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Aquatic Plant Control Research Program

Allelopathic Ability of Various Aquatic Plants to Inhibit the Growth of *Hydrilla verticillata* (L.f.) Royle and *Myriophyllum spicatum* L.

by Harvey L. Jones

Prepared for Headquarters, U.S. Army Corps of Engineers

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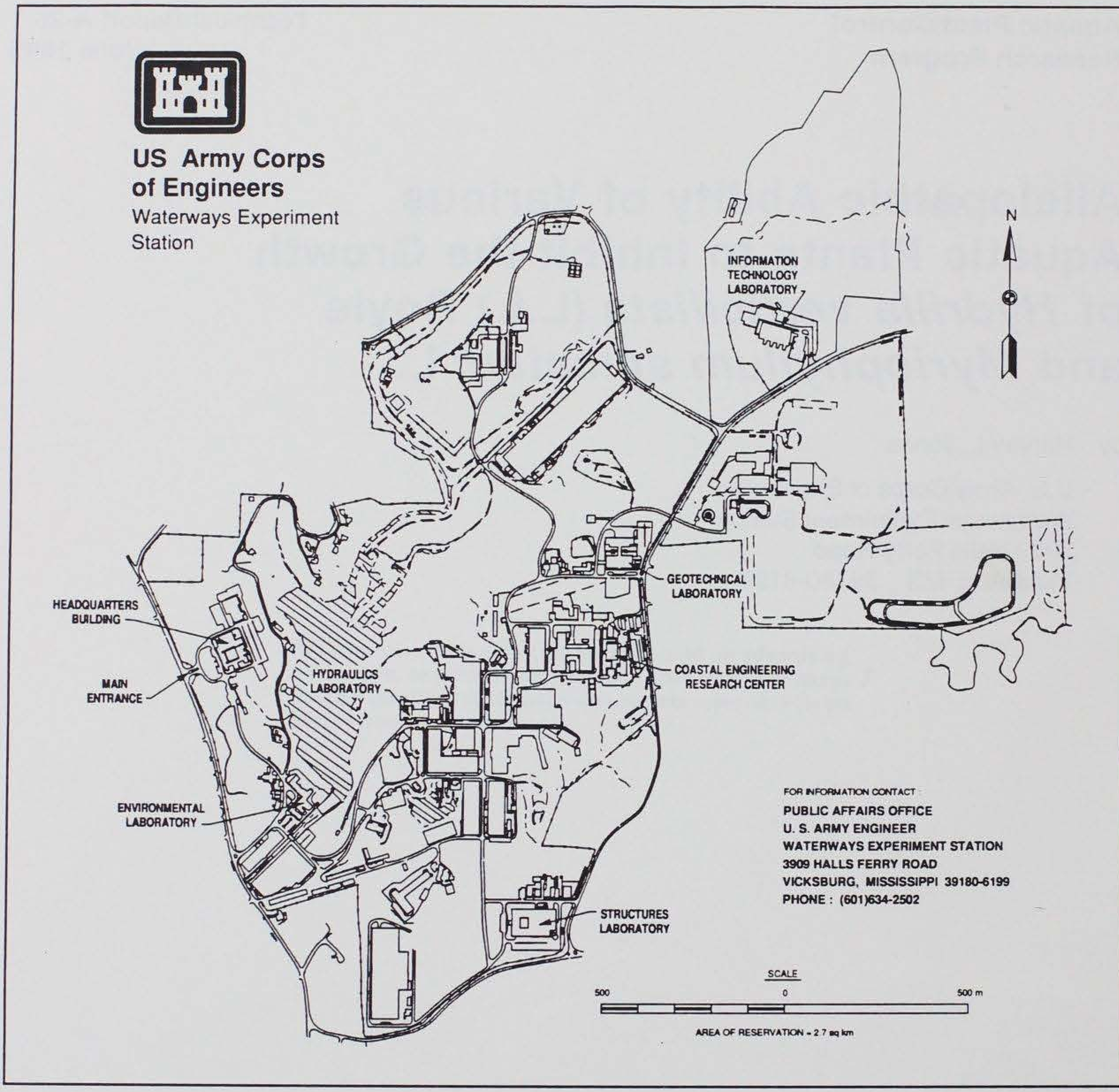

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Contents

Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 32408. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Environmental Resources Research and Assistance Programs (ERRAP), Mr. J. L. Decell, Manager. Mr. Robert C. Gunkel was Assistant Manager, ERRAP, for the APCRP. Technical Monitor during this study was Ms. Denise White, HQUSACE.

This report was prepared by Mr. Harvey L. Jones, Aquatic Ecology Branch (AEB), Environmental Resources Division (ERD), EL, WES. The study was supervised at WES by Dr. Edwin A. Theriot, Chief, AEB, and Dr. Conrad J. Kirby, Chief, Environmental Resources Division; Dr. John W. Keeley was Director, EL.

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1 Introduction

The term allelopathy was first coined by Molisch in 1937. In general, the term allelopathy refers to the detrimental effects of higher plants of one species (the donor) on the germination, growth, or development of another species (the recipient) (Putnam 1985). Specifically, allelopathy refers to the biochemical interactions that take place among plants, but its effectiveness depends on the addition of a chemical to the environment (Sutton 1986a). Rice (1974) provided us with a more functional definition as being any direct or indirect harmful effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment. Similarly, Parker (1984) defined allelopathy as the harmful effect of one plant or microorganism on another because of the release of secondary metabolic products into the environment.

Background

Hydrilla verticillata (L. fil.) Royle (common name hydrilla) is a noxious aquatic plant introduced into the United States from Africa through the aquarium industry and sold under the name "oxygen plant" or "star vine." Hydrilla has long branching stems that often fragment and form large floating mats (Tarver et al. 1978) and can grow in water depths up to 15 m. Two reproductive structures enable hydrilla to withstand extremely harsh weather conditions: (a) turions or winter buds (dense clusters of apical leaves that are produced in the leaf axils, green and ovoid-conical shaped buds) and (b) bubil-like hibernacular, commonly but incorrectly called tubers (which are formed at the ends of stolons buried in the substratum). Plants are found in lakes, rivers, drainage and irrigation canals, ponds, and streams. Severe infestations of hydrilla can restrict boat traffic and interfere with fisheries and water flow.

Myriophyllum spicatum (Eurasian watermilfoil) is a noxious aquatic plant introduced into the United States from Eurasia. It is a nuisance submersed aquatic plant that reproduces by fragmentation and is found in many southern States and California. Like hydrilla, watermilfoil is often found in lakes, rivers, drainage and irrigation canals, ponds, and streams. While severe infestations of watermilfoil can interrupt water sports and activities just as hydrilla, it can also displace important native species. Mechanical removal of both of these species tends to increase their distribution because of fragmentation. While herbicides are used to some extent in various places, there is major concern for the environment and water quality.

Because allelopathy may provide an inexpensive and more desirable method of control than more conventional methods such as the use of herbicides or mechanical removal, it may prove to be one of our best weapons for controlling hydrilla and watermilfoil as well as other undesirable aquatic plants. The results of experiments with hydrilla and watermilfoil are reported herein.

Importance of Allelopathy

Allelopathy is a potentially important mechanism in controlling undesirable nuisance aquatic plant problems. Studies have shown that some plants have the capability of eradicating other species in the same area; however, most of these studies have been conducted using terrestrial plants or plants located in the littoral zone of aquatic habitats.

Allelopathy is caused by extrinsic as well as intrinsic factors acting on the environment simultaneously. For instance, a study was conducted in Tel Aviv, Israel, to determine the interrelationships between *Najas marina* L. and two other aquatic macrophytes (*Myriophyllum spicatum* L. and *Potamogeton lucens* L.) and an emersed hygrophyte (*Scirpus litoralis* Schard.) (Agami and Waisel 1985). *Najas* dry weight yields were not affected by the presence of *Scirpus* or *Potamogeton*, but were suppressed significantly by *Myriophyllum*. *Najas* root growth was not affected as the shoot growth. Additionally, the data presented caused the author to state that the relationships between *Najas* and *Myriophyllum* are not a simple unilateral allelopathy. Those relationships suggest the existence of bilateral negative relationships between the two species.

According to a study by Cleland and Tanaka (1982), most research showing the effects of plant growth substances on flowering have been conducted using terrestrial plants and basically deal with exogenously applied substances; therefore, the significance of the physiological effects are not known. More studies are needed that are designed to look at changes in endogenous plant growth and other substances to determine if they can produce physiological changes.

Pertinent Literature Review

Allelopathic potential studies

Elakovich and Wooten (1989a) found that frond production in lemna was reduced 60 percent by five species of aquatic plants in a *Lemna minor* L. bioassay. A few studies have shown through bioassays, aquaria studies, and tank experiments that several species of aquatic plants may possess allelopathic potential to reduce the growth of other undesirable aquatic plants. The effects of different parts of *Ludwigia adscendens* Linn. and *Ipomea aquatica* Forsk on the growth of pearlmillet (*Pennisetum typhodeum* Rich.) were studied by Singhvi and Sharma (1984), who found that various parts of these plants contained different allelopathic (inhibitory) compounds. Frank and Dechoretz (1980) showed that the sod of dwarf spikerush (*Eleocharis coloradoensis*) inhibited new shoot production and reduced biomass of American pondweed (*Potamogeton nodosus* Poir.) and Sago pondweed (*Potamogeton pectinatus* L.). Sutton (1986b) found that established stands of slender arrowhead (*Sagittaria graminea* Michx.) were more effective in reducing the growth of sprouted hydrilla tubers than spikerush (*Eleocharis geniculata* (L.) R. & S. His results showed that when tubers were grown with slender arrowhead, tuber production was reduced by 90 percent when compared with controls.

A comparison of four bioassays for sensitivity to various allelochemicals showed that the *Lemna* bioassay was most sensitive; inhibition rates of cultured lemna species was detected as concentrations as low as 50 uM of the donor extract. The selection of a bioassay to be used depends on the parameters to be measured and quantity of allelochemicals available for testing (Leather and Einhellig 1985). Four bioassays were developed by Ashton, Di Tomaso, and Anderson (1985) to test the phytotoxic effects of fractionated dwarf spikerush leachated organics. Two assays used explants of the target plants (hydrilla and sago pondweed (*Potamogeton pectinatus* L.); one assay used whole lettuce seedlings; and one used tomato (*Lycopersicon esculentum* Mill.) cell suspension. The fractions, separately assayed at 250 ppm by weight, were inhibitory toward hydrilla and sago pondweed, spikerush extract, and lettuce seedling roots (3- to 85-percent inhibition) (Ashton, Di Tomaso, and Anderson 1985).

Four marsh species and three soils from a New Jersey freshwater tidal marsh were examined for their allelopathic potential (Bonasera, Lynch, and Leck 1979). Their results were that *Peltandra virginica* (L.) Schott. & Endl. and *Ambrosia trifida* (L.) reduced germination and root growth of tomato, cucumber, lettuce, and radish.

Dwarf spikerush (*Eleocharis coloradoensis*) (Britt.) (Gilly) is an aquatic plant that may displace several species of submersed nuisance aquatic plants in ponds, canals, lakes, and reservoirs (Yeo 1980). Shoot number, dry weight, and number of subterranean turions of hydrilla and tubers of sago and American pondweeds were reduced by approximately 50 percent in outdoor tank experiments when grown with dwarf spikerush from 1979 to 1980 (Yeo and Thurston 1984). Their experiments also showed that dwarf spikerush reduced the number of leaves, shoots, and/or dry weight of six of the seven species tested (*Myriophyllum spicatum* being the exception).

In India, *Ceratophyllum demersum* L. and *Ceratophyllum muricatum* Chamisso are not found growing with *Hydrilla verticillata* (Kulshreshtha and Gopal 1983). Outside tank experiments show that hydrilla inhibits the growth of these two species of *Ceratophyllum*; therefore, the growth and distribution of *Ceratophyllum* is influenced by the distribution of hydrilla.

Szczepanska (1971) established pot experiments to investigate the interrelations of aquatic plants. She found that soil type ranging from sand to peat modifies the plant-plant influences and mixed culture interrelations. Results showed that with a decrease in reed (*Phragmites communis*) production, there was generally an increase of other plant species associated with reeds. She also examined the interactions between reed cultures and a sedge (*Carex hudsonii* Bennet) between 1969 and 1972 (Szczepanska 1977). Her results showed that reed cultures grew better in a mixed stand with sedge the first year than in monoculture, whereas the reverse was true for sedge. However, in the subsequent years, sedge biomass increased when grown with reed as compared with sedge monocultures. There was a constant displacement of reed by sedge when grown together.

El-Ghazal and Reimer (1986) tested water extracts of cabomba, western elodea, American eelgrass, giant duckweed, and watermilfoil for their effect on the germination and radical growth of lettuce, barnyard grass, ivyleaf morningglory, and wheat. Radical growth of lettuce was more inhibited by the presence of the aquatic plant extracts than any of the other three target species, and ivyleaf morningglory was least affected. The effects of the eelgrass (*Zostera mariana* L.) on the growth of eight species of micro-algae and a bacterium were studied on agar plates and in liquid culture (Harrison and Chan 1980). The water soluble fraction of eelgrass extracts from fresh and slightly aged leaves (from a few days to 2 weeks) inhibited the growth and killed cells of all organisms tested. However, when the extracts were made from leaves aged in the laboratory for 35 days, the rate of inhibition decreased.

Studies of plant growth inhibitors and organic compounds

Phenolic acids have been cited as potentially allelopathic compounds when isolated from many agricultural as well as other plants. Zapata and McMillan (1979) investigated the phenolic compounds occurring in seagrasses. They found P-hydroxybenzoic acid, P-coumarin, caffeic, ferulic, vanillic, and protocatechic acids in four species of seagrasses in Texas. A survey of 43 species within 12 genera of seagrasses from diverse habitats around the world showed that they all contained flavone and/or phenolic acid sulphates

(McMillan, Zapata, and Escobar 1979). Additionally, of the 12 genera, 5 contained sulphated flavones, and 10 contained sulphated phenolic acids.

Typha seed germination was inhibited by aqueous extracts of dried cattail leaves (McNaughton 1968). When phenolic compounds were removed from the cattail leaves by extraction with Polyclar AT, the inhibitory characteristics were diminished considerably.

Anderson (1982) showed that synthetic and natural abscisic acid (ABA) induces the formation of floating type leaves in American pondweed at low concentrations. Winter buds of pondweed exposed to high concentrations of ABA exhibited lowered germinability and growth, and have inhibition of protein synthesis; however, some chlorophyll synthesis still occurred.

Whitehead, Dibb, and Hartley (1982) found that the amount of phenolic compounds differed between the species of plant grown and soil type. These authors reported that the six types of phenolic compounds found in all soil types tested were p-hydroxybenzoic, vanillic, p-coumaric, ferulic, p-hydroxybenzaldehyde, and vanillin. The soils with the highest quantities of vanillic and p-hydroxybenzoic acids were found in *Agrostis stolonifera* and *Petasites fragrans* (Whitehead, Dibb, and Hartley 1982).

Naturally occurring hydrilla inhibitors were found in the soils at Lake Starvation in Florida (Dooris, Dooris, and Martin 1988). The use of high performance liquid chromotography (HPLC) and electron microscopy allowed the detection, but not the identification of the inhibitor; attempts were made to understand its effect on the ultrastructure of hydrilla. From the first day of incubation with an inhibitor, chloroplasts from hydrilla leaves had accumulations of starch, whereas leaves without the inhibitor did not.

Ostrofsky and Zettler (1986) examined 15 species of aquatic vascular plants for presence of alkaloids. They found that all plant species tested possessed measurable quantities of alkaloids. Each of the plant species examined contained two to nine alkaloids (isolated by thin-layer chromotography). Of the 15 species, *Cabomba caroliniana* Gray had the fewest alkaloids, and *Potamogeton pectinatus* had the most. According to their results, monocotyledons appeared to have more alkaloids present than did dicotyledons.

Objectives

There were two objectives for conducting this research. The first was to conduct experiments (test-tube assays) to determine potential candidates of aquatic plants that would possibly reduce the growth, reproduction, and/or distribution of two nuisance aquatic hydrophytes, *Hydrilla verticillata* (hydrilla) and *Myriophyllum spicatum* (Eurasian Watermilfoil). The second objective of this area of research was to test the three aquatic plant species that were most inhibitory in the test-tube assays using rooted plants. Test-tube bioassays were used as a first step in determining allelopathic potential because it is a rapid screening technique to indicate allelopathic potential. Through laboratory bioassays, Elakovich and Wooten (1989) listed several aquatic plants that have the allelopathic potential to reduce or inhibit the growth of other species.

This latter study tested experiments to which plant organic matter had been added to the substrate to determine if similar results could be obtained that were found during previous test-tube assays using plant extracts. Procedures used were modified from Barko and Smart (1983).

Although there have been many studies showing allelopathic potential, most of those were conducted using terrestrial species. There are very few studies that show the allelopathic effects on submersed aquatic hydrophytes.

2 Methods and Materials

Plant Collection

Aquatic plants were field collected from Caddo Lake, LA, and J. D. Murphee Wildlife Refuge, Port Arthur, TX, and transported back to Vicksburg, MS, in ice chests with sufficient ice to keep the plants from deteriorating. Entire plants were collected whenever possible. Plants were washed to remove dirt and debris and allowed to drip to remove excess water.

Test Species Selection

Species selected for analysis were based on reports of potentially allelopathic hydrophytes from pertinent literature reviews and the publications of Elakovich and Wooten (1989a,b). In the study reported herein, the species selected were examined for their allelopathic potential to impact the growth of the target species *Hydrilla verticillata*. A list of selected species is given in Table 1.

Stock Hydrilla Cultures

Axenic cultures were grown from hydrilla tubers and turions obtained from greenhouse-grown plants. Cultures were grown in three 8-ℓ capacity nalgene cylinders in 6 ℓ of ALW media (artificial lake water). The cylinders each contained 25 to 35 tubers and were aerated with compressed air. The plants were grown for approximately 3 weeks, then clipped to 11 cm from the apical tips and recultured.

Extract Preparation

Test plants (200 g of each) were cut into small pieces, placed in a Waring commercial blender to which 200 ml of RO (reverse osmosis) water was added, and blended for 5 min on low speed and 2 min on high speed. Each

Table 1
List of Plant Species (and Plant Part) Used as Plant Extracts in the Hydrilla Assay

Common Name	Scientific Name	Plant Part
Pondweed	*Potamogeton nodosus* poir.	[1]
Fanwort	*Cabomba caroliniana* Gray var.	[1]
Common water nymph	*Najas guadalupensis* (Spreng.)	[1]
Eelgrass	*Vallisneria americana* Michx.	[1]
American lotus	*Nelumbo lutea* (Willd.) Pers.	Roots
Duck potato	*Sagittaria lancifolia* L.	Stems, leaves, and roots
Coontail	*Ceratophyllum demersum* L.	[1]
Eurasian watermilfoil	*Myriophyllum spicatum*	[1]
Fragrant waterlily	*Nymphaea odorata* Aiton	Stems and leaves
Pickerelweed	*Pontederia lanceolata* Nutt.	Stems and leaves

[1] Entire plant used as the extract.

of the 200-g aliquots were refrigerated for 24 to 72 hr to enhance extraction of the organic compounds. The aliquots were centrifuged at 10,000 rpm's for 10 min in a refrigerated centrifuge (Beckman J2-21M/E), then filtered through Whatman No. 54, 42, and GF/F filter paper, respectively. The filtrate was frozen until all plants to be tested were processed.

Experiment 1

One 2-cm-long apical tip explant of *Hydrilla verticillata* (axenic cultures) was placed in each of the eighty 90-ml capacity test tubes with 50 ml of ALW media.[1] Experimental cultures were randomly selected to receive 10 ml of one of the test plant extracts (Table 1). Test tubes were numerically arranged in test-tube racks with a vacant space on each side. Numbered aeration plugs (Figure 1) were inserted in the test tubes and compressed air supplied to each tube. Five replicates of each test plant extract were used in this study in addition to ten controls, each of which contained an additional 10 ml of ALW media in lieu of test plant extracts.

Plants were grown for 14 day before harvesting. Length and health status of plants were recorded. Plants were then placed in aluminum foil and dried

[1] Personal Communication, 1989, Dr. Craig Smith, Biologist, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

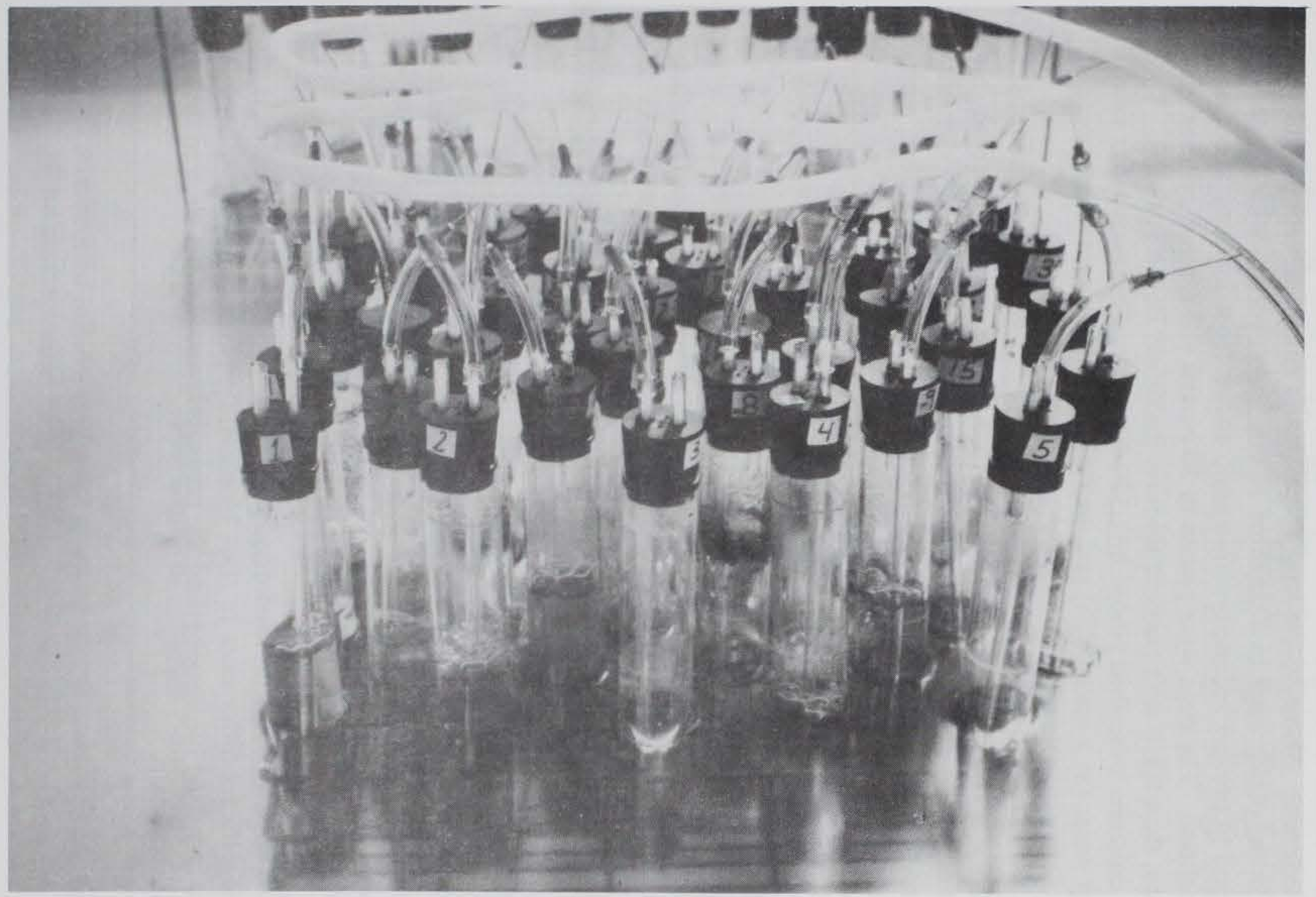

Figure 1. Photograph of experimental setup of test-tube assays

at 70 °C for 7 days, and biomass weight was recorded. The same procedure was used with *Myriophyllum spicatum* as the target species.

Experiment 2

In a greenhouse study, 5- or 20-percent organic matter from *Ceratophyllum demersum*, *Potamogeton nodosus*, and *Vallisneria americana* was added to 32-oz plastic cups containing 1,248 g of lake sediment (Brown's Lake, Mississippi) and mixed thoroughly. Controls had no additional organic matter added to the sediment. Three 15-cm-long apical tips of Eurasian watermilfoil were placed in the plastic cups to a depth of 10 cm, then overlain with a layer of silica sand to prevent sediment and organic matter from leaching into the water column. The cups were placed in 30-in.-tall (12-ℓ capacity) plexiglass cylinders, then filled with nutrient solution (modified Barko's media). The acrylic columns were then placed in 1,150-ℓ capacity fiberglass tanks filled with tap water (as a water bath) and maintained at a constant temperature of 25 °C.

Aeration was supplied to each column through the use of compressed air to prevent algal growth and ensure proper mixing of chemicals in the nutrient solution. The study was allowed to run for a period of 4 weeks, then repeated two additional times for verification.

Data Analyses

Each study was repeated three times, and all data were subjected to analyses of variance (ANOVA) and the Duncan's Multiple Range Test in SAS to determine significant differences.

Test-tube assays for hydrilla and myriophyllum

In Experiment 1 (Figure 2) test-tube assays, all extracts were significantly different from the controls; however, *pontederia lanciolata* stems and leaves increased hydrilla biomass beyond that of the controls. *Ceratophyllum demersum* showed the greatest reduction in hydrilla biomass.

In test-tube assays with *myriophyllum spicatum* (Figure 3), all extracts produced significant differences in plant biomass; however, while *Nymphaea oderata* increased biomass beyond the control, *ceratophyllum* showed the greatest inhibition.

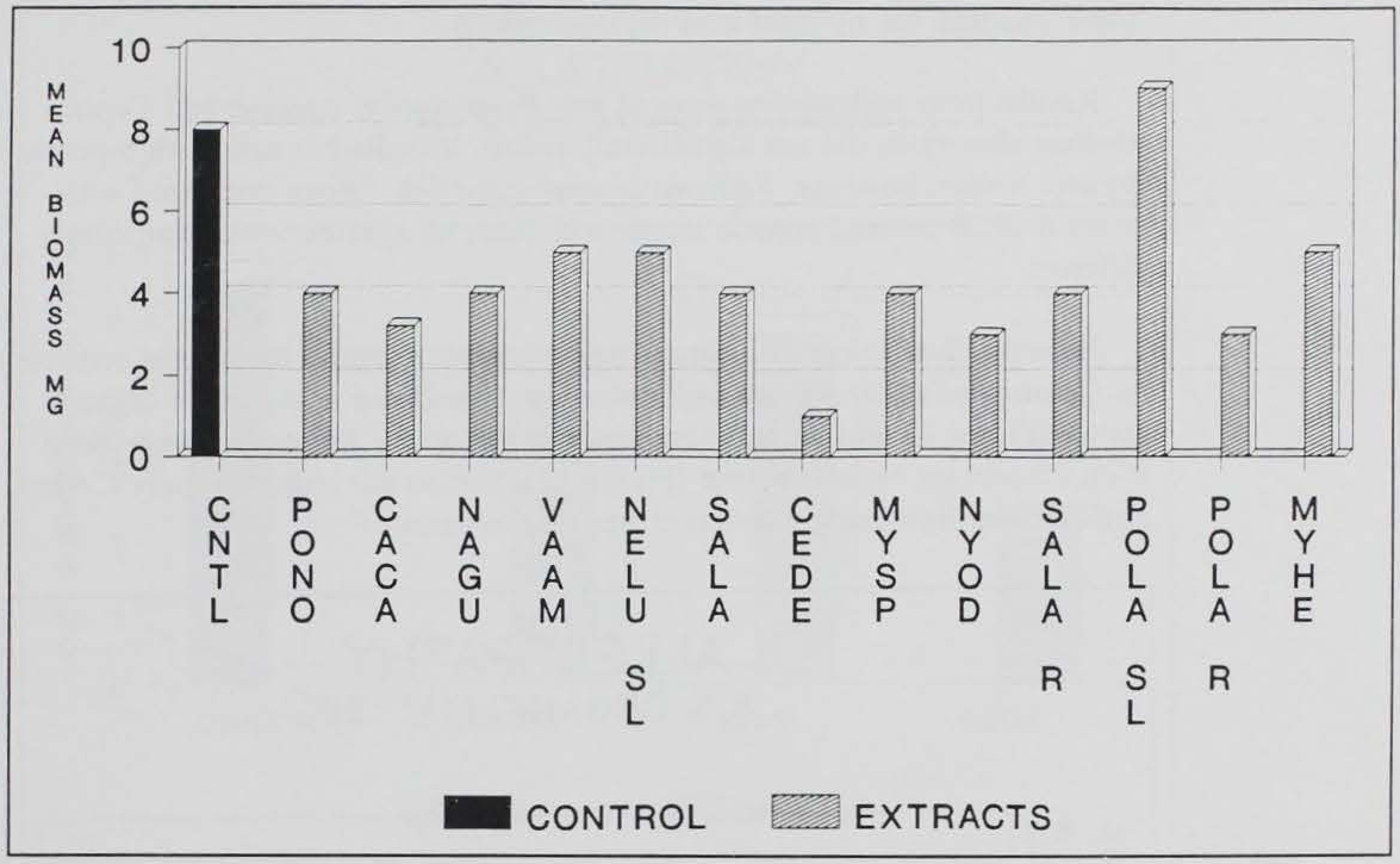

Figure 2. Allelopathic influence of plant extracts on the growth of *Hydrilla verticillata* at the 10-ml concentration

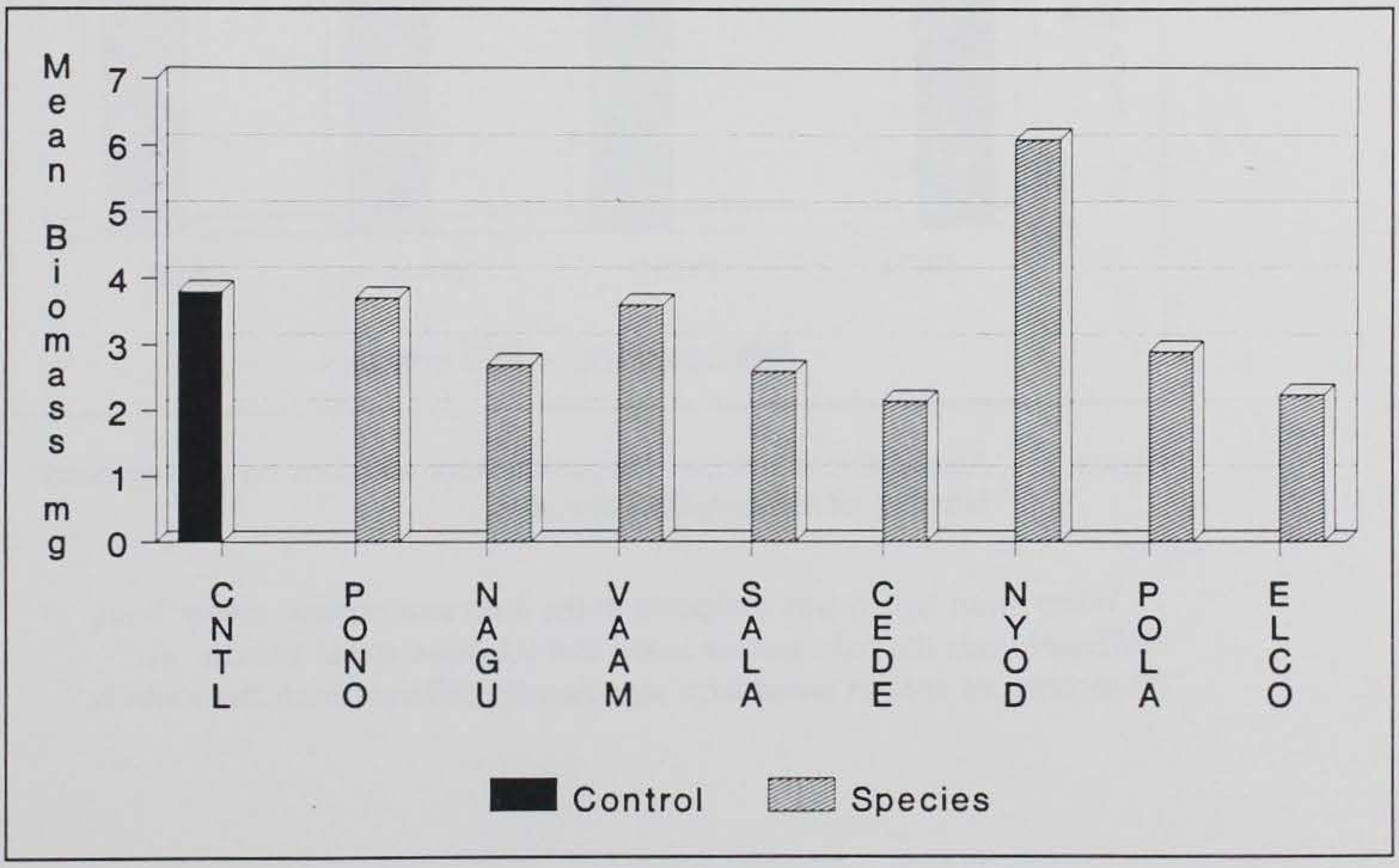

Figure 3. Allelopathic influence of plant extracts on the growth of *Myriophyllum spicatum* at 10-ml concentration

Tank studies for hydrilla and myriophyllum

Results from tank studies showed that *Potamogeton nodosus* and *Ceratophyllum demersum* did not significantly reduce hydrilla biomass with 5-percent organic matter; however, *Vallisneria americana* did. When compared with controls at 20-percent organic matter additions, all species were significantly different.

Myriophyllum spicatum biomass was stimulated beyond that of the controls by *Ceratophyllum demersum* and *Potamogeton nodosus* at 5-percent organic matter (Figure 4) and slightly less than the control by *Vallisneria americana*. With 20-percent organic matter (Figure 5) added to the sediment, only *Ceratophyllum* was significantly different from the control.

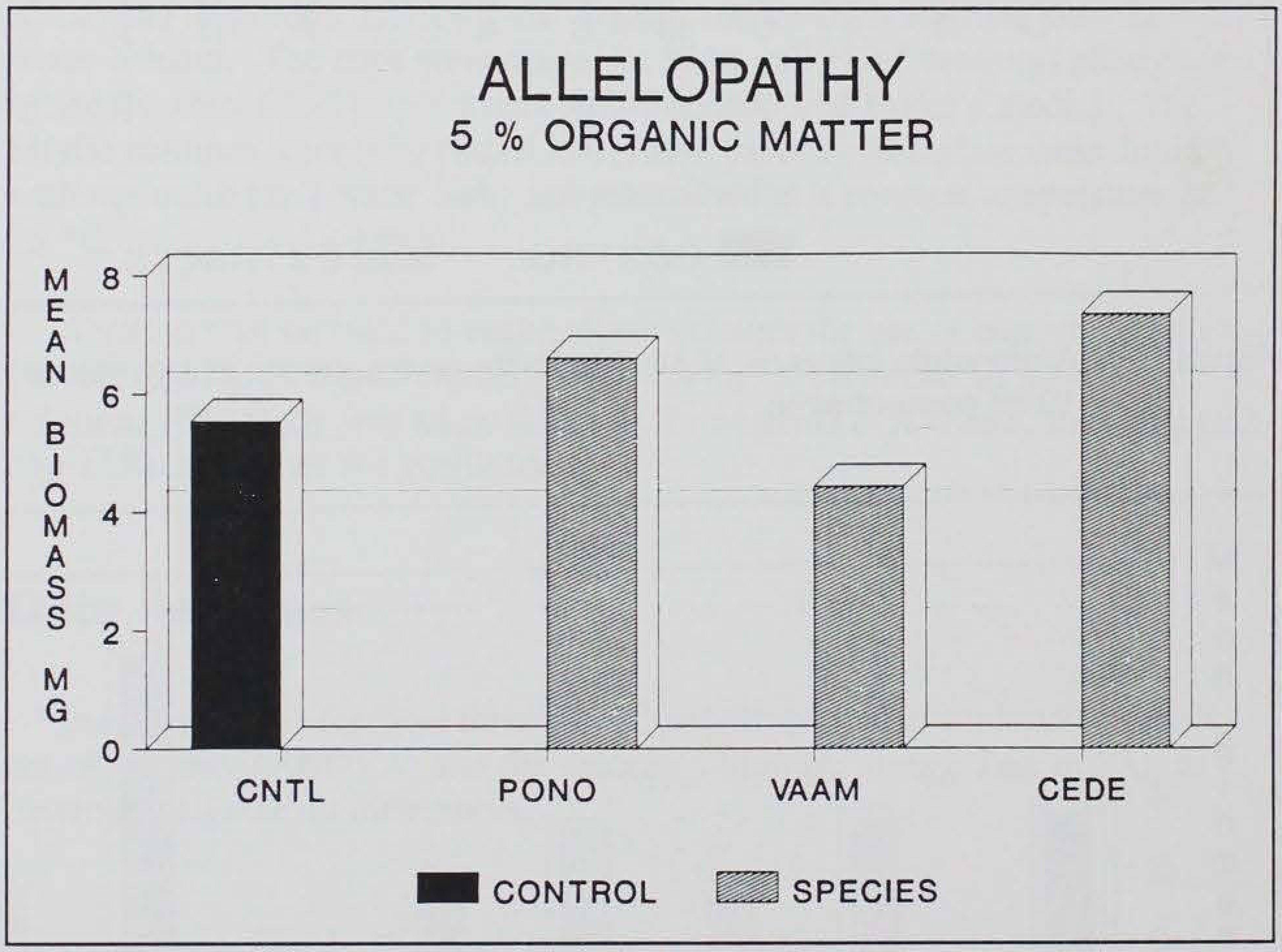

Figure 4. Allelopathic influence of organic matter additions on aboveground biomass of *Myriophyllum spicatum*

When mean length was compared at the 5-percent organic matter level, *Vallisneria* was the only species tested that inhibited apical growth. At 20-percent, all species tested were significantly different from the controls.

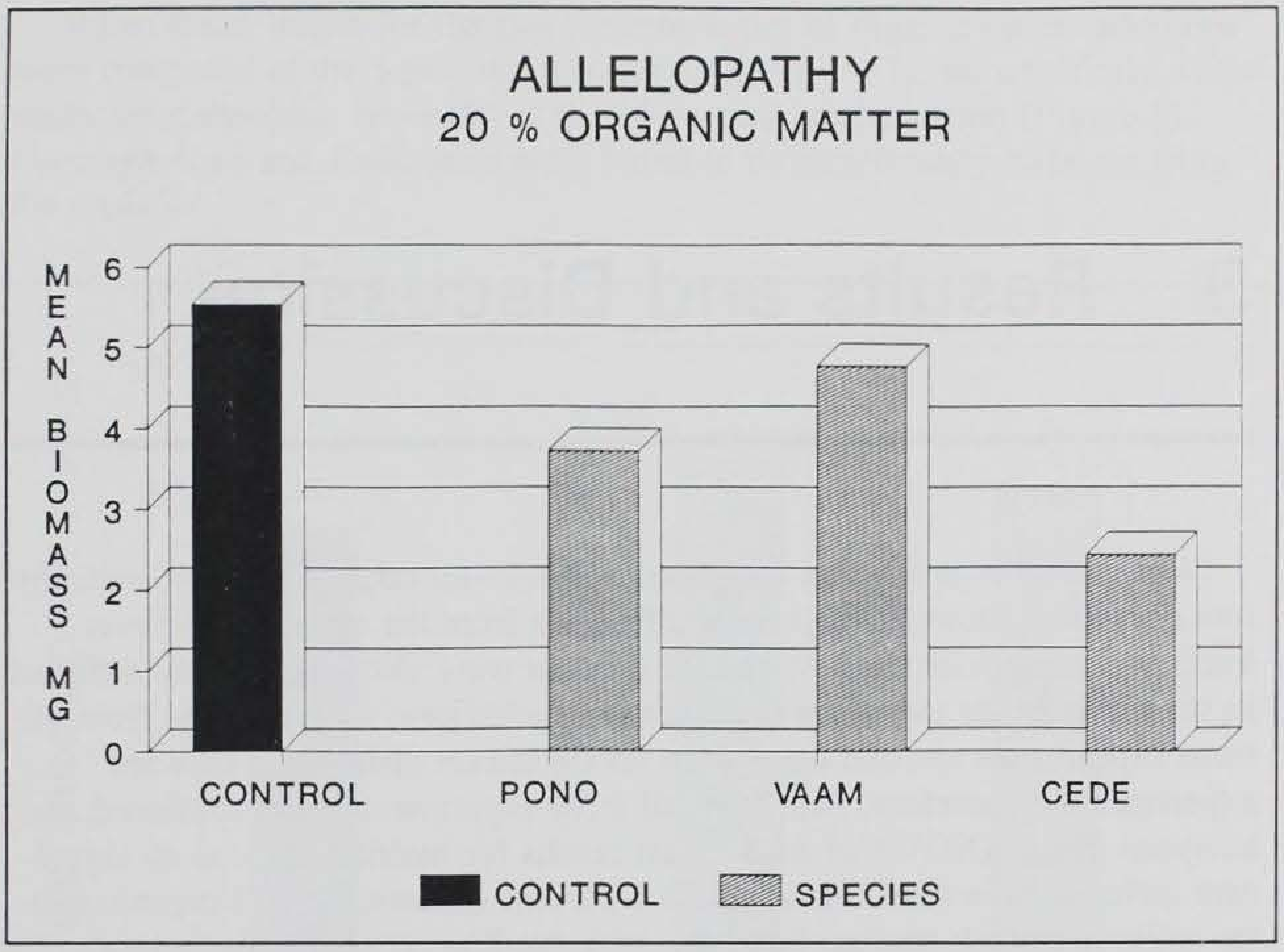

Figure 5. Allelopathic influence of organic matter additions on aboveground biomass on *Myriophyllum spicatum*

3 Results and Discussion

When mean biomass was compared at the 10-ml concentration, *Ceratophyllum demersum* showed the greatest difference from the controls; however, *Vallisneria americana* and *Nymphaea oderata* were also significantly different. In the experiments involving organic matter additions, data analyses from all three experiments showed there were no significant differences between experiments. Therefore, data from all three experiments were combined and analyzed using ANOVA of SAS. Test results for hydrilla showed no significant differences in total biomass with 5-percent concentration of organic matter addition in tank studies; however, with the 20-percent concentrations, *Ceratophyllum* showed significant differences in biomass (Figure 6) when compared with control.

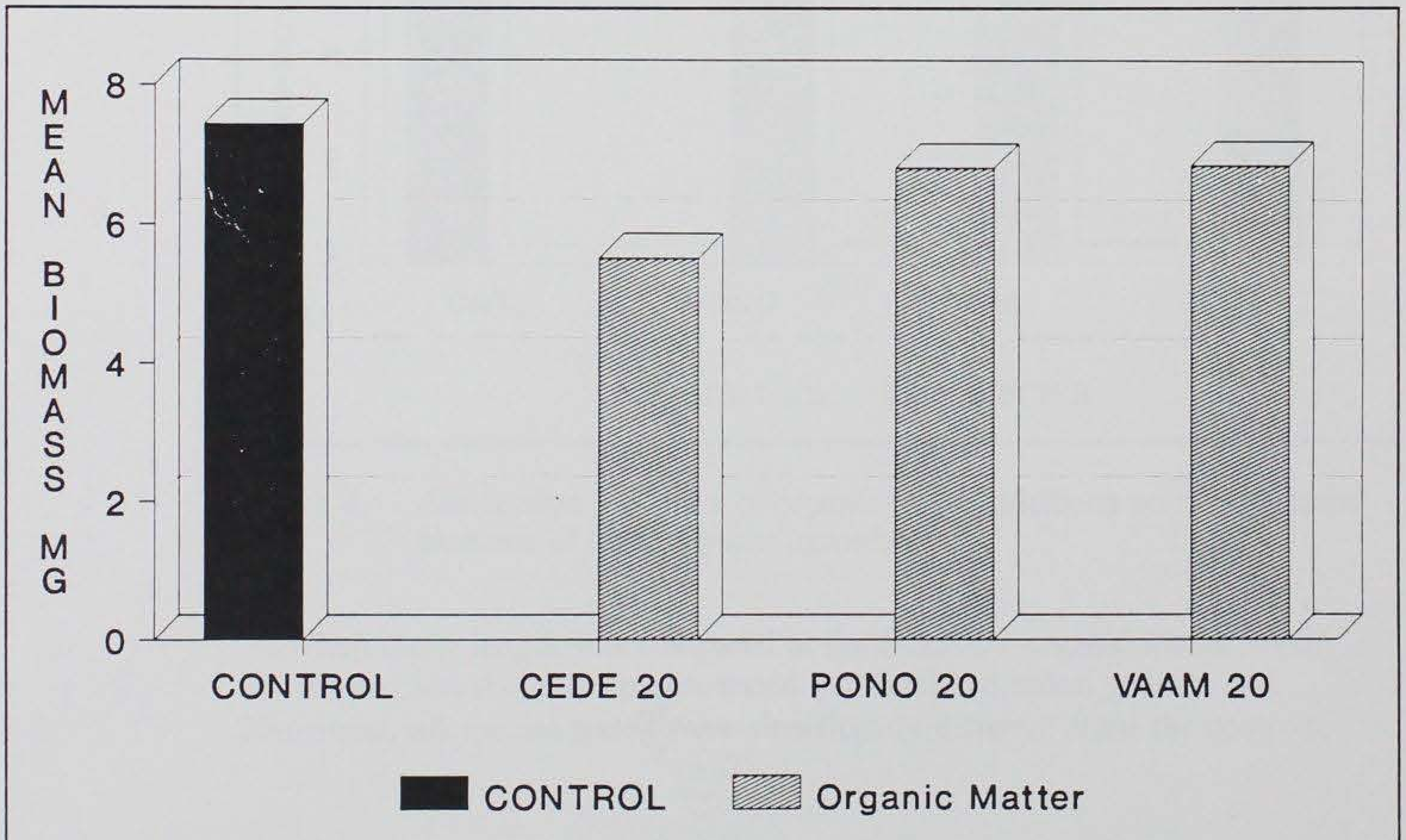

Figure 6. Allelopathic influence of 20-percent organic matter additions on aboveground biomass of *Hydrilla verticillata*

When mean length for the two concentrations of organic matter additions were compared at the 5-percent concentration (Figure 7), no significant differences were detected; however, at the 20-percent concentration (Figure 6), *Ceratophyllum* and *Vallisneria* were found to be significantly different from the control.

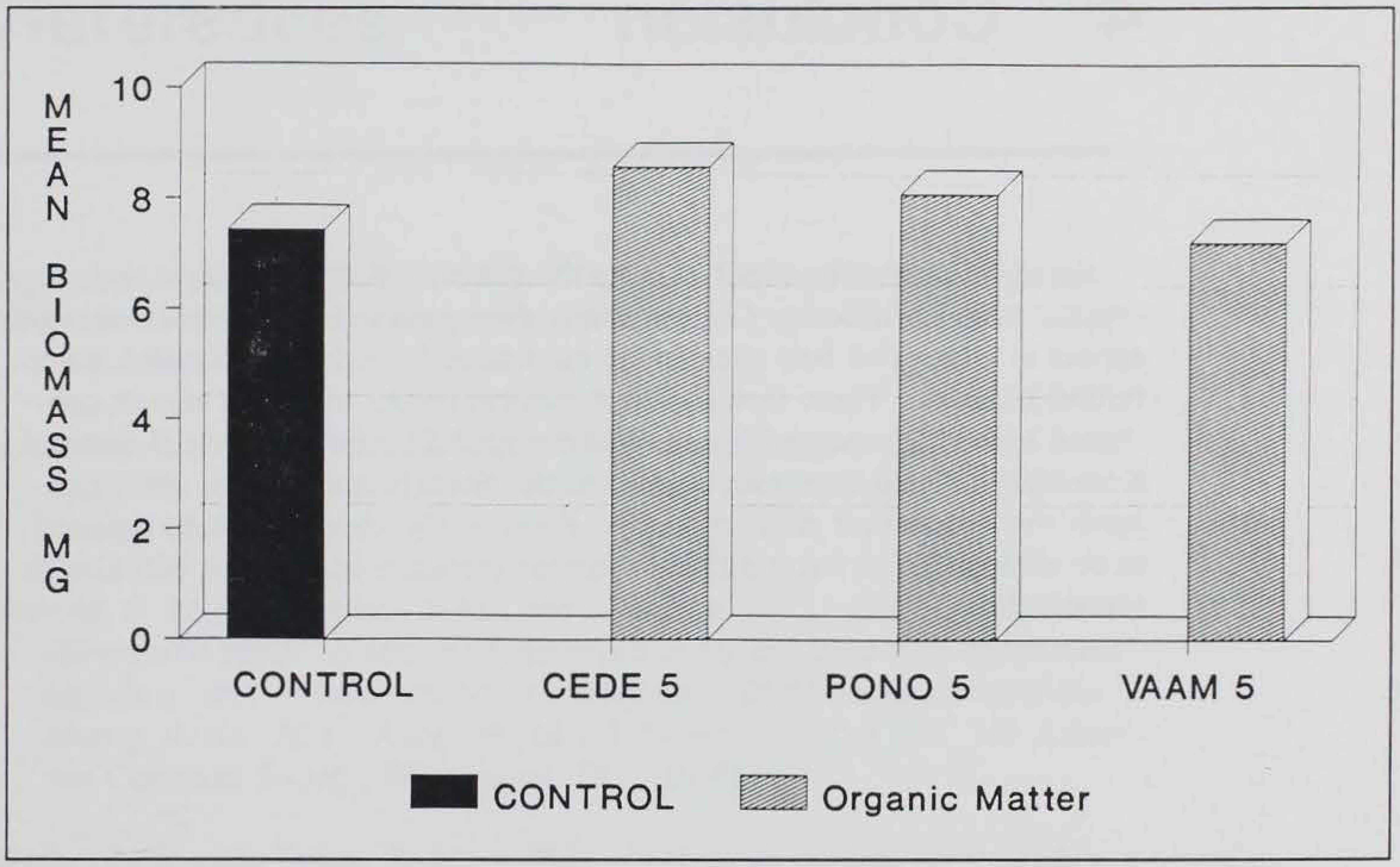

Figure 7. Allelopathic influence of 5-percent organic matter additions on aboveground biomass of *Hydrilla verticillata*

The type of organic matter present in sediments can greatly influence the growth of aquatic plants. Aquatic plants modify the underlying sediments by passively collecting organic matter from elsewhere and by their own production (Wetzel 1979; Carpenter 1981). According to preliminary data, *Ceratophyllum* has been found to be the best allelopathic potential candidate for hydrilla. Kulshreshta and Gopal (1983) investigated the allelopathic nature of hydrilla toward *Ceratophyllum* and found that hydrilla inhibited the growth of *Ceratophyllum* when grown in tanks together. There are other species that warrant additional attention because they appeared to be "borderline allelopathic" during various phases of this study or in literature reviews. These species include Southern naiad (*Najas guadalupensis* Spreng.) Magnus and dwarf spikerush (*Eleocharis coloradoensis* Britt.) because it was found to be allelopathic to several aquatic species in various literature reviews. Sutton (1986b) found that when hydrilla was grown in established stands of spikerush, hydrilla biomass production was decreased by 85 to 90 percent when compared with hydrilla plants grown alone.

4 Conclusion

Based on the results obtained from these studies (test-tube assays and organic matter additions), *Ceratophyllum demersum* and *Vallisneria americana* appear to be the two best allelopathic candidates for reducing Eurasian watermilfoil biomass. These investigations confirm results of earlier studies conducted at the U.S. Army Engineer Waterways Experiment Station (Jones and Kees 1990). Field tests with organic matter from *Ceratophyllum* and *Vallisneria* will be the next phase of study. Because *Eleocharis* has been reported to be allelopathic to watermilfoil in various literature citations, it will also be investigated.

References

Agami, M., and Waisel, Y. (1985). "Inter-relationships between *Najas marina* L. and three other species of aquatic macrophytes," *Hydrobiologia* 126, 169-173.

Anderson, L. W. (1982). "Effects of abscisic acid on growth and leaf development in American pondweed (*Potamogeton nodosus* Poir.)," *Aquatic Botany* 13, 29-44.

Ashton, F. M., Di Tomaso, J. M., and Anderson, W. J. (1985). "Spikerush (*Eleocharis* spp.): A source of allelopathics for the control of undesirable aquatic plants." *The chemistry of allelopathy: Biochemical interactions among plants*. A. C. Anderson, ed., ACS Symp., Series 268, The American Chemical Society, Washington, DC, 401-414.

Barko, J. W., and Smart, R. M. (1983). "Effects of organic matter additions to sediment on the growth of aquatic plants," *Journal of Ecology* 71, 161-175.

Bonasera, J., Lynch, J., and Leck, M. A. (1979). Comparison of the allelopathic potential of four marsh species," *Bulletin of the Torrey Botanical Club* 106, 217-222.

Carpenter, S. R. (1981). "Submerged vegetation: An internal affair in lake ecosystems succession," *American naturalist* 118, 372-383.

Cleland, C. F., and Tanaka, O. (1982). "Influence of plant growth substances and salicylic acid on flowering and growth in the Lemnaceae (Duckweeds)," *Aquatic Botany* 13, 3-20.

Dooris, G. M., Dooris, P. M., and Martin, D. F. (1988). "Effect of a naturally occurring growth inhibitor on the ultrastructure of hydrilla," *Journal of Aquatic Plant Management* 26, 72-73.

Elakovich, S. D., and Wooten, J. W. (1989a). "Allelopathic potential of sixteen aquatic and wetland plants," *Journal of Aquatic Plant Management* 27, 78-84.

Elakovich, S. D., and Wooten, J. W. (1989b). "Allelopathic aquatic plants for aquatic plant management; A feasibility study," Technical Report A-89-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

El-Ghazal, R. A., and Riemer, D. N. (1986). "Germination suppression by extracts of aquatic plants," *Journal of Aquatic Plant Management* 24, 76-79.

Frank and Dechoretz. (1980). "Allelopathy in dwarf spikerush (*Eleocharis coloradoensis*)," *Weed Science* 28, 499-505.

Harrison, P. G., and Chan, A. T. (1980). "Inhibition of the growth of micro-algae and bacteria by extracts of eelgrass (*Zostera marina*) leaves," *Marine Biology* 61, 22-26.

Jones, H. L., and Kees, S. L. (1991). "Influence of various aquatic plant extracts on the growth of *Hydrilla verticillata* Royle." *Proceedings, 25th Annual Meeting, Aquatic Plant Control Research Program*. Miscellaneous Paper A-91-3, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, 143-147.

Kulshreshtha, M., and Gopal, B. (1983). "Allelopathic influence of *Hydrilla verticillata* (L.f.) Royle on the distribution of *Ceratophyllum* species," *Aquatic Botany* 16, 207-209.

Leather, G. R., and Einhellig, F. A. (1985). "Mechanisms of allelopathic action in bioassay. *The chemistry of allelopathy: Biochemical interactions among plants*. A. C. Anderson, ed., ACS Symp., Series 268, The American Chemical Society, Washington, DC, 199-205.

McMillan, C., Zapata, O., and Escobar, L. (1979). "Sulphated phenolic compounds in seagrasses," *Aquatic Botany* 8, 267-278.

McNaughton, S. J. (1968). "Autotoxic feedback in relation to germination and seedling growth in *Typha latifolia*," *Ecology* 49(2), 367-369.

Ostrofsky, M. L., and Zettler, E. R. (1986). "Chemical defenses in aquatic plants," *Journal of Ecology* 74, 279-287.

Parker, S. P., ed. (1984). *McGraw-Hill Dictionary of Scientific and Technical Terms*. 3rd ed., McGraw-Hill Book Co., New York.

Putnam, A. R. (1985). "Allelopathic research in agriculture: Past highlights and potential." *The chemistry of allelopathy: Biochemical interactions among plants*. A. C. Anderson, ed., ACS Symp., Series 268, The American Chemical Society, Washington, DC, 1-8.

Rice, E. L. (1974). *Allelopathy*. Academic Press, New York.

Singhvi, N. R., and Sharma, K. D. (1984). "Allelopathic effects of *Ludwigia adscendens* Linn. and *Ipomea aquatica* Forsk on seedling growth of pearlmillet (*Pennisetum typhoideum* Rich.)," *Trans. Isdt. and Ucds.* 9(2), 95-100.

Smith, C. S., and Jones, H. L. (1990). "Inhibition of hydrilla growth by plant extracts and secondary compounds." *Proceedings 24th Annual Meeting, Aquatic Plant Control Research Program.* Miscellaneous Paper A-90-3, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, 91-98.

Sutton, D. L. (1986a). "Notes: Influence of allelopathic chemicals on sprouting of hydrilla tubers," *Journal of Aquatic Plant Management* 24, 88-90.

_________. (1986b). "Growth of hydrilla in established stands of spikerush and slender arrowhead," *Journal of Aquatic Plant Management* 24, 16-20.

Szcepanska, W. (1971). "Allelopathy among the aquatic plants," *Polskie Archiwum Hydrobiologii* 18, 17-30.

_________. (1977). "Interactions of *Phragmites communis* Trin. and *Carex hudsonii* Bennet," *Ekologia Polska* 25(3), 431-436.

Tarver, D. P., Rodgers, J. A., Mahler, M. J., and Lazor, R. L. (1980). "Aquatic and wetland plants of Florida," Bureau of Aquatic Plant Research and Control, Florida Department of Natural Resources, 50-51.

Wetzel, R. G. (1979). "The role of the littoral zone and detritus in lake metabolism," *Archiv fur Hydrobiologie* 13, 145-161.

Whitehead, D. C., Dibb, H., and Hartley, R. D. (1982). "Phenolic compounds in soil as influenced by the growth of different plant species," *Journal of Applied Ecology* 19, 579-588.

Yeo, R. R. (1980). "Life history and ecology of dwarf spikerush (*Eleocharis coloradoensis*)," *Weed Science* 28, 263-272.

Yeo, R. R., and Thurston, J. R. (1984). "The effect of dwarf spikerush (*Eleocharis coloradoensis*) on several submersed aquatic weeds," *Journal of Aquatic Plant Management* 22, 52-56.

Zapata, O., and McMillan, C. (1979). "Phenolic acids in seagrasses," *Aquatic Botany* 7, 307-317.

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13. ABSTRACT *(Maximum 200 words)*

Allelopathy refers to the biochemical interactions that take place among plants, but its effectiveness depends on the addition of a chemical to the environment. In general, the term allelopathy refers to the detrimental effects of higher plants of one species (the donor) on the germination, growth, or development of another species (the recipient). A more functional definition is any direct or indirect harmful effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment.

Allelopathy is a potentially important tool in controlling undesirable nuisance aquatic weed problems. Various studies have shown that some plants have the capability of eradicating other species in the same area; however, most of these studies have been conducted in using terrestrial plants or plants located in the littoral zone of aquatic habitats. There are only a few studies that show the allelopathic effects of submersed aquatic hydrophytes.

Hydrilla and myriophyllum grown (from axenic cultures) were placed in test tubes for 2 weeks at a constant water bath temperature of 25° C. Test tubes were randomly selected to receive 1 of 10 aquatic plant extracts. The extract was applied to a test tube with a 2-cm explant of the target plant (*Hydrilla verticillata* or *Myriophyllum*

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spicatum), grown for 14 days, harvested, measured, dried at 70° C, and weights recorded. The biomass of *Hydrilla verticillata* and *Myriophyllum spicatum* were significantly reduced by *Ceratophyllum demersum* extracts when compared with the controls.

In separate studies, a 15-cm sprig of *Hydrilla* or *Myriophyllum* was grown in 12-ℓ capacity acrylic columns with an additional 5- or 20-percent aquatic plant organic matter added to the sediment, filled with growth media, then placed in a 1,200-ℓ water bath at a constant temperature of 25° C. Results show that when 20-percent organic matter was added to the sediment, *Ceratophyllum demersum* significantly reduced the growth of hydrilla and myriophyllum; however at 5 percent, *Ceratophyllum* acted as a stimulant. All data were analyzed using ANOVA and the Duncan's Multiple Range Test in SAS.