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The Effect of Carbamate Insecticide on the Growth of Three Aquatic Plant Species: *Ipomoea aquatica*, *Pistia stratiotes* and *Hydrocharis dubia*

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ABSTRACT The purpose of this study was to assess the effects of carbamate insecticide on the growth of three aquatic plant species, *Ipomoea aquatica*, *Pistia stratiotes* and *Hydrocharis dubia*. These three aquatic plant species were treated with different concentrations of carbaryl and test durations. The fresh weight increase, the dry weight per fresh weight and the total chlorophyll content of the treated plants decreased with increasing concentrations of carbaryl and the test durations. The leaf injury indices of the treated plants increased with increasing concentrations of carbaryl and the test durations, while the leaf injury indices of the controls were zero. The 96-hour EC_{50} values of total chlorophyll content of *I. aquatica*, *P. stratiotes* and *H. dubia* were 0.996, 0.785 and 0.334 g/l, respectively. Moreover, chlorosis and necrosis occurred at the leaf margin of the treated plants, then they extended into the inner portion of the leaf blade. Finally, the leaves decayed and the plants died.

KEYWORDS: carbamate insecticide, growth, *Ipomoea aquatica*, *Pistia stratiotes*, *Hydrocharis dubia.*

INTRODUCTION

Carbaryl is one type of carbamate compound. Its structure comprises of aryl *N*- methylcarbamate esters of phenols.[1] Carbaryl has less toxicity to fish, birds and mammals because it is rapidly metabolized or excreted by most vertebrates. The LD_{50} values for rat, ring-necked pheasant and red-winged blackbird are 859, 707 and 56 mg/kg, respectively.[2] Carbaryl is used to control several pests such as mites on apples, pea moths and pea aphids.[1] It is applied in greatest amount because of its many advantages. Unfortunately, the insecticides that are applied as aerial sprays or dusts to foliage or directly to soil cause the pollution of water. Insecticides are deposited in water bodies by direct sprays or rain water and are toxic to aquatic plants. The accumulation or toxicity on these aquatic plants by insecticides can cause damage to animals or humans that eat them. Furthermore, the aquatic plants have various levels of resistance to insecticides and they can be used as bioindicators that can indicate the quality of water resources. Hence, three species of aquatic plants, *Ipomoea aquatica*, *Pistia stratiotes* and *Hydrocharis dubia,* were chosen for the study on the effect of carbaryl.

MATERIALS AND METHODS

Culture of aquatic plants

P. stratiotes and *H. dubia* were collected from a local pond at Nakornpathom province. *I. aquatica* were bought from the market and dipped in water for root initiation.

These three aquatic plant species were maintained in the growth media for one week (Table 1). The water quality was as follows: pH 4.0, temperature, 27±2°C, and water hardness, 113 mg/l, $CaCO_3$. Then, they were used for the experiments under the temperature of 25±2°C, cool white fluorescent lamp and photoperiod of 12 h/day.

Experimental procedures

I. aquatica (with shoots 25-30 cm long), *P. stratiotes* (with leaves 4-6 cm long) and *H. dubia* which had 3-4 leaves (3-5 cm long) were maintained in growth media which contained 0, 0.2, 0.4, 0.8 and 1.6 g/l carbaryl. The concentration of carbaryl was checked by High Performance Liquid Chromatography (HPLC) every other day. The test durations were 2, 4, 6 and 8 days. Four replicates were performed for each treatment.

During the experiments, morphological changes of the controls and treated plants were observed and recorded.

Measurement

1. *Fresh weight*

The fresh weights of the control and treated plants were measured by the equation : Fresh weight (finish)-fresh weight (start).

2. *Dry weight per fresh weight*

The dry weights per fresh weights of control and treated plants were calculated by:

$$\frac{\text{Dry weight (finish)}}{\text{Fresh weight (start)}}$$

(Aquatic plant materials were dried at 85±2°C in a hot-air oven and their dry weights were recorded).

3. *Total chlorophyll content*

Samples for each treatment were homogenized in a blender and filtered. The samples were put into a grinding tube, and two ml of 90% acetone were added. The pestle was inserted and ground to release the pigments from the cells. Then, the samples were washed with 2 ml of 90% acetone in the grinding tube. The contents of the grinding tube were washed into a screw-capped test tube and made up to a known volume. The screw-capped test tube was centrifuged at 2,500 rpm for 30 minutes. The absorbance of the supernatant was measured at 663 and 750 nm. The contents of the cuvette were poured back into the screw-capped test tube, 0.1 ml of 4 M HCl was added and the centrifugation was repeated. The absorbance of the supernatant was measured again at 663 and 750 nm. The total chlorophyll contents of the control and treated plants were calculated as previously described.[3]

4. *Leaf injury index*

After each experiment was finished, the visible leaf injury was recorded on a graph paper. The value of leaf injury index (LII) was calculated by applying the following formulae :

Percentage leaf area injured

$$= \frac{\text{Total leaf area injured}}{\text{Total leaf area}} \times 100 \text{ ....(I)}$$

Percentage number of leaves injured

$$= \frac{\text{Number of leaves injured}}{\text{Total number of leaves}} \times 100 \text{ ....(II)}$$

$$\text{LII} = \text{I} \times \text{II}$$

5. EC_{50} value of total chlorophyll content

On day 4, the percentage decrease of total chlorophyll content was plotted as the ordinate against concentration. The EC_{50} value was calculated by fitting into a probit regression line.[4]

Results

Fresh weight increase

Fig 1 (A,B,C) shows the fresh weight increases of *I. aquatica*, *P. stratiotes* and *H. dubia* treated with various concentrations of carbaryl. The fresh weight increase of the controls increased with increasing test durations. The fresh weight increases of the treated plants decreased significantly ($P<0.05$) as the carbaryl concentration was increased.

Dry weight per fresh weight

Fig 2 (A,B,C) shows the dry weight per fresh weight of *I. aquatica*, *P. stratiotes* and *H. dubia* treated with various concentrations of carbaryl. The dry weight per fresh weight of the control increased with increasing test durations. The dry weights per fresh weights of the treated plants decreased significantly ($P<0.05$) as the carbaryl concentration was increased.

Total chlorophyll content

Fig 3 (A,B,C) shows the total chlorophyll contents of *I. aquatica*, *P. stratiotes* and *H. dubia* treated with various concentrations of carbaryl. The total chlorophyll content of the control increased with increasing test durations. The total chlorophyll contents of the treated plants decreased significantly ($P<0.05$) as the carbaryl concentration was increased.

Leaf injury index

Fig 4 (A,B,C) shows the leaf injury indices of *I. aquatica, P. stratiotes* and *H. dubia* treated with various concentrations of carbaryl. The leaf injury indices of the controls were zero during the eight days of experiment. The leaf injury indices of the treated plants increased significantly ($P<0.05$) as the carbaryl concentration was increased.

EC_{50} value of total chlorophyll content

Table 2 shows the 96-hour EC_{50} values of total chlorophyll content of *I. aquatica*, *P. stratiotes* and *H. dubia* treated with carbaryl. They were 0.996, 0.785 and 0.334 g/l, in *I. aquatica*, *P. stratiotes* and *H. dubia*, respectively. These EC_{50} values showed that *I. aquatica* had the highest degree of resistance to carbaryl and *H. dubia* had the highest degree of susceptibility to carbaryl.

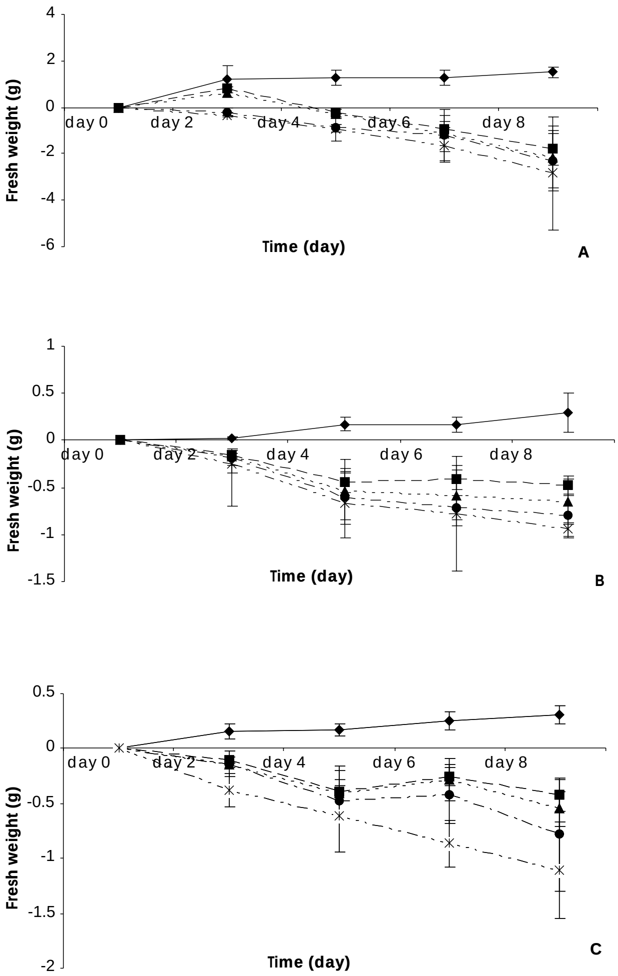

Fig 1. Effect of carbaryl on fresh weight increase of *I. aquatica* (A), *P. stratiotes* (B) and *H. dubia* (C) (—◆— 0 g/l, –■– 0.2 g/l, --▲-- 0.4 g/l, –●– 0.8 g/l, –✳– 1.6 g/l of carbaryl).

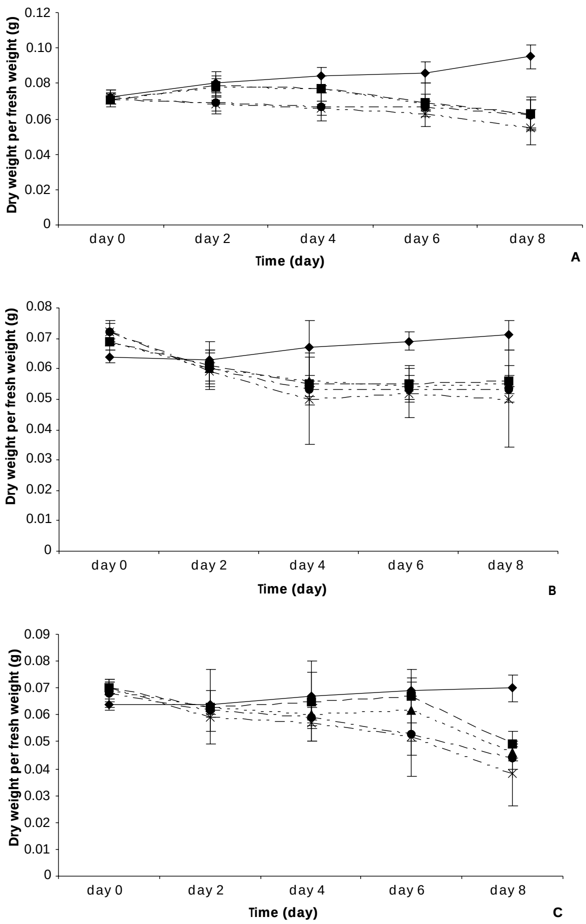

Fig 2. Effect of carbaryl on dry weight per fresh weight of *I. aquatica* (A), *P. stratiotes* (B) and *H. dubia* (C) (—◆— 0 g/l, –■– 0.2 g/l, --▲-- 0.4 g/l, –●– 0.8 g/l, –✳– 1.6 g/l of carbaryl).

Morphological Study

The effects of carbaryl were more pronounced in the leaves of treated plants than in other organs. Similar morphological changes were found in *I. aquatica, P. stratiotes* and *H. dubia*. These changes started from the leaf margins and extended toward the inner portion of the blade. The visible signs of injury were chlorosis and then necrosis.

Discussion

From this study, it is clear that the fresh weight increase and the dry weight per fresh weight of untreated plants, *I. aquatica*, *P. stratiotes* and *H. dubia* which could grow in the nutrient media increased with increasing test durations. However, the fresh weight increase and the dry weight per fresh weight of these plants treated with carbaryl decreased with increasing concentrations of carbaryl and test durations. Thus, it indicates that carbaryl can decrease the growth of these three aquatic plant species. The results on the effect of carbaryl on the growth of *I. aquatica*, *P. stratiotes* and *H. dubia* agreed with those studied in *Nitzschia* sp., *Scenedesmus quadricauda*, *Selenastrum capricornutum*, *Microcystis flosaquae*, *Cyclotella meneghiana*, *Lemna minor*,[5] *Westiellopsis prolifica*,[6] *Ankistrodesmus* sp., *Euglena* sp. and *Chlorella pyrenoidosa*[7]. Moreover, carbaryl can decrease the fresh weight and the dry weight in *Pisum sativum* and *Vigna sinensis*.[8]

The other carbamate pesticides can also inhibit the growth of phytoplanktons and vascular plants. Thiobencarb decreases the growth of *Scenedesmus acutus*, *Chlorella saccharophila* and *Pseudanabaena*

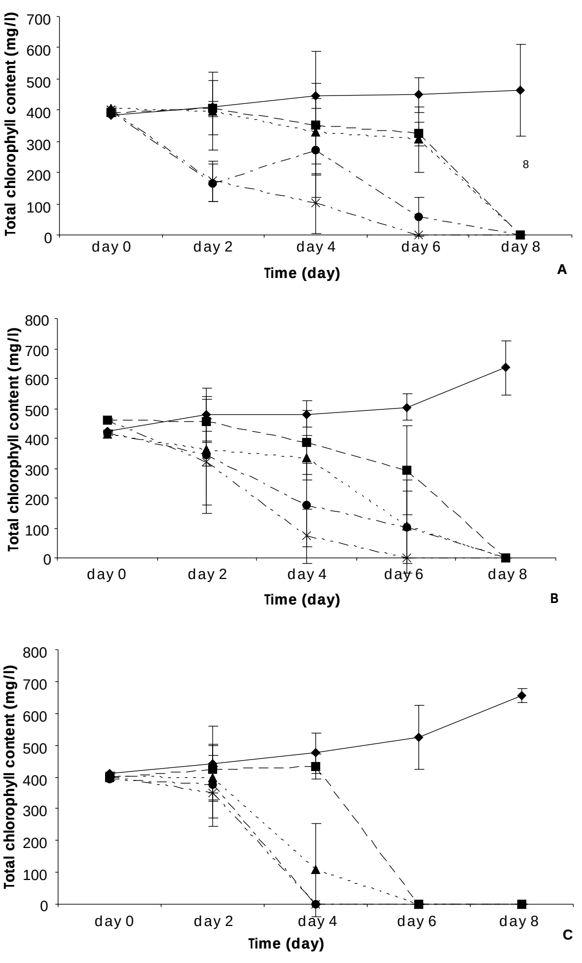

Fig 3. Effect of carbaryl on total chlorophyll content of *I. aquatica* (A), *P. stratiotes* (B) and *H. dubia* (C) (—◆— 0 g/l, – ■ – 0.2 g/l, - -▲- - 0.4 g/l, – ● – 0.8 g/l, – ✳ – 1.6 g/l of carbaryl).

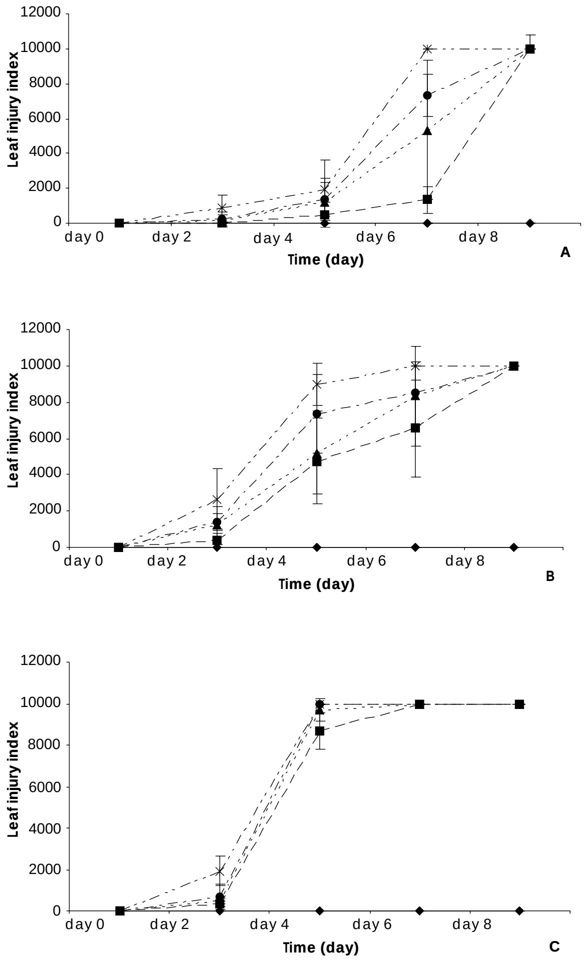

Fig 4. Effect of carbaryl on leaf injury index of *I. aquatica* (A), *P. stratiotes* (B) and *H. dubia* (C) (—◆— 0 g/l, – ■ – 0.2 g/l, - -▲- - 0.4 g/l, – ● – 0.8 g/l, – ✳ – 1.6 g/l of carbaryl).

galeata.[9] Benthiocarb can decrease the growth of *Nostoc muscorum*[10] and carbofuran can also decrease the growth of *S. quadricauda*, *Microcystis aeruginosa*, *L. minor*,[5] *Anabaena fertilissima* and *Nostoc commune*.[11] Furthermore, dimetilan decreases the fresh weight and dry weight of *P. sativum* and *V. sinensis*.[8] Chlorpropham and sulfallate decrease the shoot dry weights of *Medicago sativa* and *Triticum vulgare*. Phenmedipham also decreases the shoot and root dry weights of *M. sativa*, *T. vulgare* and *Sorghum vulgare*.[12] However, some carbamate pesticides can promote the growth of phytoplankton and seedlings of some vascular plants. Aminocarb promotes the growth of *C. pyrenoidosa*[13] and carbofuran promotes the growth of *Oryza sativa*, *Gossypium hirsutum* and *Arachis hypogea* seedlings.[14]

The total chlorophyll contents of the untreated plants, *I. aquatica*, *P. stratiotes* and *H. dubia* increased with increasing test durations. The total chlorophyll content of these plants treated with carbaryl decreased with increasing concentrations of carbaryl and test durations. Therefore, carbaryl could decrease the total chlorophyll content of these three aquatic plant species. This result agreed with those studied in *Anabaena* sp. and *W. prolifica*.[6] Moreover, carbaryl can decrease the chlorophyll a contents of *A. fertilissima*, *A. variabilis*, *Nostoc sphaericum*, *N. linckia*, *N. muscorum*, *Calothrix parietina*, *Scytonema multiramosum* and *Westiellopsis* sp.[15] Other carbamate pesticides such as benthiocarb also reduce the chlorophyll a content of *N. muscorum*[10].

The 96-hour EC_{50} values of *I. aquatica*, *P. stratiotes*, and *H. dubia* were 0.996, 0.785 and 0.334 g/l, respectively. The results of this study indicated

Table 1. Compositions of the growth media for three aquatic plant species.

Chemical	**Concentration (g/l)**		
	I. aquatica	***P. stratiotes***	***H. dubia***
Major elements			
Urea fertilizer	0.20	–	0.05
$KNO_3 \cdot H_2O$	-	0.013	-
$(NH_4)_2(SO_4)_2 \cdot H_2O$	-	0.011	-
$KH_2PO_4 \cdot H_2O$	-	0.136	-
$CaSO_4 \cdot 2H_2O$	-	0.015	-
$MgSO_4 \cdot 7H_2O$	-	0.241	-
$FeSO_4 \cdot 7H_2O$	-	0.0028	-
Chemical	**Concentration (mg/l)**		
	I. aquatica	***P. stratiotes***	***H. dubia***
Minor elements			
$H_3BO_3 \cdot H_2O$	2.86	2.86	2.86
$MnCl_2 \cdot 4H_2O$	1.81	1.81	1.81
$ZnSO_4 \cdot H_2O$	0.22	0.22	0.22
$CuSO_4 \cdot 5H_2O$	0.08	0.08	0.08
$H_2MoO_4 \cdot H_2O$	0.02	0.02	0.02

Table 2. The 96-hour EC_{50} values of total chlorophyll content of three aquatic plant species treated with carbaryl.

Plant species	**96-hour EC_{50} value (g/l)**
I. aquatica	0.996
P. stratiotes	0.785
H. dubia	0.334

that *I. aquatica* had the highest degree of resistance and *H. dubia* had the highest degree of susceptibility to carbaryl. Nevertheless, the 96-hour EC_{50} values of *Chlorococcum* sp., *Nitzschia angularum*, *Skeletonema costatum* and *Chlorella* sp. are 1.8, 1.0, 0.9 and 0.6 ppm, respectively[16]. The 15-day EC_{50} values of *A. fertilissima*, *A. variabilis*, *N. sphaericum*, *N. linckia*, *N. muscorum*, *C. parietina*, *S. multiramosum* and *Westiellopsis* sp. are 7.4, 5.1, 9.0, 15.4, 22.5, 28.1, 18.3 and 9.6 ppm, respectively[15]. The 96-hour EC_{50} values of this study may have differed from those observations because of several factors such as the test species, the experimental procedures and the parameters used to calculate the EC_{50} values.

The treated plants, *I. aquatica*, *P. stratiotes* and *H. dubia* were maintained in the growth media which contained different concentrations of carbaryl. Carbaryl adsorption occurs along the entire length of the root, but it is maximal in the apical portion.[17] Carbaryl molecules diffuse through the cortex and the endodermis into the stele, where the vascular tissue is located. These molecules enter the phloem and xylem and move to the shoots and leaves. Carbaryl tends to accumulate at the leaf tip and along the leaf margin.[17,18] Thus, the first leaf injury should appear at the leaf margin, then at the other parts of leaf . These patterns of leaf injury occurred in the leaves in this study. The first chlorosis appeared at the leaf margin and extended toward the inner portion of the blade. The visible signs of injury, chlorosis and necrosis, appeared in a few days. The leaf injury indices of the treated plants increased with increasing concentrations of carbaryl and test durations while the leaf injury indices of the control were zero in every test duration. Therefore, carbaryl is toxic to these three aquatic plant species. Chlorosis and necrosis occurred in the leaf of *H. dubia* faster than those of *I. aquatica* and *P. stratiotes*. This result was in agreement with the 96-hour EC_{50} values and it is cleared that *H. dubia* had the highest degree of susceptibility to carbaryl.

From the morphological studies of these three aquatic plant species, chlorosis and necrosis appeared at the leaf margin and extended toward the inner portion of the blade. These visible signs of injury occurred in the mature leaves, then they appeared in the young leaves. The translocation and accumulation of carbaryl in these three aquatic plant species appears to be similar to those of carbofuran in *A. hypogea*.[14] Furthermore, the roots and the shoots of the treated plants stopped growing completely in a few days. There were some studies which indicate that carbaryl could inhibit the mitotic cell division of *Allium cepa*[19] and the meiotic cell division of *Vicia faba*[20]. The other carbamate pesticides, such as urethane and avadex also inhibit mitosis.[21, 22] Therefore, the growth of roots and shoots of the treated plants may be inhibited by carbaryl.

From this study, these species of aquatic plants can be used as bioindicators of the quality of water resources. The morphological changes occurred when they were maintained in the toxic water. The severity of plant injuries can indicate the degree of toxicity of the water resource.

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