# Cultivation and utilization of tropical seaweed *Kappaphycus alvarezii* in warm waters of Japan

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Large-scale joint investment project between the Ministry of Agriculture, Forestry and Fisheries and the private sector was established in 1991. A decade of research to extract and commercialize has begun. Because the lectin is a protein, the study required *K. alvarezii* raw samples throughout the year. K. *alvarezii* did not have a Japanese name and was named Nettai Kirinsai ( Yano,M.et al.2007).

The cultivated green type and reddish brown type materials of 2kg wt. K.alvarezuii fronds were transplanted from Philippines. They were grown in the sea from May to November for 12 years since May 1992 ( Ohno & Largo, 2020), and used as experimental samples. Their materials were cultivatied in the sea for summer season).

From December to spring of the following year, the temperature their matrieals were culture in the aquatron which is adjusted to 23°C in aquatron (50 cm x 50 cm x 50 cm constant temperature, closed, aerated acrylic tank with occasional water changes) owned by Kochi University.*K. alvarezii* strains managed at Kochi University since 2020 when the project.

These seedlings were kept on ropes in fish pen frames. The seedings had started in the sea in May. Sea water temperature reached excellent 25-30 ℃ in August. Tank culture was performed when the water temperature reached 22°C in December.

The tank cultured *K.alvarezii s*eedlings were kept at 22°C during the winter season. outdoor (roofed) tank culture under natural light, which was adjusted with a heater and a temperature control device. *K.alvarezii* cultivation was successfully established using small cages and net for 30 years in Tosa Bay, warm sea in Japan.

## Cultivation with fish cultivated cage

*K.alvarrezii* cultivation was done by ropes with 10mx10m fish net floating cage and 20 ropes were used. The cultivated ropes were sanked at a depth of about 50 cm with anchors attached to both ends. The water temperature in the cultivation area reaches over 20°C in early May, 23°C in June, 24-28°C in July and 29-32°C in August. The water temperature dropped from September to 25-28°C, and gradually dropped from October to December until it reached 22°C, when the sea surface culture was finished.

In June, the growth rate of frond increased with the rise of water temperature, and the daily growth rate reached 3-4 %. In September, the daily growth rate was more than 5%, and the leaf length and weight more than doubled in one week. Rainfall and lack of light slowed the growth, and the daily growth rate also fluctuated during the rainy season in June, the salinity dropped significantly to 19-24 psu due to rainfall, and the growth rate slowed down. Salinity increased to 29 ~32 psu in summer. Their growth slows when water temperatures exceed 30°C in summer. The highest growth rate became during the period from September to November, when the water temperature is 23-28°C.

This period was split with harvest and again palm-sized fronds were attached to the rope. In the past sea aquaculture, when 1 kg of overwintered seedlings were cultured in the sea, the yield was 70 to 100 kg at the end of the aquaculture in December. The decrease in salinity in the rainy season and the high temperature in summer greatly affected the production.

**Net cultivation method**

Eucheumatoid cultivation are using ropes currently. This method is carried out using the long line method in the open sea, but a large amount of fronds fall off the rope due to the waves. Therefore, The method cultivated *K. alvarezii* by putting the leaves in a net bag has tried for the open sea. New leaf bodies elongated outside the net. The problem was the material of the net. If the thread (material of cotton thread and synthetic thread) is thick, it will get very dirty. Nori net have been used *K. alvarezii c*ultivation, but the net is less dirty, so it is better to insert larger seedlings.

In future open sea *K.alvarezii* cultivation, the development of cultivation methods that insert seedings into nets will be an issue.

**Over winter tank culture**

Overwintering tank culture of seedlings from the end of November, when the water temperature drops below 20 degrees, the growth of algae stops and the leaves become softer and lose color. It becomes yellowish green. Every year when the sea surface temperature approached 20°C, they started preparing for wintering. Approximately 20 kg of alga bodies were stored in a one-ton tank installed in a breeding building with a roof, and seawater was added little by little. The water temperature was kept at 23°C by a heater, and the water was aerated. The fronds were cultured in an outdoor tank culture in winter in a state in which the fronds grew slightly. Every year, when the seawater temperature reaches 20 ℃ in May, the fish are transferred to the sea surface culture method, and the preserved stock has been maintained. Prospects for tropical *K,alvarezii* in Japan The growth rate of them has doubled in a week when the water temperature reaches 23-28 °C in Tosa Bay for many years, and the daily growth rate often exceeds 4%. The daily growth rate in commercialized euchuematoid cultivation in the Philippines was 4-5%, similar to the growth rate of *K,alvarezii in* Tosa Bay (Trono & Ohno,1989)

It was confirmed that the growth rate of them slows down when the water temperature drops below 23°C in November, and the green color from alga bodies decreases. However, it was found that even at a water temperature of 18°C in winter, the growth was retarded or grew normally. Therefore, if seedlings are secured by tank culture, it will be possible to cultivate *K.alvarezii* in many sea areas in Japan where the temperature is over 20℃ from May to November. However, the growth is better in a state of high salinity. It was found that they can survive even at low salinity of 15 psu and continue to grow. Sandy sea areas where algae-eating fish do not exist are suitable for farming ( Yano &Ohno,2007)

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**Fig.1. Cultivation methods of Kappapycus alvarezuii with sishe cage in Tosa Bay in Japan**

A: Brown type frond of *K.alvarezii* B, Green type frond cultivated of *K.alvarezuii*
C: Green type frond of *K.alvarezii* D: Fish cage ( 10m X10m size)
E: Hanging seeding with anchor F: seeding ropes hanged at one m depth from surface water
G: *K.alvatezii* seeding fixed interval of 20cm
H: Seeds grown aftter about two weeks in June (rain season)
I;. seeding ropes fixed each 20 lines ( in summer season)

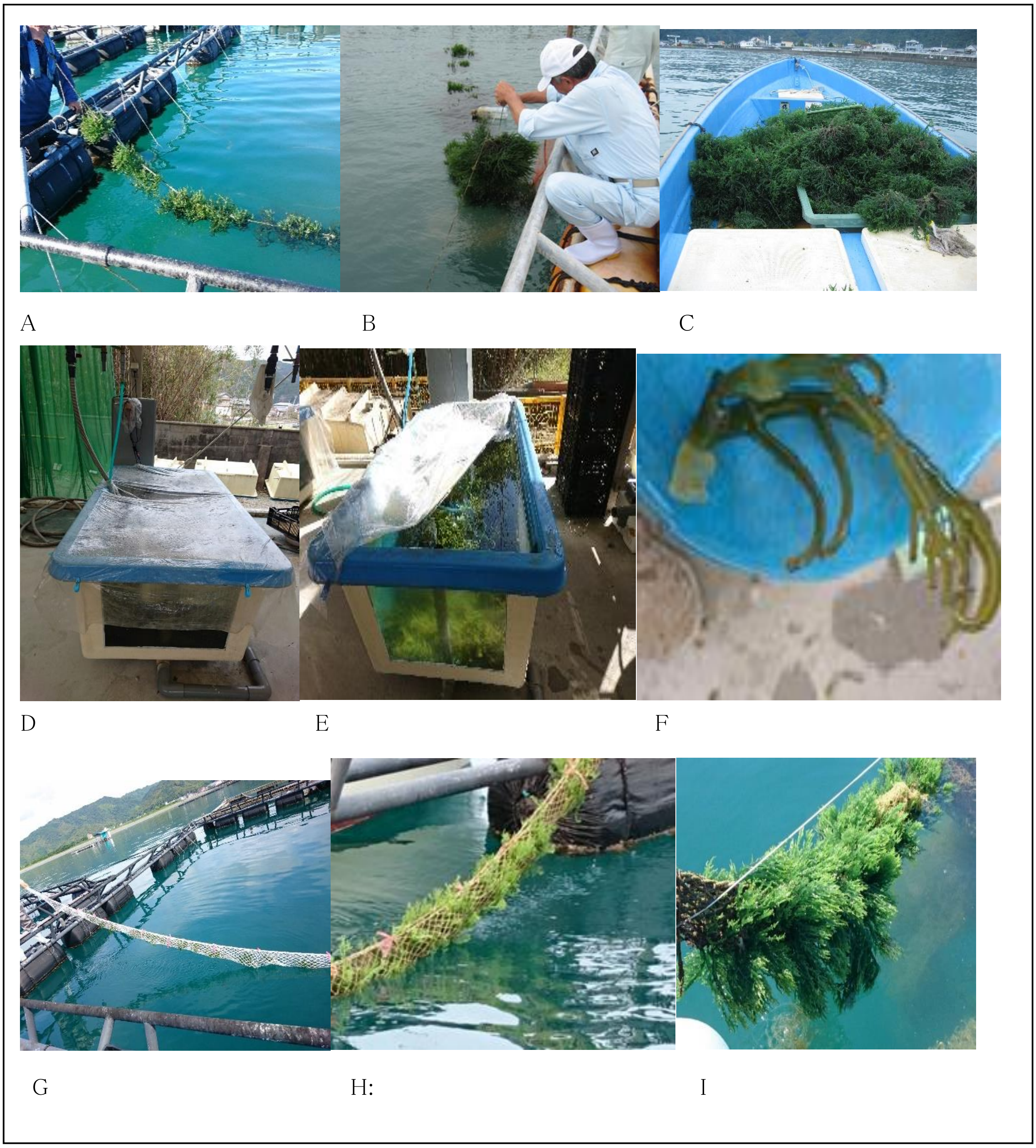


Fig.2 **Cultivation methods of *Kappapycus alvarezii* with fish cage in Tosa Bay in Japan**

A:*Kappaphycus* products of the ropes in autumn. B: Harvested products of the ropes in Decmber

C:　Harvested products in the boat

D, E: The tank culture of *K.alvarezii* fronds with control of water temperature

F: The disease frond which the epidermis has pee off (white color)

G: Net method of *Kappaphycus alvarezii* cultivation.　H: Growing fronds from the nets after two weeks after seeding. H: Growing frond from the net after one month after seeding.

## Uses of Japanese euchuematoid products

**Fields of use as ingredients**

The reason why *K.alvarezuii* cultivation has been able to continue in Tosa Bay is because of the demand for it as an ingredient in Italian and French cuisine. Adding a small amount of the dry powder to kamaboko (fish paste) and jelly is popular for its viscosity and texture, so it is likely that applications in this field will be developed in the future. Currently, it is also recognized as effective as a seaweed fertilizer. If demand for these increases, *K.alvarezii* cultivation in Japan will continue as fields of use as ingredients in the sea in Japan.

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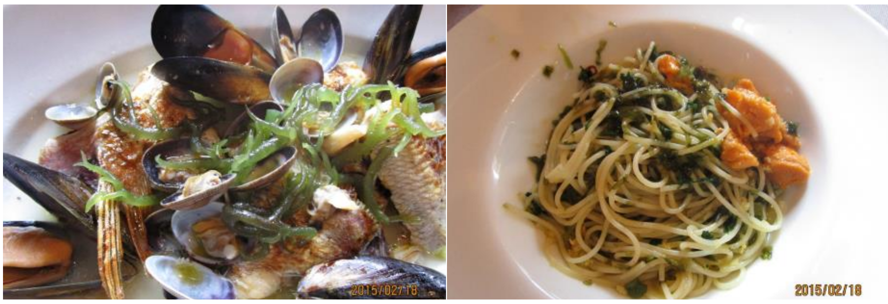

Fig 3 Italian1dishes with *K.alvaresii* materials

**Effects of seaweed liquid fertilizer (Bio-Stimulant)**

Kelp species live in cold waters and thrive in large areas of the world's oceans. Since the 1990s, liquid seaweed fertilizer has been produced by squeezing *Ascophyrum notodum* and *Ecklonia maxima*, which have grown in Northern Europe and South Africa. Became *K.alvarezii* juice was prepared by the following method.

About 60% of juice could be obtained by squeezing raw *K.alvarezii* fronds, and about 80% or more of juice could be obtained from their frozen fronds. The juice that was filtered from the raw material became clear and pale yellow-green. Excess juice from the frozen leaf becomes a pink liquid. This pink pigment is sensitive to light, and when stored at room temperature, it gradually fades to a pale, transparent yellow-green color similar to the juice of raw *K.alvrezii*. There was not difference in the fertilizing effect in the test examples up to row *K.alvarezii* liquid prepared in this way was transparent with a slight odor when stored at room temperature, and could be stored for a long period of time.

**Effect of *K. alvarezii* liquid**

**Flowers:**It was compared daisies of similar size with 100 cc of *K.alvarezii* liquid fertilizer (500-fold dilution) added once a week with no addition. The added strains had thicker stems and slightly longer leaves. The condition after 3 weeks is shown in the photograph(Fig. 4). A florist who sells cut flowers commented that adding a small amount of *K.alvarezii* liquid fertilizer to cut flowers would make them last longer.:

**Melon**: juice (500-fold dilution) was added to melon cultivation by greenhouse farmers from the time of planting seedlings. In melon cultivation, a plastic tube is run along the base of the melon, and a moderately small hole is opened in the tube. This time, *K.alvarezii* liquid fertilizer was added to the conventional liquid fertilizer once a week. As for the melon fruit, in the area to which juice was added, the fruits were enlarged more than in the area to which no juice was added. The pericarp was thin, sweet and soft enough

**Tomato:** Add K/alvarezii juice( 500-fold dilution )to the conventionally used liquid fertilizer and sprinkle it on the roots. Tomatoes have become sweeter and richer.

**Sweet potato**: Since the spring of 2020, an agricultural corporation in Okayama has been experimenting with sweet potato cultivation in sandy fields using *K.alvaezii* liquid fertilizer diluted 500 times. The growth of sweet potatoes is better than the non-fertilized area. The growth of the vine is remarkably good, and the growth of the rhizome is also having a good effect. Sweetness and umami are judged after the potatoes are dried and fully ripe (4-6 months after digging), but good results are expected.

*K.alvarezii* liquid fertilizer contains more potassium than other seaweed fertilizer, as shown in the table 1. It featured quite a few things. It is presumed that the fact that the potassium content is higher than that of kelp and sargassum species promotes the growth of roots, stems, and leaves as a liquid fertilizer, and increases the sweetness component. Iron is a component of chloroplasts and activates photosynthesis.Alginic acid from brown seaweed, which is a viscous polysaccharide, and carrageenan from red alga *K.alvarazi*i iswell when they absorb water, and have the property of forming a highly viscous aqueous solution. This property works effectively when soil improvement is carried out. When the content of the viscous polysaccharides in the soil increases, the water holding capacity increases, the soil moisture is stabilized, the propagation of useful microorganisms is promoted, and the soil becomes a good environment. In ordinary fertilizers, amino acids and enzymes are synthesized in the plant body from nitrogen components absorbed from the roots, and then phosphoric acid is combined to synthesize proteins and the like. After the amino acids of seaweed are absorbed as they are from the leaves and roots, they are immediately involved in the synthesis of proteins and the like. Seaweed liquid fertilizer containing amino acids promotes the growth of crops more efficiently than inorganic fertilizers. Plant hormones involved in plant growth play a very important role in the growth of plants in general, such as increasing flowering, promoting branching and elongation of roots and stems, improving cold resistance, promoting fruit maturation, and preventing aging. ing. Plant hormones are also necessary ingredients to produce amino acids. The kelp (*Ecklonia maxima)* liquid fertilizer collected in Cape Town is described as auxin (11 mg/L) and cytokinin (0.03 mg/L) (Rotmann KWG.2001)). Eucheuma muricatum grows at almost the same growth rate as these species, so it is speculated that *K.alvarezii* also contains a large amount of plant hormones.

Table. 1 Composition table of seaweed liquid fertilizer

1000g unit

| Content | *K.alvarezuii* | JapaneseKelp, *Laminaria* | *Sargassum hornri* | *Ascophylum nodosum* |
|---|---|---|---|---|
| moisture | 88.8g | 95 g | * | * |
| protein | 1.2g | 1.1 g | 1.62 g | 0.53 g |
| Lipid | 0.1 g | 0.1 g | 0.15 g | 0.24 g |
| carbohydrate | 5.3g | 6.4 g | 5.11 g | 7.33 g |
| Ash | 4.6g | 2.0 g | 3.0 g | 1.9 g |
| Sodium | 280 mg | 260 mg | 51.9 mg | * |
| Potassium | 1600 mg | 610 mg | 117 mg | * |
| Calcium | 13 mg | 78 mg | 18 mg | * |
| Magnesium | 32 mg | 53mg | 19 mg | * |
| Phosphorus | 16mg | 18mg | * | * |
| Iron | 0.7 mg | 0.3mg | * | * |

* :no data (*According to the materials of each fertilizer company)*

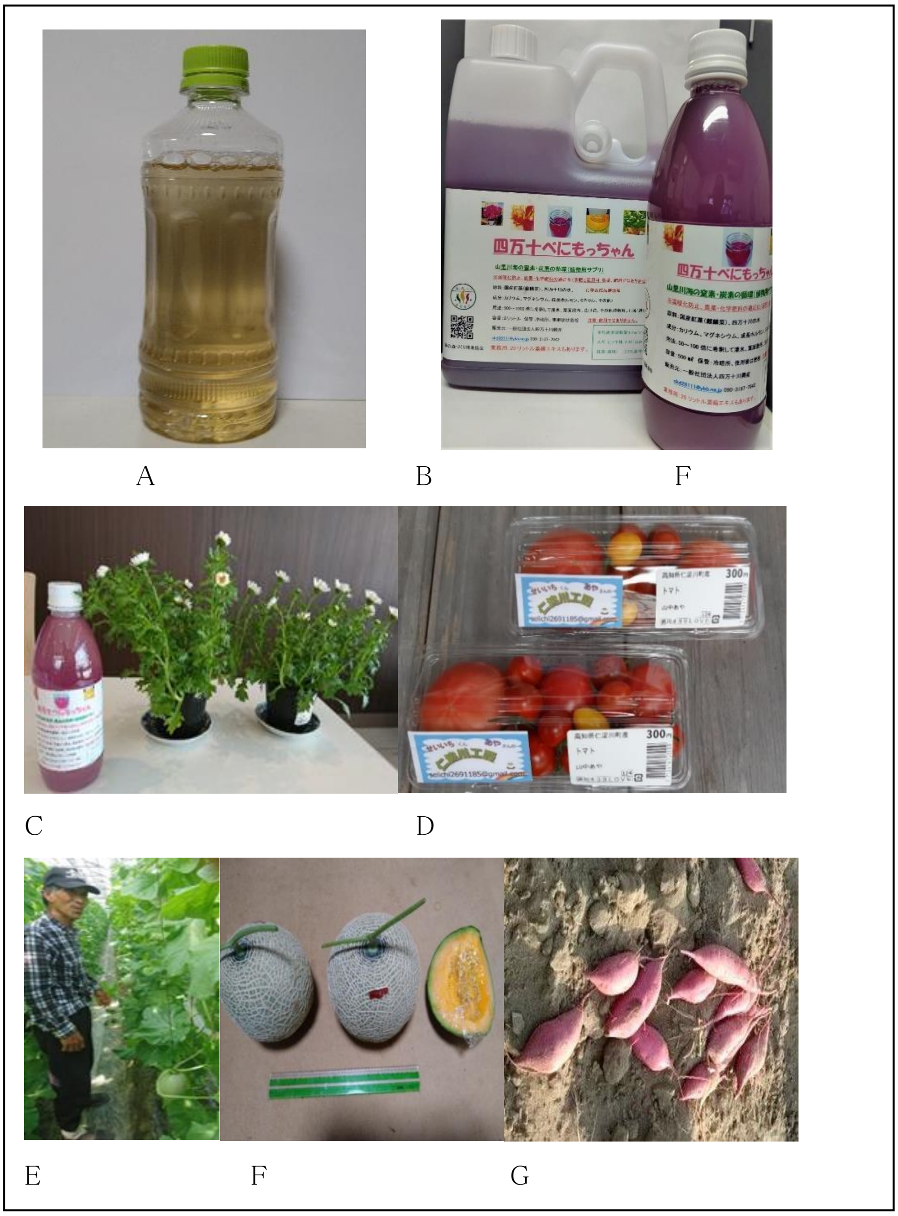


Fig. 4. *K.alvarezuii* juice: A; filtered *K.alvarezuii* juice; B; filtered juice of frozen *K.alvarezuii* fronds

C: Flowers: daisies, characterized by large leaves

D: Tomatoes: dark fruit color, sweeter

E; Greenhouse farming: Injecting liquid fertilizer at the base of the tube

D: Melon: Larger in size and sweeter

E; Sweet potato: bigger and sweeter

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