Journal of Environmental Sciences Vol. 13, No. 4, pp. 481—484, 2001

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2001)04-0481-04 **CLC number**: X173; Q145 **Document code**: A

Allelopathic effect of *Acorus tatarinowii* upon algae

HE Chi-quan[1], WANG Chang-ke[2]

(1. Department of Environmental Science & Engineering, Shanghai University; Shanghai 200072, China. E-mail: hecq @ China. com; 2. Changchun Institute of Geography, Chinese Academy of Sciences, Changchun 130021, China)

Abstract: Besides competing with algae for light and mineral nutrients (i.e. N, P, etc.), the root system of *Acorus tatarinowii* excretes some chemical substances, which injure and eliminate alga cells, to inhibit the growth of the algae. When the algae cells were treated in "*A. tatarinowii* water", some of the chlorophyll a were destroyed and the photosynthetic rate of algae decreased markedly and the ability of alga cells to deoxidize triphenyltetrazolium chloride (TTC) reduced greatly. Then alga cells turned from bright red to bluish green under fluorescence microscope. These showed that the allelopathic effects of *A. tatarinowii* on algae were obvious and planting *A. tatarinowii* can control some green algae. The experiment on the extractions of the secretions of the root system showed that the inhibitory effect had a concentration effect. If the concentration of the root secretion was below 30 μl/disc, the inhibitory rate was negative; if it was over 45 μl/disc, the inhibitory rate was positive. This proved that the influence of the root secretion on the same acceptor was a kind of concentration effect. When the concentration of the root secretion was low, it promoted the growth of algae; when the concentration reached a definite threshold value, it restrained the growth of algae. In present case, the threshold value was between 30 μl/disc and 45 μl/disc.

Keywords: *Acorus tatarinowii*; allelopathy; algae; wetlands

Introduction

A. tatarinowii Scott, a perennial immerged herb in wetlands, belongs to Araceae, Acorus. Its florescence was from April to July and its rhizome was fragrant. It is distributed over the South part of China, East Indian, Vietnam and North Thailand. It was common in wetlands, brooks or thick forests in 20 – 2600 meters above the sea level. The rate of naphtha in its fresh leaf and rhizome was 0.5% – 0.8%, and asarum aether was the essential composition of naphtha.

Allelopathy was the effect of a plant to other plants or microbe through secondary metabolite which was the carrier of transitional information among plants (Rice, 1984). From the 1980's on, it has been established that allopathic influences occur among some plants, this in turn is determined by the biological and chemical characteristics of soil, such as pH, organic matter, nutrients and microbes(Dalton, 1983; Inderjit, 1995; Grime, 1976; Robinson, 1972; Blum, 1988). For example, *Dicranopterwas dichotoma* on the other plants all around (Ye, 1987), *Pedicularwas resupinata* on the seed germinatation of *Vicia faba* and *Triticum sativum* (Zhang, 1981), and *Eichhornia crassipes* on algae (Sun, 1992). However, the work about allelopathic effect of *A. tatarinowii* on algae is less. The present study was aimed at doing so.

1 Materials and methods

1.1 Collection of the experimental materials

A. tatarinowii was collected from a clear brook in a suburb of Nanchang City, Jiangxi Province.

Algae were collected from a pond in the campus of Nanchang University. They consist of 3 phyla, 15 genera, and 32 species. The three phyla were *Chlorophyta*, *Bacillariophyta*, and *Cyanophyta*. The dominant genus was *Scenedesmus* and *Scenedesmus obliquus*, *S. dimophus* and *S. Quadricauda* were common. *Chlorella spp.* and *Pediastrum spp.* could also be found.

The Alga Laboratory of Hydrobiology Institute, Chinese Academy of Science, provided *Chlamydomonas reinhardtii*.

1.2 Compounding the culture solutions and mediums

A. tatarinowii was cultured in 1/10 mol/L modified Hoagland solution (Redyy, 1983), Bwaschoff and Bold Medium (BBM)(Bold, 1987), and normal beef cream peptone medium.

1.3 The culture conditions

A. tatarinowii was cultured near a window in a room with a northern exposure. The temperature of the room was 25 ± 3℃, and the humidity was 70% – 80%.

The algae were cultured under exactly the same condition as described above, except that they were only cultured in a modified Hoagland solution.

Foundation item: The National Natural Science Foundation of China and the "Ninth-five" significant B item (No. KZ-951-B1-201) of the Chinese Academy of Sciences.

1.4 Analysis methods

Algae densities were indicated by the OD_{650} value of the culture solution(Vusak, 1985), which was assayed with a 721 spectrophotometer made by the Third Instrument Factory of Shanghai.

Chlorophyll a was assayed using colorimetric method (American Public Health Institute, 1985).

The deoxidization ability of Algae cells was tested using TTC deoxidization methods (Steponkus, 1967).

The algae death situation was detected using a fluorescence microscope (OPTON model, made in German)

2 Result analysis

2.1 Test about the inhibitory effect of "the water with *A. tatarinowii*" on algae

Filled a 300ml conical flask with 150 ml "*A. tatarinowii* water" filtered with 0.45 μm millipore filter and added just the right amount of culture solution. Then inoculated alga seeds, making sure that the original density of algae (OD_{650} value) should be 0.10. The control group was treated exactly in the same described above except filling no "*A. tatarinowii* water". Because the algae deposited during the course of the culture, the OD_{650} value was measured through two ways: waving and not waving.

The alga density of the control group rose and that in the treated group descended. More algae deposited in the treated group than in the control group (Fig.1).

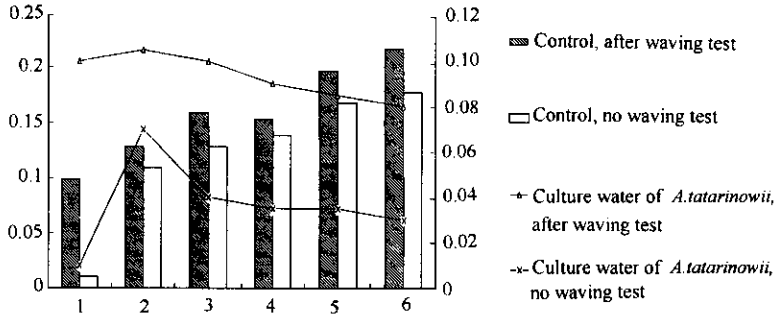

Fig.1 Allelopathic effect of cultured water of *Acorus tatarinowii* on algea

20 ml "algae water" was used to determine the content of chlorophyll a. After 0.2 ml $MgCO_3$ suspension was added to it, the sample was centrifuged, extracted. The content of chlorophyll a was calculated after colorimetric analysis. The chlorophyll a content in the control group was 356 mg/m^3 and 87.2 mg/m^3 in the treated group. The calculation of the deoxidization ability of Algae cells showed that 2.4×10^{-4} mg/ml of triphenylte trazolium chloride (TTC) was reduced into deoxy-triphenylte trazolium chloride and 1.27×10^{-3} mg/ml in the control group-namely the ability of the algae cell to deoxidize TTC was bigger in the control group than in the treated group.

The chlorophyll a was destroyed, so the photosynthesis intensity decreased distinctly and the ability of alga cells to deoxidize triphenylte trazolium chloride (TTC) reduced greatly. The alga cells in the treated group were light cyan under a florescence microscope manifested that they were close to death or already died. This proved that "*A. tatarinowii* water" contained some chemical compounds, which inhibited algae.

2.2 A comparison between the inhibitory effects of the whole plants and the rhizomes and roots of *A. tatarinowii*

First, three glass jars(Φ8 × 15 cm), covered with white paper, were filled with 3000 ml nutrient solution (original OD_{650}, 0.09) in which the algae grew. Then some whole plants of *A. tatarinowii* (fresh weight, 150g) were planted into the first jar. The roots and rhizomes of another 150g fresh *A. tatarinowii* (in the similar size and form as that in the first jar) were planted into the second jar. *A. tatarinowii* was not planted in the third jar. Both the second jar and the third jar were in simulated shading, that is, a 1000 ml beaker, in which *A. tatarinowii* grew, was put in the water.

This test showed that both the roots and rhizomes and the whole plants of *A. tatarinowii* could reduce the OD_{650} and restrain algae, but the former was slower to act than the latter. The curve of whole plants had a peak in 1 – 2 days because the CO_2, exhaled by the root system, could accelerate the growth of the algae. This manifested that the roots and rhizomes of *A. tatarinowii* released most of the inhibitory substances (Fig.2).

2.3 A comparison between the allelopathic effect of shading and that of the secretion of the roots and rhizomes

In natural conditions, if water was all covered by *A. tatarinowii*, the roots of *A. tatarinowii* were almost under dark condition. If their coverage was small, their roots were in weak light condition. The following experiment was carried out in order to find the allelopathic effects of shading and the secretions of root and rhizome in two different conditions. Three glass jars (Φ28 × 15cm) were filled with 3000 ml "algae water", the original alga density(OD_{650}) of which was 0.04. Each glass jar was covered by a piece of white paper to simulate the weak light condition. These glass jars were treated in three different ways: (1) the first jar: no *A. tatarinowii*; (2) the second jar: *A. tatarinowii* was planted; (3) the third jar: no *A.*

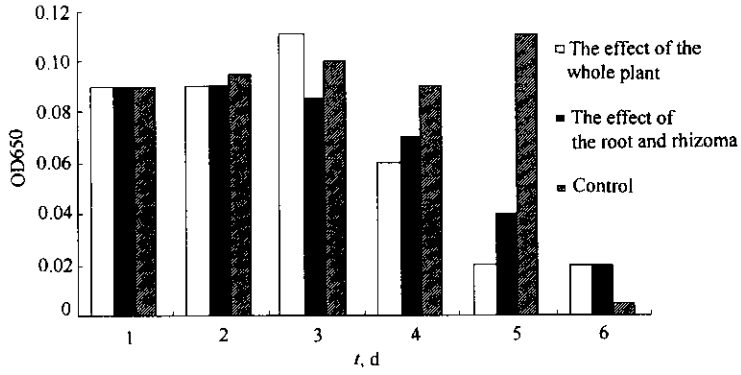

Fig.2 Comparison of inhibitory affect between the whole plant, root and rhizome of *A. tatarinowii*

tatarinowii, but under simulated shading. Three opaque enamel jars (in the similar size and form to the glass ones) were used to simulate the almost dark condition, and they were treated exactly as the glass ones. After seven days, the result is shown in Table 1.

When the roots of *A. tatarinowii* were in dark condition-corresponding to the water were all covered by *A. tatarinowii* (the inhibitory effect of shading of the leaves and stems was bigger than that of secretions of the root and rhizome. When the roots were in weak light condition) corresponding to the water were partly covered by *A. tatarinowii*, the inhibitory effect of secretions of the root and rhizome was bigger than that of shading of the leaves and stems. In a word, the shading of the leaves and stems and secretions of the root and rhizome restrained algae simultaneously, but their relative contribution varied with the coverage of *A. tatarinowii*.

Table 1 Allopathic effects of the shading of the leaves and stems and the secretion of the root and rhizome

	OD_{650}		Shading			Secretion	
	Control	With *A. tatarinowii*	Shading no plant	Quantity of inhibition	Rate of inhibition	Quantity of inhibition	Rate of inhibition
Enamel jars	0.10	0.01	0.02	0.08	80	0.01	10
Glass jars	0.11	0.01	0.10	0.01	9	0.09	81

2.4 Extraction of the secretions of the root system

200 ml rarefied ether was added to 200 ml "*A. tatarinowii* water two times" to extract the secretions of *A. tatarinowii*. After the extraction had been dried with anhydro-$CaCl_2$, it was put in 40℃ water to evaporate the dissolvent (ether). Then it was adjusted to 100 μl with absolute ether. Five different concentrations, namely 0, 15, 30, 45 and 75 μl/disc, of the extraction were dropped onto five round filter papers (Φ11 mm) with a micro-pipette. After the dissolvent had volatilized, the five round filter papers were transferred onto five piece of agar-BBMs (Φ12 mm, 2.5 mm in thickness) within a culture plate (Φ60 × 15 mm). Then 15μl "Chlamydomonas reinhardtii water" (OD_{650}, 0.12) was dropped into the culture plate. The culture plate was kept within a temperature of 24 - 26℃ and irradiated with a fluorescent lamp (80 μmol/(m^2 · s)). After 96 h, the chlorophyll a contents of alga cells in each filter paper were calculated and the inhibitory rates were counted out (Table 2).

Table 2 Allelopathic effects of secretions from the root of *A. tatarinowii* on algae

Item	Inhibitory effects of different concentration of root secretions from *A. tatarinowii* on alga				
Root secretion, μl/disc	0	15	30	45	75
Inhibitory rate, %	0	- 8.2	- 2	+ 31	+ 86

Notes: + inhibitory action; - auxo-action

If the concentration of the root secretion was below 30 μl/disc, the inhibitory rate was negative; if it was over 45 μl/disc, the inhibitory rate was positive. This proved that the influence of the root secretion on the same acceptor was a kind of concentration effect. When the concentration of the root secretion was low, it promoted the growth of algae; when the concentration reached a definite threshold value, it restrained the growth of algae. In present case, the threshold value was between 30 μl/disc and 45 μl/disc.

3 Discussion and conclusions

In the natural waters where aquatic vascular plants were common, there were few algae and the water was very clear. At first this phenomenon was attributed to the competition between the vascular plants and the algae for light and mineral nutrients, but later Sun *et al.* thought that chemical secretions of the vascular plants restrain algae population (Sun, 1989). The author have completed above experiment about the inhibitory effect of *A. tatarinowii* on algae and this also proved that the

competition for nutrients also was not the main cause of the restraint on algae. The experiment on the extractions of the secretions of the root system showed that the inhibitory effect had a concentration effect. If the concentration of the root secretion was below 30 μl/disc, the inhibitory rate was negative; if it was over 45 μl/disc, the inhibitory rate was positive. This proved that the influence of the root secretion on the same acceptor was a kind of concentration effect. When the concentration of the root secretion was low, it promoted the growth of algae; when the concentration reached a definite threshold value, it restrained the growth of algae. In present case, the threshold value was between 30 μl/disc and 45 μl/disc. The experiments of the root system secretions and their inhibition on the algae proved that the inhibition of *A. tatarinowii* on algae resulted from the fat-soluble organic compounds excreted by *A. tatarinowii*. But the physical-chemical qualities of those compounds were not known. The experiments with the whole plant and the root and rhizome of *A. tatarinowii* showed that the organic substances were released by the root and rhizome of *A. tatarinowii*. The experiment on the effects of shading and the secretions proved that the cause of the inhibitory effect of *A. tatarinowii* on algae was a synthetic action of shading, the competition for the mineral nutrients and the secretions of the root and rhizome. But the inhibitory effects of them varied with space-time conditions.

In some fresh aquatic ecosystem, the algae propagate rapidly in eutrophic water and reduce the content of the dissolved oxygen. This will threat the survival of the aquatic creature and give rise to the so-called "deserts in the waters" (Ye, 1993). Those algae may adhere to the surface of the immerge-plants and the roots of the emerge-plants. This was harmful to the growth of the higher plant. But some higher plants may have some mechanisms of protection or counterattack. The above experiments have confirmed this deduction. The secretions of the roots and rhizome of *A. tatarinowii* on algae can be used to purify swimming pool and other waters of entertainment. And it was safer to use these secretions than to use inorganic compounds, such as, cupric sulfate (He, 1999). By making through study of the chemical composition of the secretions and the method to synthesize them, we can produce algaecide.

References:

American Public Health Association, 1985. The assay of phytoplankton chlorophyll[M]. The normal checking methods of water and waste water. Beijing: The Chinese Architecture Press. 901—904.

Blum U, Shafer R, 1988. Microbial populations and phenolic acids in soil[J]. Soil Biol Biochem, 20: 793—800.

Bold Wynne, 1987. Introduction to the algae[J]. Structure and Reproduction, 572—574.

Dalton B R, Blum U, Weed S B, 1983. Allelopathic substances in ecosystems: effectiveness of sterile soil component in altering recovery of ferulic acid[J]. J Chem Ecol, 9:1185—1201.

Grime J P, Curtis A V, 1976. The interaction of drought and mineral nutrient stress in calcareous grasslands[J]. J Ecol, 64:975—988.

He C Q, 1999. Allelopathic effect and its use in wetland study[J]. Chinese Journal of Ecology, 18(3):

Inderjit, Dakshini K M M, 1995, On laboratory bioassays in allelopathy[J]. Bot Rev, 61:32—48.

Reddy K R, Tucker J C, 1983. Productivity and nutrient uptake of water hyacinth *Eichhornia crassipes* I. Effect of nitrogen Source[J]. Economic Bot, 37:237—247.

Rice El, 1984. Allelopathy[M]. Orlando Florida: Second Academic Press Inc. 382.

Robinson R K, 1972, The production by roots of *Calluna vulgaris* of a factor inhibitory to growth of some mycorrhizal fungi[J]. J Ecol, 60: 219—224.

Steponkus P L, Lanphear F O, 1967. Refinement of the triphenyl tetrazolium chloride method of determining cold injury[J]. Plant Physical, 42:1423—1426.

Sun W H, 1991. The biological assay of the allelopathic compound of *Eichhornia* crassipes[J]. Plant Physiology Communication, 27(6): 433—436.

Sun W H, 1989. The biological government of the eutrophic waters in city and the mechanism of the allelopathic effect of Eichhornia crassipes on algae[J]. Environment Science Journal, 9(2):188—195.

Sun W H, Yu S W, 1992, Allelopathy and its application[J]. Plant Physiology Communication, 28(2):81—87.

Ye J X, He C Q, 1996. *A. tatrinowii*——a plant to purify waters[J]. Plant Magazine, 30(2):10—11.

Ye J X, Hong R C, 1987, The effect of the extraction of *Dicranopterwas dichotoma* on the growth of several plants[J]. Plant Ecology and Geo-Botany Journal, 11(3):203—210.

Ye J X, 1993. The awake of the 21st century——Earth, a lonely boat in the ecological coordinates[M]. Nanchang: The 21st Century Press. 243—248.

Zhang B C, 1981, Preliminary study on the biochemical inhibition of the extraction of *Pedicularis resupinata*[J]. Ecology Journal, 1(3): 227—230.

(Received for review September 11, 2000. Accepted March 5, 2001)