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THE EFFECT OF HERBICIDES ON GROWTH AND PHOTOSYNTHETIC PIGMENTS OF THREE SUBMERGED MACROPHYTES

By

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ABSTRACT

An undesirable side-effect of the agricultural use of herbicides is that they may damage the flora of the adjacent water bodies. The possibility that results from such hazard was investigated by evaluating the response of the three isolated submerged macrophytes; *Ceratophyllum demersum*, *Potamogeton pectinatus* and *Elodea canadensis* to acetochlor and paraquat. Plants in laboratory bioassays were exposed to 11, 16 and 22 ug/l acetochlor and 100, 300 and 500 ug/l paraquat. *Ceratophyllum* lost its weight (at using 11 & 16 ug/l acetochlor) but recovered again at higher concentration (22 ug/l), while the plant was injured by all paraquat levels. Its chlorophyll a and b contents fluctuated between increases and decreases without respect to herbicide levels. *Potamogeton* showed increases in its fresh weight yield at exposure to 11 & 16 ug/l acetochlor. It lost its weight at exposure to 300 ug/l paraquat, but recovered at 500 ug/l paraquat. Its chlorophyll a and b contents showed increases at the lowest herbicides levels after which they were reduced at higher concentrations. *Elodea* showed increases in biomass as well as chlorophyll a and b contents, particularly at 11 ug/l acetochlor and 100 ug/l paraquat. Generally, carotenes decreased in tested plants at all herbicidal concentration levels. The results indicated that any plant was not

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predictively most sensitive than the other concerning both growth performance together with photosynthetic pigments variations.

INTRODUCTION

Modern agriculture has greatly influenced freshwater ecosystems in large parts of the world by using herbicides for crop protection in agricultural areas. These chemicals may enter adjacent freshwater ecosystems such as ponds and drainage ditches, e.g. by spray drift, leaching, run-off and/or accidental spills. Although increased levels of herbicides contamination are reported to affect shallow freshwaters, hardly any information is available on the effect of these pollutants in aquatic ecosystems. The quantification of herbicide action in plants is important for the assessment of the ecological consequences of their use. This assessment includes the consideration of the aquatic plants that are not the target of the herbicide application. Macrophytes must be considered because herbicides are washed from the fields into creeks and ponds. Although the macrophytes are subjected to comparably low herbicide concentrations, an effect on those plants as a part of the aquatic ecosystem is possible. Doub *et al.* (1988) found out that, continuous use of herbicides may induce species composition shifts. In addition, Fox and Murphy (1990) studied the ecological impacts of herbicides on the submerged plant communities of British rivers. They reported that the macrophyte (Rununculus plants) were removed or damaged for considerable distances downstream of the point of herbicide application. Also, Spawn *et al.* (1997) suggested that the herbicide (alachlor) inputs can alter both algal community composition and biomass in agricultural streams. Many authors studied the comparative sensitivity of algae and aquatic macrophytes to many herbicides (Feurtet-Mazel, *et al.*, 1996; Fairchild *et al.*, 1997 and Forsyth *et al.*, 1997). They studied the toxicity affects of paraquat, atrazine, alachlor, metolachlor, tordon and acetanilides on growth and photosynthetic pigments of phytoplankton and aquatic macrophytes.

The present study was conducted to assess the effects of different concentrations of the two herbicides; acetochlor and paraquat on the growth and chlorophyll a, chlorophyll b and carotenoids of three submerged macrophytes (*Ceratophyllum demersum, Potamogeton pectinatus and Elodea canadensis).*

MATERIALS AND METHODS

Plant material : *Elodea canadensis, Potamogeton pectinatus* and *Ceratophyllum demersum* were collected from Idku lagoon, during October 1999, away from direct exposure of agricultural drainage.

Herbicides : Acetochlor and paraquat were chosen, because they have increased importance in agriculture. Preliminary experiments had been conducted using both herbicides at high concentration levels (lethal dozes), but lower dozes were chosen for the present experiment. Commercially available herbicides were used at concentrations 11, 16 and 22 ug/l acetochlor and 100, 300 and 500 ug/l paraquat. Indoor microcosms (glass aquaria), of length 70 cm, width 90 cm; depth 50; water depth 30 cm; sediment depth 5 cm and the conditions in the climate room were: temperature 20 °C; photoperiod 14h. Each concentration was conducted in triplicate.

Cultivation : The test plants were grown in dechlorenated tap water supplied with the different concentrations of herbicides. The used media were discarded at intervals of 5, 4 and 3 days exposure to acetochlor and 7, 6 and 5 days exposure to paraquat, and new media with another concentrations were replenished. The test plants were maintained at one gram fresh weight per liter medium and the excess yield (if detected) was harvested. Sometimes the test plant had to be substituted with new fresh inoculum at the beginning of the experimental trial. Control media (without herbicides) were set up parallel to the treated media.

To check whether the herbicides affect the growth of macrophytes, growth of plants was detected by determining the differences between the final and the initial fresh weights every trial. Laboratory experiments were also performed to study the acute effects of the series of herbicides dozes on the photosynthetic pigments. Chlorophyll a and b were determined according to Goodwin (1965), using acetone (80%) for pigment extraction and their absorbances were measured spectrophotometrically.

The concentrations were calculated as follows :

Concentration of chlorophyll-a (mg/g tissue) =

$$12\,(E663) - 2.69\,(E\,645) \times V/\,(W \times 100)$$

Concentration of of chlorophyll-b (mg/ g tissue) =

$$22.9\,(E\,649) - 4.68\,(E\,663) \times V\,(W/100)$$

Where :

E = extinction at 663 or 645 nm.
V = Volume.
W = weight of sample.

Carotenes were determined according to Official methods of analysis (1970) using acetone and petroleum ether for extraction and the absorbances were measured spectrophotometrically at 452 nm. The concentrations were calculated as following :

ug of carotene/ 100g = Concentration of carotene in solution as read form standard curve (mg/ml) x final volume x dilution x 100/ Weight of sample

(Standard curve was constructed using available pure, B-carotene ampoules to prepare different concentrations).

RESULTS AND DISCUSSION

Effect of herbicides on plant growth :

Ceratophyllum demersum was obviously affected by acetochlor. This was indicated by a small loss (0.5%) of its initial fresh weight inoculum after 5 days exposure to 11 ug/l acetochlor, followed by a pronounced inhibition in its growth with a reduction of 4.44% of its initial weight upon the addition of 16 ug/l acetochlor within 4 days exposure. The test plant *C. demersum* was not uniformly sensitive, since the highest concentration used in the experiment (22 ug/l acetochlor) stimulated its growth with an increase of 1.07g /g initial fresh weight. This increase was still lower than the untreated control plant which showed increases in its fresh weight from 2.28 to 4.59 g/ g initial weight along the investigation period. Mazzeo *et al.* (1998) explained that the broad phenotypic plasticity, probably reduced ecotoxicological variability between clones. On the other hand, Spawn *et al.* (1997) pointed out that some algal taxa were affected at levels of >10 ug/l alachlor within 21 days exposure, while others recovered from exposure by day 7 or longer or did not recover. However, Fairchild *et al.* (1998), in their studies on comparative sensitivity of five macrophytes, ranked *C. demersum* the most susceptible species to alachlor and metolachlor herbicides.

Acetochlor affected also the growth of *P. pectinatus* and *E. canadensis* but to a lesser extent than it did with *C. demersum*. Low fresh weight yield increases (1.2 and 1.02 g/g initial fresh plant inoculum) were detected for *P. pectinatus* and *E. canadensis* respectively after exposure to 11 ug/l acetochlor Table (1). These were still lower compared with the untreated control plant yield (2.2 and 3.8 g/g initial weight) for the respective plants. Both test plants could withstand higher concentration (16 ug/l acetochlor) with an increase of 1.21 g/g initial plant for *P. pectinatus* but with no yield for *E. canadensis*. Acetochlor at concentration level of 22 ug/l inhibited the growth of both plants with a reduction of the initial fresh weights by 13.1 and 4.4% loss for *P. pectinatus* and *E. canadensis* respectively.

Paraquat damaged strongly *C. demersum* at all concentration levels, particularly at 500 ug/l where it showed cell lysis. *Potamogeton pectinatus* and *E. canadensis* yielded 1.14 and 1.29 g/g initial fresh weight respectively at exposure to 100 ug/l paraquat within 7 days treatment (Table 1).

The growth of *P. pectinatus* was inhibited with a 16% loss in fresh weight when exposed to 300 ug/l paraquat during 6 days treatment.

Table (1) : Effects of acetochlor and paraquat on biomas yield and chlor-a, chlor-b and carotenes contents in *C. demersum, P. pectinatus* and *E. canadensis* grown in laboratory microcosms.

Plant species	Herbicides	Conc. µg/l	Duration of exposure	Plant yield g/g fresh weight	Chlor-a mg/g tissue	Chlor-b mg/g tissue	Carotenes mg/g tissue
C. demersum	Paraquat	00	Start	1g/l medium	11.76	1.66	0.017
		100	7	No yield	5.20	2.68	0.004
		300	6	No yield	4.73	2.25	0.009
		500	5	Cell lysis	2.25	1.31	0.008
	Acetochlor	11	5	0.5% loss	3.99	2.24	0.006
		16	4	4.44% loss	3.99	2.24	0.006
		22	3	1.07	4.56	2.48	0.008
P. pectinatus	Paraquat	00	Start	1g/l medium	6.67	2.54	0.006
		100	7	1.14	1.82	0.87	0.005
		300	6	16% loss	2.20	0.96	0.004
		500	5	1.08	2.98	1.58	0.005
	Acetochlor	11	5	1.20	8.02	3.20	0.014
		16	4	1.21	4.92	1.98	0.008
		22	3	13.1% loss	3.24	1.32	0.006
E. canadensis	Paraquat	00	Start	1g/l medium	3.34	1.82	0.012
		100	7	1.28	3.42	2.13	0.009
		300	6	1.21	2.59	1.58	0.004
		500	5	3.2% loss	2.59	1.50	0.006
	Acetochlor	11	5	1.02	4.91	3.86	0.009
		16	4	No yield	2.51	1.31	0.004
		22	3	4.4% loss	2.41	2.41	0.006

The plant was recovered within 5 days exposure to 500 ug/l paraquat, with an increase of fresh weight by 1.08 g/g initial.

Elodea canadensis continued to grow with an increase of 1.21g/g initial when exposed to 300 ug/l paraquat, but the plant lost about 3.2% of its initial weight at 500 ug/l level. The increased yields of all test plants were still lower than detected in the control (untreated) plants as mentioned before.

The recovery of plants fresh matters exhibited in the present investigation could be explained as postulated by Hofmann and Whinkler (1990) that, tolerance against a stressor depends on the contaminations at the plants site of growth during the growth period. Jeffries and Mills (1990) confirmed that *P. pectinatus* and *E. canadensis* are characterized by hardy species and that *E. canadensis* had many growing points and recovers quickly from disturbances. However, sometimes herbicide does not affect plant growth as Forsyth *et al.* (1997) mentioned since he found that clopyralid herbicide did not inhibit, but stimulated tuber production of *P. pectinatus*.

Effect of herbicides on photosynthetic pigments :

Ceratophyllum demersum contaminated with 11 ug/l acetochlor showed a slight decrease in chlorophyll-a content (from 4.76f to 3.99 mg/g tissue), but chlor-a increased again by the addition of higher acetochlor dozes (Fig. 1). Addition of acetochlor resulted into obvious increases in chlor-b content by approximately 1.4 times its initial content (untreated plant) at all concentration levels. As for carotenes, there was a drop in their content by the addition of 11ug/l acetochlor, followed by slight rises along with the addition of higher acetochlor dozes. The elevation of the photosynthetic pigments was not accompained by a corresponding yield increases.

As shown in Fig. (1), chlor-a and b increased in *P. pectinatus*, compared with their initial contents, during 5 days exposure to 11 ug/l acetochlor, followed by a reduction along with continuous addition of higher acetochlor dozes. Carotenes exhibited a steady reduction with continuous increases in acetochlor inoculations. The lowest photosynthetic pigments attained by exposure to 22 ug/l acetochlor was accompanied by loss in fresh weight.

Elodea canadensis exhibited different trend towards acetochlor addition. Thus, after the first increases in chlor-a and b by addition of 11 ug/l in five days exposure, their contents dropped within four days of the second treatment (16 ug/l), followed by another rise during three days of the third treatment (22 ug/l) attaining higher values than their initials. Carotenes showed a continuous decrease along with increases in herbicide concentrations. It was noticed that pigments concentration was independent of biomass yield.

Wejnar (1989) in his study on the effect of a herbicide on *Lemna gibba,* concluded that sometimes the herbicide causes a concentration – dependant increase in the pigment content. He found that, after 6 days a marked, and after 27 days a maximum increase in pigment content was evident. The study of the previously mentioned author was not in accordance with the present one since the decrease or increase in pigment contents of test plants were not correlated with herbicide concentrations. However, Wejnar *et al.* (1992) concluded that, herbicide application frequently leads to stimulation effects or positive stress on photosynthetically active pigments, which might confirm the present results.

Addition of 100 ug/l paraquat to culture media of *C. demersum* resulted into an increase in chlor-a and b contents. A decrease in these contents was detected following the addition of 300 and 500 ug/l paraquat (Fig. 2). Carotene contents decreased considerably by the end of the first paraquat addition, but rised again by the addition of higher concentrations. No yield was detected at first and second treatment, while cell lysis accompanied the third one.

The photosynthetic pigments of *P. pectinatus* showed a pronounced reduction by treatment with paraquat at all concentration levels if compared with their initial contents as shown in Fig. (2). Thus, it could be concluded that paraquat had bleaching effect on the photosynthetically active pigments (chlorophylls a & b and carotenes) as Wejnar *et al.*

(1992) mentioned, although accompanied, sometimes by biomass increases (1st & 3rd treatments).

Elodea canadensis contaminated with 100ug/l paraquat showed a slight increase in chlor-a and b within 7 days exposure, while higher concentrations (300 and 500 ug/l) resulted into a small reduction in chlorophyll contents within 6 and 5 days respectively compared with initials. On the other hand carotenes showed decreases in their values by the contamination with all paraquat concentrations levels. Vàradi *et al.* (1998) recorded that leaves of *E. canadensis* responded strongly to paraquat and lost their photosynthetic functions after 4 hrs of treatment with 81 ug/l paraquat. Compared the result given by Vàradi *et al.* (1998) with those recorded in the present experiment it could be concluded that *E. canadensis* was able to withstand 100 ug/l paraquat. Such concentration migh be considered a sublethal doze which was accompanied by increases in cholr.-a and b contents as well as biomass yield (Table 1).

On the other hand, Cseh *et al.* (1998) examined the effect of paraquat on both susceptible and resistant biotypes of *E. canadensis*, he concluded that the susceptible biotype displayed a continuous deterioration in photosynthetic parameters after paraquat treatment whereas the resistant biotype displayed 20 min after treatment thereafter exhibited a recovery. According to Cseh *et al.* (1998), *E. canadensis* used in the present experiment, then, might be considered a resistant biotype.

The general loss of plant biomass, during exposure to different acetochlor and paraquat concentrations, which was accompanied by either stability or recovery of the photosynthetic pigments could be due to the effects of hebricides on other metabolic processes as reported by Young (1991). Thus, Fujiwara *et al.* (1998) supported Young's (1991) report mentioning that paraquat had an effect on the activity of defensive enzymes in *Chlamydomonas reinhardtii*, since it inhibited its growth.

On the other hand, Hofmann and Winkler (1990) postulated that, the chemical contaminations existing during growth period of a plant are of eminent importance to the plant's stress tolerance. However, resistance of a plant also could arise from decreased uptake as well as decreased degradation of herbicides.

It should be noticed that, there were increases in chlorophyll a and b contents in all test plants subjected to 11 ug/l acetochlor, beside their increases in *C. demersum* exposed to 100 & 300 ug/l paraquat as well as in *E. canadensis* treated with 100 ug/l paraquat. Those increases could be referred to the increase of chloroplast granum stability induced by sublethal concentration of these herbicides as stated by Simard *et al.* (1991).

In conclusion, the three test plants were not uniformly more sensitive than one another across the concentration ranges of herbicides tested. In spite that acetochlor is a cell division inhibitor and paraquat is a photosynthetic electron flow divertor (Worthing, 1991), their effects on growth as well as the photosynthetic pigments were not predictable when considering their obvious differences in their modes of action and also, plant phylogeny.

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الملخص العربى

تأثير مبيدات الحشائش علي نمو وأصباغ التمثيل الضوئي لثلاثة نباتات مائية مغمورة

د. ماري جندي غبريال

من التأثيرات الجانبية الناتجة عن استخدام مبيدات الحشائش أنها تؤدي إلي تدمير البيئة النباتية في المسطحات المائية المجاورة للأراضي الزراعية. الاحتمالات التي تنتج عن هذه العشوائية قد طرحت للبحث وذلك باختبار استجابة ثلاث نباتات مائية مغمورة وهي سيراتوفيلليم ديمرسم *Ceratophyllum demersum*، الحامول *Potamogeton pectinatus* والإيلوديا *Elodea camadensis*.

أجري مسح بيولوجي معملي وذلك بتعريض هذه النباتات لتركيزات ١١، ١٦، ٢٢ ميكروجرام لكل لتر من الأسيتوكلور و١٠٠، ٣٠٠، ٥٠٠ ميكروجرام لكل لتر من البراكوات.

نبات السيراتوفيلم فقد وزنه الحي عند استخدام ١١، ١٦ ميكروجرام لكل لتر أسيتوكلور ولكنه استعاد حيويته عند معاملته بتركيز اكبر (٢٢ ميكروجرام/لتر). ولكن هذا النبات قد أضير عند معاملته بكل تركيزات البراكوات. محتوي النبات من كلوروفيل أ، ب تأرجح بين الزيادة والنقصان بدون أي علاقة ارتباط بتركيزات المبيد.

نبات الحامول أظهر زيادة في الكتلة الحيوية عند تعرضه لتركيزات ١١، ١٦ ميكروجرام / لتر أسيتوكلور. وفقد النبات من كتلته الحيوية عند تعرضه لتركيز ٣٠٠ ميكروجرام/ لتر براكوات ولكنه استعاد وزنه عند تركيز أعلي (٥٠٠ ميكروجرام/لتر براكوات).

نبات الإيلوديا حقق زيادة في وزنه وأيضا في محتوي كلوروفيل أ، ب عند استخدام ١١ ميكروجرام/لتر أسيتوكلور، ١٠٠ ميكروجرام / لتر براكوات.

ولوحظ أن الكاروتينات عامة تناقص محتواها في كل النباتات وعند استخدام كلا المبيدين.

أشارت النتائج إلي أنه ليس أي من النباتات المختبرة يكون أكثر حساسية متوقعة عن الأخر عند استخدام المبيدين، إذا أخذ في الاعتبار الوزن الحيوي أو المحتوي الكلوروفيل