($P<0.05$) at different concentrations of each compound

mg/L, respectively. The carotenoid content in the fronds of *S. cucullata* significantly decreased when exposed to 2.5–10.0, 5.0–10.0, and 5.0–10.0 mg/L of deltamethrin, cypermethrin, and a combination of deltamethrin and cypermethrin (1:1), respectively. The proline content in the fronds of *S. cucullata* was not affected by cypermethrin, and the combination of deltamethrin and cypermethrin (1:1), while 2.5–10.0 mg/L deltamethrin decreased the proline content significantly (Table 4).

A decrease in the chlorophyll content was seen clearly in the fronds of *S. cucullata*. All synthetic pyrethroid pesticides could decrease their chlorophyll content significantly. On the other hand, synthetic pyrethroid pesticides did not affect the chlorophyll content in the leaves of *S. polyrrhiza* and tended to increase some pigments in the fronds of *A. microphylla*. Decreasing pigment content and increasing antioxidant enzyme activity have been reported in plants exposed to cypermethrin. For example, a decrease in the chlorophyll content in the leaves was found in three plant seedlings, *Z. mays*, *A. cepa*, and *L. sativus*, exposed to 0.2–0.8 g/L cypermethrin before germination (Aveek et al., 2019). Exposure to 5–15 mg/L cypermethrin was reported to increase antioxidant enzymes in A. pinnata. (Prasad et al., 2015). However, in this study, exposure to 2.5–10 mg/L cypermethrin or the combination of cypermethrin and deltamethrin decreased the proline content in the fronds of *A. microphylla*. The response of the carotenoid content in the fronds of *A. microphylla* to deltamethrin was the same as the response of *A. pinnata* to cypermethrin and increased when pyrethroid was present in the water (Prasad et al., 2015).

Accumulation of Synthetic Pyrethroid in Aquatic Plant Tissue

After seven days, *S. cucullata* and *A. microphylla* could accumulate deltamethrin and cypermethrin. The concentrations of deltamethrin within the biomass of *A. microphylla* and *S. cucullata* were 988.2±64.3 and 623.0±28.7 mg/kg, respectively, while cypermethrin was found in the biomass of *A. microphylla* and *S. cucullata*at 593.7±43.4 and 316.7±45.0 mg/kg, respectively (Table 5). The bioconcentration factor of both species shows the capacity to accumulate deltamethrin and cypermethrin, and *A. microphylla* could significantly accumulate

Table 4
Chlorophyll, carotenoid, and proline content in fronds of Salvinia cucullata *growing in pyrethroid-contaminated water for 7 days*

Pyrethroid concentration (mg/L)	Chlorophyll *a* (mg/g FW)	Chlorophyll *b* (mg/g FW)	Total Chlorophyll (mg/g FW)	Carotenoid (mg/g FW)	Proline (mg/g FW)
Deltamethrin					
0	0.19±0.008a	0.12±0.029a	0.32±0.04a	81.2±4.23a	1.0±0.07a
2.5	0.18±0.016a	0.10±0.018ab	0.27±0.03a	65.9±1.43b	0.1±0.10c
5.0	0.12±0.005b	0.04±0.004b	0.16±0.01b	65.4±5.04b	0.4±0.21bc
7.5	0.10±0.004b	0.04±0.013b	0.14±0.01b	66.7±4.19b	0.7±0.20ab
10.0	0.10±0.020b	0.05±0.009b	0.14±0.03b	41.2±3.16c	0.4±0.05bc
Cypermethrin					
0	0.19±0.008a	0.12±0.029a	0.32±0.04a	81.2±4.23a	1.0±0.07a
2.5	0.17±0.014ab	0.07±0.004a	0.24±0.01ab	73.4±7.28a	0.5±0.25a
5.0	0.16±0.014ab	0.08±0.011a	0.24±0.02ab	46.8±2.11b	0.6±0.18a
7.5	0.12±0.026b	0.06±0.013a	0.18±0.04b	41.9±0.39bc	0.7±0.15a
10.0	0.12±0.016b	0.06±0.008a	0.18±0.02b	33.0±1.53c	0.8±0.33a
Deltamethrin + Cypermethrin (1:1)					
0	0.19±0.008a	0.12±0.029a	0.32±0.04a	81.2±4.23a	1.0±0.07a
2.5	0.18±0.019ab	0.06±0.006b	0.24±0.02ab	79.6±1.50a	0.6±0.51a
5.0	0.14±0.010bc	0.06±0.003b	0.20±0.01b	65.2±0.80b	0.3±0.26a
7.5	0.15±0.002abc	0.06±0.004b	0.22±0.01b	63.0±0.37b	0.8±0.15a
10.0	0.12±0.022c	0.06±0.010b	0.18±0.03b	59.4±2.18b	0.9±0.20a

Note. Different small case letters showed a significant difference ($P<0.05$) at different concentrations of each compound

Table 5
Pyrethroid remaining in water and plant biomass after growing with aquatic plant for 7 days

Treatment	Cypermethrin	Deltamethrin
Water concentration (mg/L)		
Starting concentration	9.6±0.65	8.4±0.31
Azolla – Day 7	0.3±0.01	0.2±0.02
Salvinia – Day 7	1.6±0.38	1.2±0.47
Plant concentration (mg/kg)		
Azolla – day 0	0.03±0.00	B.D.
Salvinia – day 0	0.03±0.00	0.2±0.04
Azolla – Day 7	988.2±64.30	593.7±43.40
Salvinia – Day 7	623.0±28.70	316.7±45.00
Bioconcentration factor		
Azolla	3508.8±172.90a	2323.5±199.60a
Salvinia	453.0±115.30b	381.7±176.10b

Note. Different small case letters showed a significant difference ($P<0.05$) at different treatments of each compound; B.D. = Below detection limit

both pyrethroid compounds more than *S. cucullata*.

Aquatic plants have been reported to remove pyrethroid contamination in water. For example, *Eichornia crassipes*, *Pista stratiotes*, and algae could remove pyrethroid (permethrine, cypermethrine, deltamethrine, and bifenthrine) at 76, 68, and 70%, respectively, within seven days, but the mechanism of pollutant removal was not indicated (Riaz et al., 2017). *Lemna* sp. was also reported to decrease cypermethrin in water by 99.1% (initiation concentration = 10 µg/L), while in the *Lemna*-free treatment, cypermethrin was decreased by 98% within 12 days. The adsorption of the pesticide by the biological surface was assumed to be the main mechanism of *Lemna* sp., but the cypermethrin amount in the plant tissue was not reported (Mugni et al., 2011). In our study, both plant species could accumulate cypermethrin at 94.6 and 90.8%, while deltamethrin was accumulated at 65.0 and 52.8% for *A. microphylla* and *S. cucullata*, respectively, within 7 days. However, all the dried tissue of the plants was sent for analysis of the pyrethroid content, and the adsorption and absorption mechanisms could not be separated. These results show that *A. microphylla* is an effective aquatic plant for pyrethroid phytoremediation compared to other species.

CONCLUSION

Synthetic pyrethroids were more toxic to the pigment content in the leaves of aquatic plants than the plant's fresh and dry weight. Among the three species, *S. cucullata* was the most sensitive, and *A. microphylla* was the most tolerant to synthetic pyrethroids when considered with pigment content. Based on plant dry weight, *S. polyrrhiza* was the most sensitive, and *S. cucullata* was the most tolerant to synthetic pyrethroids.

However, both species have the capacity to accumulate deltamethrin and cypermethrin within the plant biomass. It is interesting to use both plant species for phytoremediation of pyrethroid-contaminated water, but using *A. microphylla* biomass from pyrethroid-contaminated sites as green manure in agricultural situations may be a concern.

ACKNOWLEDGEMENTS

We thank Nakhon Sawan Rajabhat University and Thailand Science Research and Innovation (TSRI) Grant Year 2022 under Grant No. FRB650025/0199 Project No. 2558225 for the financial support.

REFERENCES

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris. Plant Physiology*, *24*(1), 1–15. https://doi.org/10.1104/pp.24.1.1

Aveek, S., Jyoti, P. S., Jaydeb, J., & Somashree, M. (2019). Effect of cypermethrin on growth, cell division and photosynthetic pigment content in onion, maize and grass pea. *Research Journal of Chemistry and Environment*, *23*(8), 126-129.

Ayad, M. A., Fdil, M. A., & Mouabad, A. (2011). Effects of cypermethrin (pyrethroid insecticide) on the valve activity behavior, byssal thread formation, and survival in air of the marine mussel *Mytilus galloprovincialis*. *Archives of Environmental Contamination and Toxicology*, *60*, 462-470. https://doi.org/10.1007/s00244-010-9549-7

Ensminger, M., Bergin, R., Spurlock, F., & Goh, K. S. (2011). Pesticide concentrations in water and sediment and associated invertebrate toxicity in Del Puerto and Orestimba Creeks, California, 2007–2008. *Environmental Monitoring and Assessment*, *175*, 573-587. https://doi.org/10.1007/s10661-010-1552-y

Iha, D. S., & Bianchini Jr., I. (2015). Phytoremediation of Cd, Ni, Pb and Zn by *Salvinia minima*. *International Journal of Phytoremediation*, *17*(10), 929-935. https://doi.org/10.1080/15226514.2014.1003793

John, R., Ahmad, P., Gadgil, K., & Sharma, S. (2008). Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. *Plant Soil and Environment*, *54*(6), 262–270. https://doi.org/10.17221/2787-PSE

Karmakar, S., Mukherjee, J., & Mukherjee, S. (2016). Removal of fluoride contamination in water by three aquatic plants. *International Journal of Phytoremediation*, *18*(3), 222-227. https://doi.org/10.1080/15226514.2015.1073676

Kooh, M. R. R., Lim, L. B. L., Lim, L., & Malik, O. A. (2018). Phytoextraction potential of water fern (*Azolla pinnata*) in the removal of a hazardous dye, methyl violet 2B: Artificial neural network modelling. *International Journal of Phytoremediation*, *20*(5), 424-431. https://doi.org/10.1080/15226514.2017.1365337

Maneepitak, S., & Cochard, R. (2014). Uses, toxicity levels, and environmental impacts of synthetic and natural pesticides in rice fields – A survey in Central Thailand. *International Journal of Biodiversity Science, Ecosystem Services and Management*, *10*(2), 144-156. https://doi.org/10.1080/21513732.2014.905493

Mugni, H., Demetrio, P., Bulus, G., Ronco, A., & Bonetto, C. (2011). Effect of aquatic vegetation on the persistence of cypermethrin toxicity in water. *Bulletin of Environmental Contamination and Toxicology*, *86*, 23–27. https://doi.org/10.1007/s00128-010-0143-5

Prado, C., Chocobar-Ponce, S.,Pagano, E., Prado, F., & Rosa, M. (2021). Differential effects of Zn concentrations on Cr(VI) uptake by two *Salvinia* species: Involvement of thiol compounds. *International Journal of Phytoremediation*, *23*(1), 10-17. https://doi.org/10.1080/15226514.2020.1786796

Prasad, S. M., Singh, A., & Singh, P. (2015). Physiological, biochemical and growth responses of *Azolla pinnata* to chlorpyrifos and cypermethrin pesticides exposure: A comparative study. *Chemistry and Ecology*, *31*(3), 285-298. https://doi.org/10.1080/02757540.2014.950566

Rahman, M. A., & Hasegawa, H. (2011). Aquatic arsenic: Phytoremediation using floating macrophytes. *Chemosphere*, *83*(5), 633-646. http://doi.org/10.1016/j.chemosphere.2011.02.045

Rai, P. K. (2008). Technical note: Phytoremediation of Hg and Cd from industrial effluents using an aquatic free floating macrophyte *Azolla pinnata*. *International Journal of Phytoremediation*, *10*(5), 430-435. https://doi.org/10.1080/15226510802100606

Riaz, G., Tabinda, B. A., Iqbal, S., Yasar, A., Abbas, M., Khan, A. M., Mahfooz, Y., & Baqar, M. (2017). Phytoremediation of organochlorine and pyrethroid pesticides by aquatic macrophytes and algae in freshwater systems. *International Journal of Phytoremediation*, *19*(10), 894-898. https://doi.org/10.1080/15226514.2017.1303808

Sangchan, W., Bannchan, M., Ingwerson, J., Hugenschmidt, C., Schwadorf, K., Thavornyutikarn, P., Pansombat, K., & Streck, T. (2014). Monitoring and risk assessment of pesticides in a tropical river of an agricultural watershed in northern Thailand. *Environment and Monitoring Assessment*, *186*, 1083–1099. https://doi.org/10.1007/s10661-013-3440-8

Somtrakoon, K., & Chouychai, W. (2023). Phytoremediation potential of *Ceratophyllum* sp. on arsenic-contaminated conditions. *Journal of Agricultural Sciences - Sri Lanka*, *18*(2), 183-192. http://doi.org/10.4038/jas.v18i2.10252

Steinwandter, H. (1985). Universal 5 min on-line method for extracting and isolating pesticide residues and industrial chemicals. *Fresenius' Zeitschriftfuer Analytische Chemie*, *322*, 752-754. https://doi.org/10.1007/BF00489393

Su, C., Jiang, Y., Li, F., Yang, Y., Lu, Q., Zhang, T., Hu, D., & Xu, Q. (2017). Investigation of subcellular distribution, physiological, and biochemical changes in *Spirodela polyrhiza* as a function of cadmium exposure. *Environmental and Experimental Botany*, *142*, 24-33. https://doi.org/10.1016/j.envexpbot.2017.07.015