

# ALLELOPATHY AND TOXICITY TO FISH OF AQUATIC WEEDS

Jiro Harada  
National Institute of Agro-Environmental Sciences  
3-1-1 Kannondai, Tsukuba 305, Japan

## ABSTRACT

*Piscicidal plants have been widely used to catch fish in different parts of the world. Various types of piscicidal substances have been isolated from these plants, and are reviewed in this paper. It was found that the piscicidal substances in some aquatic weeds might have an adverse effect on fish populations. One of these,  $\alpha$ -naphthoquinone, was isolated from *Ammannia baccifera* L. Incorporation of this plant into the soil reduced the emergence of weeds.*

## INTRODUCTION

Piscicidal plants have been widely used by traditional societies all over the world as a means of catching fish. Various types of piscicidal substances have been isolated from these plants (Kawazu 1972) and are discussed in this paper.

Recently, *Polygonum hydropiper* L., an aquatic weed, has been found to contain polygodial, which showed strong piscicidal activity (Harada and Yano 1983). This finding is very important because such weeds may be a potential source of environmental pollution, especially in Southeast Asian countries where only small amounts of agrochemicals are used.

From this, we began to consider the possibility that piscicidal substances contained in aquatic weeds might be a cause of fish deaths. Hence, we examined the piscicidal activity of weed species growing in a tropical aquatic environment. We isolated the active substance from *Ammannia baccifera*, an aquatic weed common in Thailand, which showed the strongest activity. It was also found that incorporating this weed into the soil reduced the emergence of weeds. The results are presented in this paper.

## PISCICIDAL PLANTS

Piscicidal plants have been widely used in the past to catch fish. From these plants, various

piscicidal substances have been isolated (Fig. 1). Rotenone has been isolated from *Derris elliptica*, *D. montana* and *D. pubipetala* (belonging to the Leguminosae), the roots of which were widely used in Southeast Asia; juglone (5-hydroxynaphthoquinone) from *Juglans mandshurica* (Juglanaceae), the roots and fruits of which were used in Japan; justicidin A and B from *Justicia hayatai* var. *decumbens* (Acanthaceae), the whole plant of which was used in Taiwan (Ohta *et al.*, 1969, 1971); callicarpone from *Callicarpa candicans* (Verbenaceae), the leaves of which were used in the Caroline and Philippine islands; maingayic acid from *Callicarpa maingayi* (Verbenaceae) (Nishino *et al.* 1971); huratoxin from *Hura crepitans* (Euphorbiaceae), latex from which was used in South America (Sakata *et al.* 1971); vibsanine A from *Viburnum awabuki* (Caprifoliaceae), the leaves of which were used in Okinawa, Japan (Kawazu and Mitsui 1974); inophyllolide from *Calophyllum inophyllum* (Guttiferae), the leaves and seeds of which were used on the Malay peninsula; and ichthyothereol from *Ichthyothere terminalis* (Compositae), the leaves of which were used in the Amazon (reports without citation, reviewed in Kawazu 1972).

Although all these plants show very strong piscicidal activity, most of them do not seem to be any threat to the aquatic environments of Southeast Asia because they are found in distant areas.

Key words: Aquatic weeds, piscicidal substances, *Ammannia baccifera* L.,  $\alpha$ -naphthoquinone, allelopathy

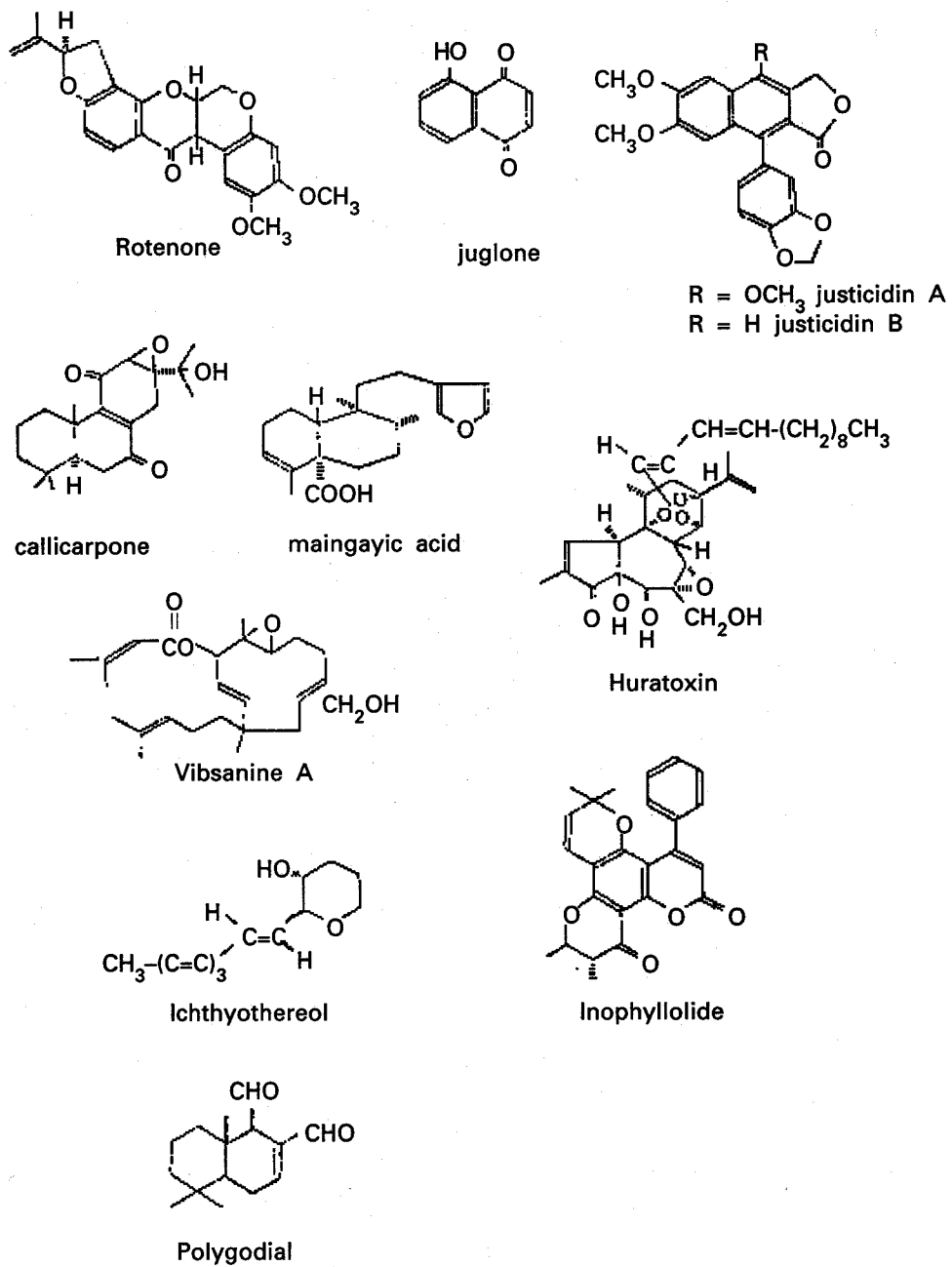


Fig. 1. Chemical structure of piscicidal substances isolated from plants

## PISCICIDAL AQUATIC WEEDS

We have already mentioned in this paper the finding that the aquatic weed *P. hydropiper* contains polygodial, which has strong piscicidal activity (Harada and Yano 1983). However, we were not at first aware of the significance of this fact, because aquatic environments such as rivers, lakes and paddy fields in Japan are too polluted with agrochemicals and factory wastes to serve as an important source of edible fish.

In most Southeast Asian countries, however, including Thailand, the aquatic environment is very important as a site of fish production. Most farmers where fish culture is commonly practiced control aquatic weeds by plowing the soil and then flooding it. Fish reared in such areas often die from unknown causes, even though no agrochemicals at all may have been used.

We were thus interested in the possibility that piscicidal substances contained in aquatic weeds might be a cause of fish deaths. Hence, we examined the piscicidal activity of 54 weed species growing in a tropical aquatic environment on guppy fish, and found that 12 of these species showed piscicidal activity (Table 1).

### ISOLATION OF PISCICIDAL SUBSTANCES

A piscicidal substance was extracted using various solvents from the shoots of *Ammannia baccifera* (Fig. 2), the weed which showed the strongest activity. Most extracts except those using water as a solvent showed strong piscicidal activity

(Table 2). It seems clear that the active substance is easily soluble in non-polar to polar organic solvents. Methanol extract, however, killed the fish earlier than the other extracts used, so was thereafter used for extraction.

Purification of the active substance(s) was carried out using charcoal-celite column chromatography and a water/acetone step elution system. Active substance(s) are shown as a 0/100 (water/acetone, v/v) fraction. The active fraction extracted from 1 g fresh material was developed on a TLC plate, using a n-hexane/ethylacetate mixture (9/1, v/v). A dark violet band was found at Rf 0.65 under UV light, as shown in Fig. 3. This zone was scraped off and eluted with methanol, then tested as to its piscicidal activity and other characteristics. It showed strong piscicidal activity.

The UV-absorption spectrum of the active zone at Rf 0.65 is shown in Fig. 4. The  $\lambda$  max. in methanol was 244 nm. This pattern is similar to that of a-naphthoquinone. GC and GC-MS analyses were carried out in order to identify the substance.

The GC pattern is shown in Fig. 5. The active zone gave three peaks, one major and two minor. A mass spectrogram of the major substance (A) gave fragment peaks  $158M^+$ , 130, 104, and 102, as shown in Fig. 6. Fragment peaks of first (B) and second (C) minor substances were  $174M^+$ , 158, 146, 130, 118, 105, 102, 89, 76, and  $188M^+$ , 174, 158, 130, 116, 102, 89, 76, respectively. On the basis of these results, the major active substance contained in *A. baccifera* was identified as a-naphthoquinone. The two minor substances, however, have not yet been identified.

Table 1. Piscicidal weeds growing in aquatic environments

Weed species	Family
<i>Ammannia baccifera</i>	Lythraceae
<i>Polygonum tomentosum</i>	Polygonaceae
<i>Dysophylla stellata</i>	Labiatae
<i>Sphenoclea zeylanica</i>	Sphenocleaceae
<i>Bacopa monnieri</i>	Scrophulariaceae
<i>Mimosa pigra</i>	Mimosaceae
<i>Neptunia natans</i>	Mimosaceae
<i>Sesbania javanica</i>	Papilionaceae
<i>Ipomoea aquatica</i>	Convolvulaceae
<i>Centrostachys aquatica</i>	Amaranthaceae
<i>Potamogeton malaianus</i>	Potamogetonaceae
<i>Pistia stratiotes</i>	Araceae

Source: Zungsonthiporn *et al.*, unpublished data



Fig. 2. *Ammannia baccifera* plant

Table 2. Piscicidal activity of various extracts from 0.1, 0.5 and 1.0 g fresh weight of *Ammannia baccifera* shoots

Solvent	Mortality (%)		
	0.1	0.5	1.0
Cold water	0	0	0
Hot water (80°C)	0	0	20
Methanol	100	100	100
Ethyl acetate	100	100	100
Benzene	100	100	100
n-Hexane	100	100	100

Source: Zungsonthiporn *et al.* 1986

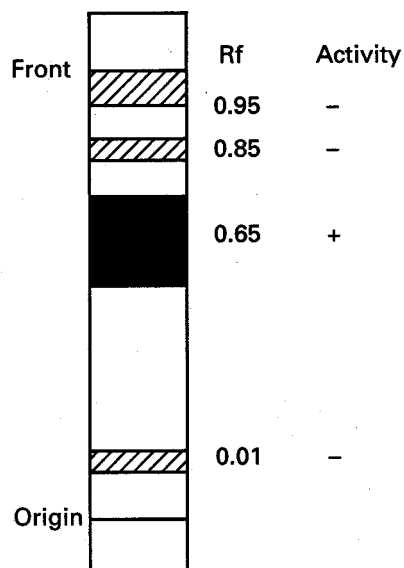


Fig. 3. Thin layer chromatogram of the active fraction from charcoal-coal-celite column chromatography

Source: Zungsonthiporn *et al.* 1986

Silica gel plate --- Whatman LK 6,5 x 20 cm  
 Developing solvent -- n-Hexane : Ethyl acetate 9:1 (v/v)

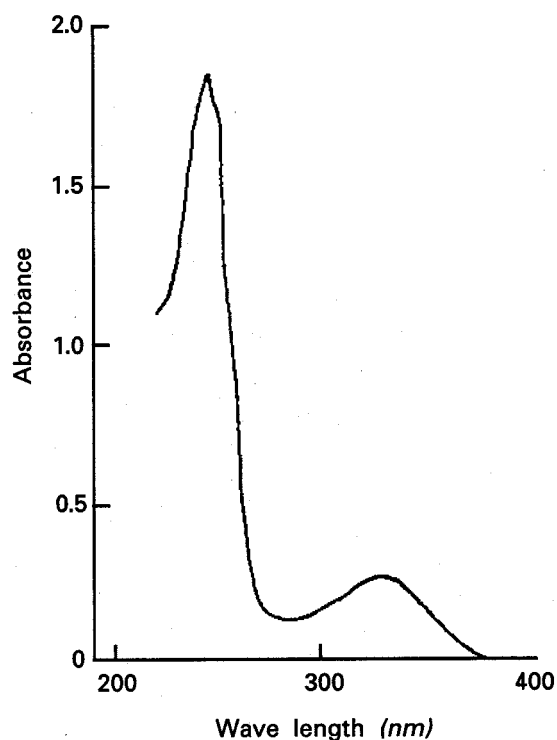


Fig. 4. UV-absorption spectrum of methanol solution of piscicidal substance isolated from *Ammannia baccifera*

Source: Zungsonthipora *et al.* 1986

The piscicidal activity of synthetic a-naphthoquinone was examined, using guppies. As Fig. 7 shows, the TLm (24 h) was about 0.09 ppm. This value is only moderate compared to that of other much more toxic piscicidal substances, such as huratoxin (0.0014); justicidin A and B (0.02 and 0.04); callicarpone (0.04); vibsanine (0.1); cis-dehydromatricaria ester (2); maingayic acid (4.7); and inophyllolide (5 ppm) (reviewed in Kawazu 1972).

Quantitative analysis using the UV absorption spectrum revealed that 1 g fresh weight of *A. baccifera* shoots contained 78 µg of a-naphthoquinone. In terms of availability, the present population of *A. baccifera* contains enough of the toxic substance(s) to pollute aquatic environments all over Thailand.

Our field observations in Buri Ram, Surin and Si Sa Ket, Northeast Thailand, in December 1984 revealed heavy infestation with piscicidal aquatic weeds, including *A. baccifera*, in areas where large numbers of fish had died (Harada 1989). Further studies, however, are essential to clarify the

relationship between piscicidal aquatic weeds and large-scale fish deaths under natural conditions.

## EFFECT OF PISCICIDAL AQUATIC WEEDS ON WEED EMERGENCE

During this study of piscicidal aquatic weeds, we found that Thai farmers are using *A. baccifera* incorporated into paddy soil in order to reduce weed emergence. To analyze this effect, pot experiments were conducted. *A. baccifera* was found in fact to reduce the emergence of weeds (Fig. 8), (Harada and Sumiyoshi 1991). The chemical structure of a-naphthoquinone, the piscicidal substance contained in *A. baccifera*, is similar to that of ACN, which is a known paddy herbicide (Fig. 9). From this point of view, a-naphthoquinone seems to possess both types of biological activity, herbicidal and piscicidal.

## CONCLUSION

Isolation and identification of piscicidal substances from aquatic weeds is of great importance, not only to reveal possible sources of environmental

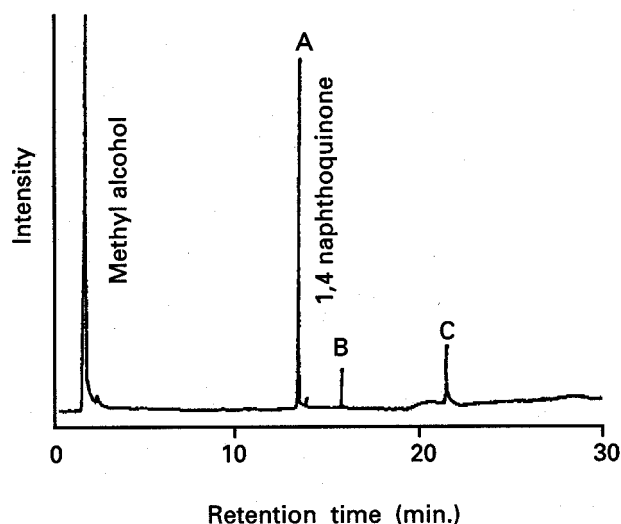


Fig. 5. Gas-chromatogram of active fraction from TLC

Source: Zungsonthiporn *et al.* 1986

Column: 25 m L, 0.2 mm I.D.

Temp: 100 - 280°C (6°C/min)

Liquid phase: 5% phenylmethyl silicone

Carrier Gas: He.

Flow rate: 50 ml/min, column 0.5 ml/min

Vent: 50 ml/min, split ratio: 1:100

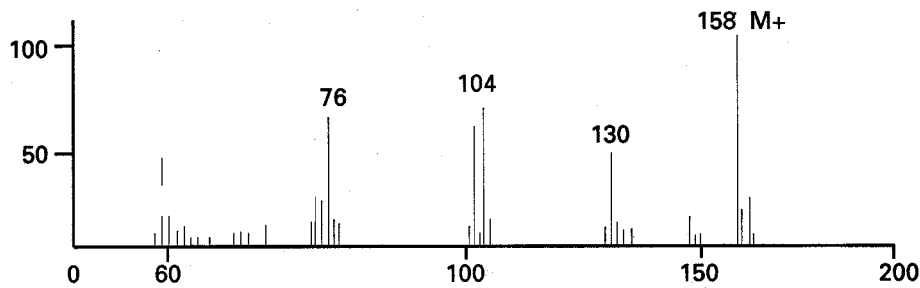


Fig. 6. Mass spectrogram of piscicidal substance isolated from *Ammannia baccifera*

Source: Zungsonthiporn *et al.* 1986

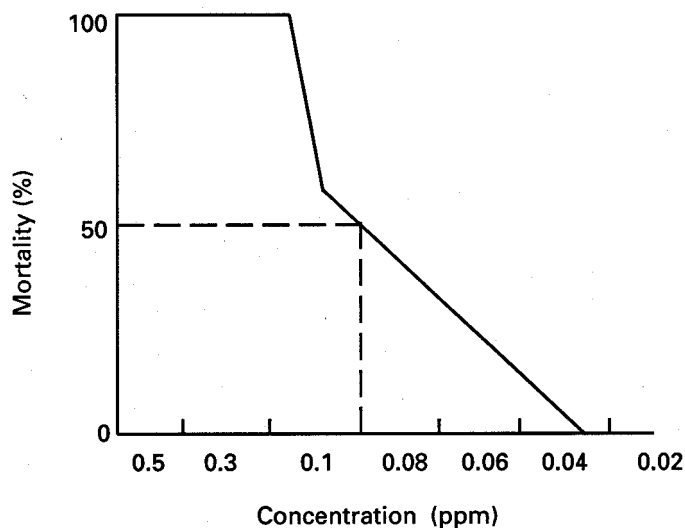


Fig. 7. Effect of synthetic  $\alpha$ -naphthoquinone on guppy fish

Source: Zungsonthiporn *et al.* 1986

pollution, but also to find new biologically active substances for future practical use. Such work should focus on the tropics, where abundant plant resources exist.

## REFERENCES

- Harada, J. 1989. Piscicidal aquatic weeds in Thailand. *Shokucho* 23: 166-172.
- Harada, J. and M. Yano. 1983. Plant growth inhibiting substances contained in *Polygonaceae* weeds. *Proceedings of the 9th Asian-Pacific Weed Science Society Conference*, pp. 71-75.
- Harada, J. and T. Sumiyoshi. 1991. Inhibitory effect of mulching using plant materials on the emergence of weeds. *Weed Research, Japan* 36 (Extra issue 1): 148-149.
- Kawazu, K. 1972. Active constituents of piscicidal plants. *Yuukigoseikagaku* 30: 615-628.
- Kawazu, K. and T. Mitsui. 1974. Novel diterpene esters with biological activity from the leaves of *Viburnum awabuki*. *18th Symposium on the Natural Products (Abstracts)*, pp. 77-84.
- Nishino, C., K. Kawazu and T. Mitsui. 1971.

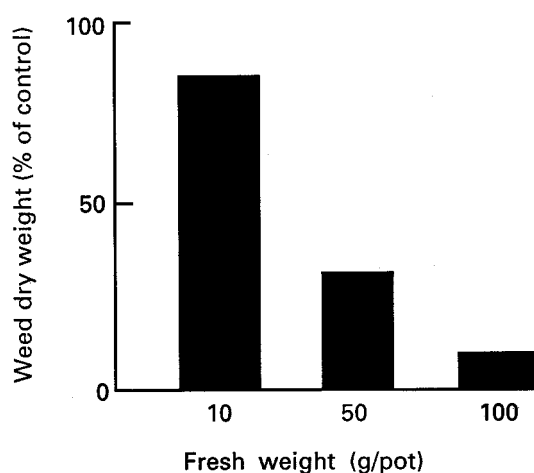


Fig. 8. Effect on weed emergence of incorporating *Ammania baccifera* into paddy soil

Source: Harada and Sumiyoshi 1991

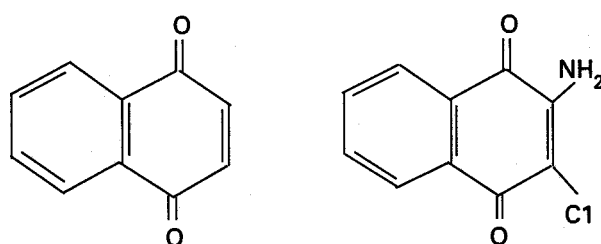


Fig. 9. Chemical structures of  $\alpha$ -naphthoquinone (left) and ACN (right)

- Isolation and structure of maingayic acid, a piscicidal constituent of *Callicarpa maingayi*. *Agricultural and Biological Chemistry* 35: 1921-1930.
- Ohta, K., Y.L. Chen, S. Marumo and K. Munakata. 1969. Studies on the piscicidal components of *Justicia hayatai* var. *decumbens*. I. Isolation and piscicidal activities of justicidin A and B. *Agricultural and Biological Chemistry* 33: 610-614.
- Ohta, K., S. Marumo, Y.L. Chen and K. Munakata. 1971. Studies on the piscicidal components of *Justicia hayatai* var. *decumbens*. II. The structures of justicidin A and B, and synthesis of justicidin B and related compounds. *Agricultural and Biological Chemistry* 35: 431-438.
- Sakata, K., K. Kawazu and T. Mitsui. 1971. Studies on a piscicidal constituent of *Hura crepitans*. I. Isolation and characterization of huratoxin and its piscicidal activity. *Agricultural and Biological Chemistry* 35: 1084-1091.
- Zungsonthiporn, S., C. Premasthira and J. Harada. 1986. Piscicidal substance contained in *Ammania baccifera* L. *Proceedings of 10th Asian-Pacific Weed Science Society Conference*, Vol. 2: 452-457.

## DISCUSSION

Dr. Fujii was interested in the similar herbicidal effects of a-naphthoquinone and ACN. Since the two chemicals have similar characteristics, he asked which of the two has the stronger herbicidal effect. Dr. Harada replied that the two substances had not been given a comparison test, but pointed out that the ACN used as a herbicide in Japan is also toxic to fish, and that farmers must take care in using it.

Dr. Kim pointed out that although research on the allelopathic role of aquatic weeds had mainly been looking for water soluble allelopathic substances, in practice it had mainly used solvent. He asked whether an allelopathic plant might accumulate allelopathic material as it grows, to be released after the plant dies and decays. As its organic matter broke down, he asked whether it might combine with allelopathic substances and interfere with emergence. Dr. Harada answered that there was no evidence of whether *Ammannia baccifera* acts in this way under natural conditions. He explained that all piscicidal aquatic weeds had been identified as such in the laboratory, and he did not know whether they were active under field conditions. However, he recommended that farmers should handle them carefully, especially near an aquatic environment or fish pond, because of the possibility that they might contain toxic substances which kill fish.

He agreed that substances extractable with water were more closely related to field conditions. He suggested that in areas without rain, water soluble substances might be good for causing allelopathy. However in Japan and Southeast Asian countries, any substance readily soluble in water would wash out in the rain, so that the nature of the solubility was not of much importance.

Dr. Bayot pointed out that *Ipomoea aquatica* is one of the most important vegetables in the Philippines. He asked whether the chemical substance responsible for its piscicidal activity had been identified, and whether it had any toxic effect on human beings after the vegetable had been cooked. Dr. Harada replied that the substance had not yet been isolated, but it did not have a strong piscicidal effect on fish. He thought there were no health problems if people ate the plant as a vegetable, and pointed out that they had been doing this for a very long time.