A THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

Development of growth-promoting substance for the healthy growth of diatom *(Navicula sp.)*

Junseong Kim

Department of Marine Life Sciences

GRADUATE SCHOOL

JEJU NATIONAL UNIVERSITY

08, 2015

CONTENTS

국문초록……....4

LIST OF FIGURES……6

LIST OF TABLES……..8

ABSTRACT…….……..9

INTRODUCTION…….…..……11

MATERIALS AND METHODS…….…….13

Diatom culture…….....13

Preparation of sample…….…..13

Measurement of chlorophylly-a……..…….14

Proximate composition of EBE…….…….……..14

Measurement of minerals from EBE……17

Separation the active ingredient from EBE……..17

Develop growth-promoting substances of diatom……...…….17

Statistical analysis……...…….18

RESULTS……20

Feed efficiency of benthic diatom ……23

Development of growth-promoting substance of benthic diatom……28

Growth confirmation of NPGS ……28

Confirmation of Separation the active ingredient from EBE……31

DISCUSSIONS……34

REFERENCES……37

국문초록

Navicula sp.는 전복치패 먹이로 사용 되어지는 대표적인 부착규조류의 하나로 양식장에 먹이부족으로 인한 전복 치패의 폐사율을 줄이기 위하여 국립수산과학원에서 현재 대량으로 배양중에 있다. 부착규조류는 전복 치패에 먹이로 전복치패가 파판에 부착하여 먹이를 먹는 시기부터 각장이 5mm가 되는 시기까지 규조 현존량이 큰 영향을 미치는 것으로 알려져 있어 꼭 필요한 요소이다. 현재 전복 양식장에서 부착규조류를 배양하는 방법으로는 PVC 파판에 자연 발생시켜 먹이로 사용하고 있다. 이는 부착규조뿐만 아니라 다른 종들이 같이 서식하기에 치패 성장에 많은 어려움을 겪고있다. 규조류는 전복치패 성장에 가장 중요한 성장 먹이로써, 규조류의 질과 양은 부착률, 변태율, 생존율 및 성장율의 가장 큰 영향을 미친다. 이에 전복치패에 건강한 성장을 위해 필수 요소인 규조류의 성장촉진제를 개발을 위해 연구 하였다. 규조류를 성장시키기 위해 선정한 첨가물질로 Alginic acid, fucoidan, *Scyosiphon lomentaria*, *Ecklonia cava*, *Ecklonia cava* boiled extracts (EBE), *Porphyra tenera*를 정하여 실험에 사용하였다. 가공 부산물인 EBE가 그 중 가장 뛰어난 활성을 보였으며, 규조류 성장 촉진 물질로서 충분히 가능성이 있었다. 또한 EBE에 eckol이 규조류에 함유되어 성장하는 것을 확인 할 수 있었다. 이

어지는 실험을 통해 현재 양식장에서 사용하고 있는 시판대조구들과 비교해도 규조류의 성장이 증가하는 것을 확인 하였다. 이에 EBE를 선정하여, 기존에 규조류 성장에 효과가 있는 질소, 인, 규소를 대체하는 인산비료, 질소비료, 규산나트륨을 EBE와 혼합하여 성장촉진 물질 개발에 사용한 결과, 더 효율 좋은 규조류의 성장을 확인 하였다. 이를 *Navicula* sp. growth-promoting substance (NGPS)라 임의로 명명하였다.

따라서, 이 연구에서는 직접적으로 규조류 성장에 영향을 미치는 규조류의 성장촉진 물질을 찾아 전복치패 단계에서의 건강한 성장과 폐사율을 줄일 수 있는 방안을 밝히고자 했다.

LIST OF FIGURES

Figure. 1. Extraction approaches of EBE using solvent-solvent partition chromatography

Figure. 2. In order to confirm growth of diatom, each samples were added into Petri dishes following as: 200µg of each sample (1 mg/ml), 20ml of f/2 medium and 200 µl of diatom. Valueshaving different superscripts are significantly different at P< 0.05.

Figure. 3. EBE treated-diatom were increased the growth. The growth of non-treated (control), EBE-treated (40, 100, 200, and 400x) diatom were analyzed in an microscope.

Figure. 4. To confirm the effect on growth of *Navicula* sp. with various concentrations of EBE, chlorophyll-a was analyzed. Valueshaving different superscripts are significantly different at P< 0.05.

Figure. 5. To confirm the effect on growth of *Navicula* sp. with various concentrations of PF, NM and SS, chlorophyll-a was analyzed. Standard error bars are shown

Figure. 6. In order to confirm growth of diatom, various concentrations of Mixture each samples were add. Standard error bars are shown.

Figure. 7. The growth effects of *Navicula* sp. of NGPS comparing with control (non-treated), MP 1, MP 2 and EBE.

Figure. 8. The growth of diatoms treated carbohydrate, protein, polyphenol and minerals purified from EBE. The growth effects of *Navicula* sp. of EBE comparing with control (non-treated), carbohydrate, protein, polyphenol and minerals

Figure. 9. Compare with treated EBE diatom and non-treated EBE diatom through UPLC (Ultra Performance Liquid Chromatography).

Fig. 10. *Ecklonia cava* analyzed by UPLC for confirm 6,6 - bieckol

LIST OF TABLES

Table 1. Chemical compositions of EBE (%)

Table 2. Minerals concentrations of the EBE

Abstract

Navicula sp. is one of the famous benthic diatom consumed by abalone. Currently the *Navicula* sp is cultivated in large scale by the National Fisheries Research & Development Institute (NFRDI) in order to decrease the mortality of abalone spat caused by prey scarcity.

Benthic diatom is known to have a strong influence on survivability between the stage of dashboard attached to the feeding and become shell length 5mm. Recently, benthic diatom are cultivated in abalone farms accretionary on PVC. But the efficient growth of abalone spat are affected due to the growth of the other unexpected species on the PVC. Thus, we explored the possibilities of growth-promoting substances of diatoms which allows the cultivation of the healthy abalone spats. Alginic acid, fucoidan, *Scyosiphon lomentaria*, *Ecklonia cava, Ecklonia cava* boiled extracts (EBE), *Porphyra tenera* were used this study to assess the growth promoting effect of *Navicula* sp..

Among the growth promoting substances used in his study, EBE exhibited the best growth promoting activity. With comparison to the market products used as growth promoters of diatom, EBE exhibited a significant increase in the growth of diatom. The effects of Phosphate Fertilizer (PF), Nitrogenous manure (NM) and Sodium silicate (SS) have previously been reported in other studies.

During this study attempts were made to identify the optimal composition of mixtures of PF, NM and SS with that of EBE to identify in the increase of growth of the diatom.

It was observed that the growth of *Navicula* sp. was enhanced by the *Navicula* sp. growth promotuing substance (NGPS) mixture compared with the existing market products.

Successfully, we were able to find a growth-promoting substance of diatoms that enables the cultivation of healthy abalones with reduced mortality.

1. INTRODUCTION

Abalone is an important and valuable aquaculture species throughout the world (Watson eta al., 2004). Among the estimated 100 abalone species 20 species are known as the catchable macrospecies which inhabit temperate waters. Mainly 5 species of abalone are cultivated in Korea, that includes *Haliotis divwesicolor* (relatively small in size), *H. gigantean*, *H. discus* and *H. sieboldii* that inhabit warm water currents, and *H. discus hannai* that grows in cold water. Abalone aquaculture has developed very rapidly in Korea. During the mid 1990s, abalone aqua culture have given an annual production of about 100 tons. Since then the yield has increased to an estimate of 9,000 tons by the 2012. Modernization of abalone aquaculture in Korea began during early 1970s, since then diatom feed stocks have been cultured by allowing diatom to naturally grow on PVC plates. According to recent studies, the major problems in abalone aqua culture have been identified as mass mortality and reduced productivity, etc. (NA., 1996). In spite of the increasing number of studies caring out about the nutrition of settled abalone juveniles, the growth and survival rate of abalones during the early juvenile stages still have some un solved issues (Gordon et al., 2006; Baek et al., 2003). One of the cause is that the growth of the juvenile abalones are affected based on their feed of diatoms. Success of commercial abalone production depends on their growth and survival. Recently interest has been devoted to improve success in culturing the highly vulnerable postlarval and early juvenile stages by focusing on their particular feeding requirements (Takamin et al., 1997; Kawamura et al., 1998 a,b,c; Daume et al., 2000; Watson et al., 2004). Benthic diatoms are considered as the main source of principal food for settled abalone and juveniles until they develop up to 3 mm shell length at which they can start to consume macroalgae (Kawamura et al., 1995; Daume et al., 2000; Watson et al., 2004;). Naturally

occurring benthic diatoms are cultivated in seed production hatcheries of abalone on plastic plates. These are then used as a primary source food for juvenile abalone (Kawamura et al., 1995). Benthic diatoms can be cultured and grow rapidly in certain conditions provided with necessary nutrition such as nitrogen, phosphorus and silica as well as the basic ecological factors such as illumination, water temperature and so on (Uriarte et al., 2006; Yang et al., 2014). Despite the importance of diatoms in abalone culture, the relationships between nitrate enrichment, protein content and dietary value have only been examined for a single benthic diatom species and a single species of abalone post-larvae. The edible marine algae including *Ecklonia cava*, *Porphyra* sp, and *Scyosiphon lomentaria* contains a high nutritional value with vitamins, minerals and other functional components such as phlorotannins, sulfated polysaccharids, and sterols. During this study, evaluations were made on the growth effect of benthic diatom administrated with various marine algae extracts as promoters of growth with certified chemical compounds that provide basic nutritional requirements of diatoms.

2.Materials and methods

2.1 Diatom Culture

Marine microalgae *Navicula* sp. was purchased from the Korea Marine Microalgae Culture Center (KMMCC). Medium used was f/2 (Guillard and Ryther, 1962), that was sterilized for 30 minutes at 121℃ prioriar to use. Diatom was cultured in under following conditions: 20℃, Lux 5,000, on a 12:12 h L: D photo cycle (Watsom et al,.2004).

2.2 Preparation of samples

Alginic acid, fucoidan, *Scyosiphon lomentaria*, *Ecklonia cava*, *Ecklonia cava* boiled extracts (EBE), *Porphyra tenera*, market product 1 (MP 1) and market product 2 (MP 2) were used as the Benthic diatom feed. Each 200 μg of sample was treated into Petri dishes following, 20ml of 1 mg/ml f/2 medium and 200 μl of diatom.

2.3 Measurement of chlorophyll-a content

To determine the chlorophyll-a, first, diatoms are collected using centrifugation. After the centrifugation, supernatants were removed and 10 mL of 90% acetone was added into each of the collected diatoms. Then, the samples were sonicated for 1 hour in order to break down the cell walls and kept under 4℃ in dark for 24 h. After the incubation period, the fluorescence was observed at 642.5/660 nm using black well plates as an indication of diatoms with removed the cell walls.

2.4 proximate composition of EBE

Approximate chemical composition of the marine microalgae was determined according to AOAC method (1990). Crude carbohydrate was determined by phenol-sulfuric acid reaction (absorbance at 480 nm, using glucose as the calibration standard), crude lipid content was determined using Soxhlet method and the crude ash content was determined by incinerating at 550℃ in the dry-type furnace for 6 hours. The amount of crude protein was determined by Lowry method, measuring absorbance at 540 nm using bovine serum albumin as the calibration standard (Lowry et al., 1951) (Table. 1).

2.5 Measurement of minerals composition of EBE

First, EBE was freeze-dried for 24 h and the minerals content was measured using wet digestion method. Minerals were detected with the use of ICP (Inductively Coupled Plasma: Lactain 8440 Plasmalab) (Kim et al.,1997) (Table. 2).

Components	Moistures	Ash	Carbohydrates	Proteins	Lipids
Contents (%)	**4.1**	**20.2**	**66.2**	**9.4**	**0.1**

Table .1 Chemical composition of EBE (%)

Test items	Standard	Results	Unit	Method
Boron	**-**	**18.94**	**mg / 100g**	**Korean Food StandardsnCodex9.1.2.1**
Phosphorus	**-**	**74.14**		
Zinc	**-**	**2.62**		
Copper	**-**	**1.56**		
Magnesium	**-**	**728.45**		
Manganese	**-**	**4.62**		
Iron	**-**	**8.94**		
Potassium	**-**	**3115.95**		
Calcium	**-**	**532.21**		
Sodium	**-**	**2104.73**		

Table 2. Concentrations of minerals in EBE

2.6 Separation of active ingredients from EBE

EBE was separated to determine the impact on diatom growth. The EBE (500 mL) was mixed well with 1.0 L of 99.5% ethanol at 4 ℃. After 24 h, the supernatant liquid was collected by centrifugation at 12,000 × g for 20 min at 4℃ (Reference). To obtain the polyphenols and minerals, the supernatant liquid was mechanically stirred with ethyl acetate for 24 h at room temperature. In addition, sediment fraction was mixed with ammonium sulfate to obtain the proteins and carbohydrates, respectively. Proteins and carbohydrates were lyophilized for further experiments.

2.7 Developing growth-promoting substances of diatom

Phosphate fertilizer (PF) (Haifa Chemicals Ltd.), Nitrogenous manure (NM) (Namhae chemical) was purchased from NH (NongHyup), Sodium silicate (SS) was purchased from the Daejung Chemicals & Metals LTD. ESL was purchased from Taerim LTD. Synthesis of growth-promoting substances for optimal condition.

2.8 UPLC analysis

A Dionex UltiMate 3000 UPLC system (Dionex, Sunnyvale, CA, USA) and a Thermo Scientific Q Exactive mass spectrometer with a heated electrospray ionization source (HESI) (Thermo Fisher Sci-entific, Bremen, Germany) were employed for the analysis of theoligosaccharide. Chromatographic separation was performed using a Waters ACQUITY UPLC BEH Amide column (2.1 mm × 150 mm; Waters Corporation, Milford, MA, USA).

(Tie et al., 2015). The mobile phase was acetonitrile – water in gradient mode as follows : acetonitrile – water (0 min ~ 5 min : 95:05 v/v, ~ 10min : 70:30 v/v, ~ 15min : 60:40 v/v, ~ 18min : 40:60 v/v, ~ 25min 100:0 v/v).

2.8 Statistical analysis

All the measurements were made in triplicate and all the values were represented as mean ± SEM. The results were analyzed using an ANOVA test. $P < 0.05$ was considered significant

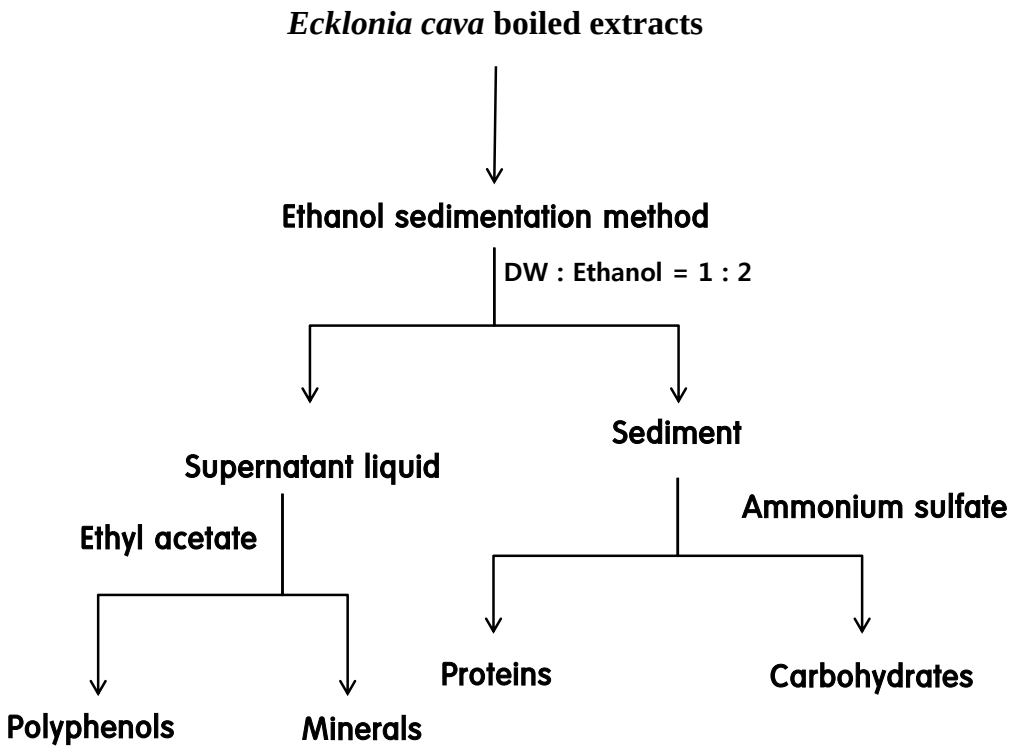

Fig. 1. Extraction approaches of EBE using solvent-solvent partition chromatography.

3. Results

3.1 Feed efficiency of benthic diatom

Benthic diatom *Navicula* sp. was prepared at KMMCC and Wando for a comparative analysis of growth efficiency as fed benthic diatom. (Fig. 2). *Navicula* sp. was purchased from at KMMCC. As shown in Fig 2A, Alginic acid and EBE indicated a higher growth rate than that of control. Among them, EBE showed the highest growth rate of 154% compared with the control group. Especially, this value is better with comparison to the market product of growth-promoting substance. These data indicate that EBE affects the growth of benthic diatom.

As shown in Fig 2B, Navicula sp. harvested from wando growth rate of *Scytosiphon lomentaria*, EBE, *Porphyra tenera*, and market control increased higher than that of control. Among them, EBE showed the highest growth rate of 184% compared with the control group. Especially, this value is better than that of the MP1, growth-promoting substance. These data suggests that EBE affects the growth of benthic diatom. This tendency equally identified EBE affects the growth of benthic diatom at KMMCC and Wando.

According to the microscopic observations made using magnification levels of 40x, 100x, 200x, 400x EBE exhibited the highest growth rate (biomass and density) compared with the control group (fig. 3). These results suggests that EBE, possess growth-promoting activity on benthic diatoms.

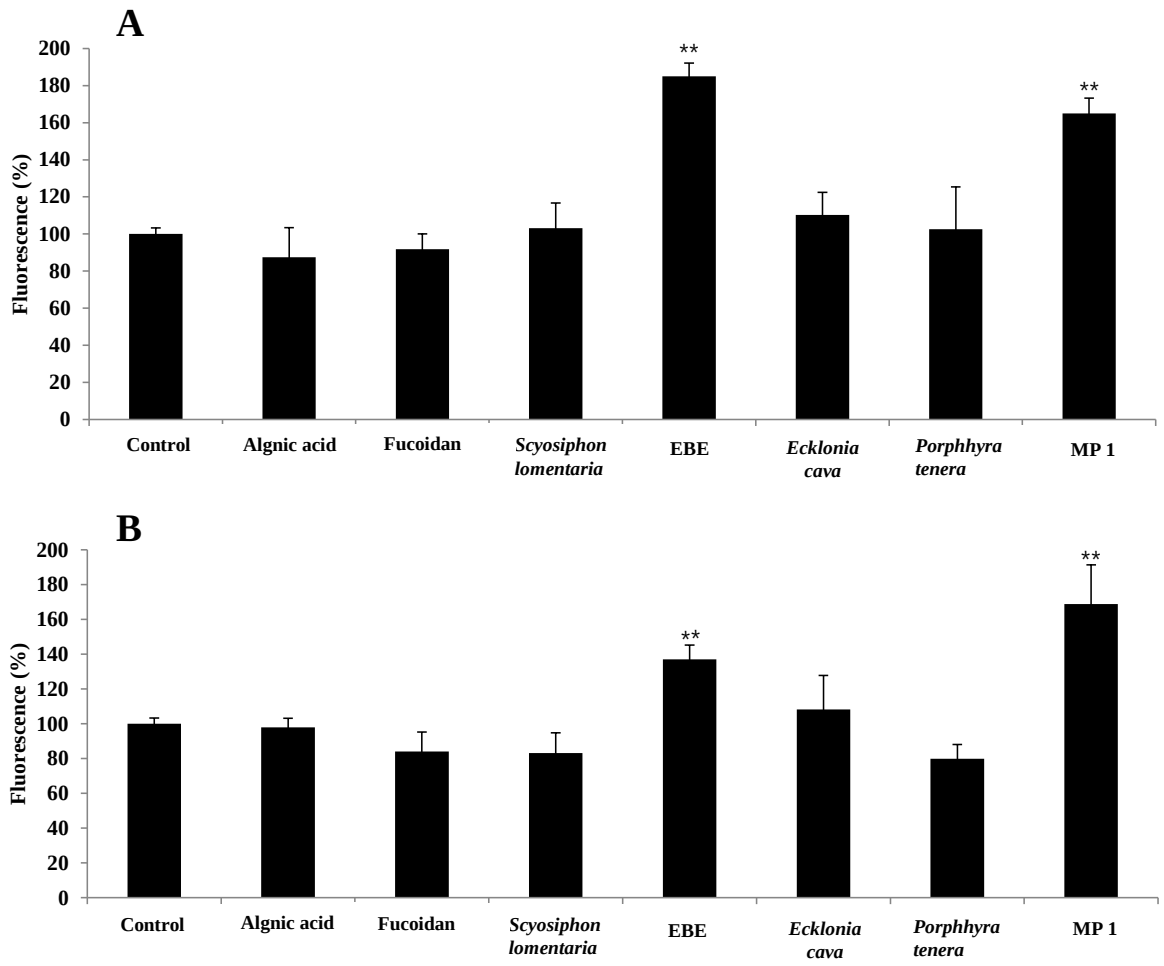

Fig. 2. In order to confirm growth of diatom, 200µg of each sample was treated into Petri dishes with 20ml of 1 mg/ml f/2 medium and 200 µl of diatom. Values having different superscripts are significantly different at $P< 0.05$.

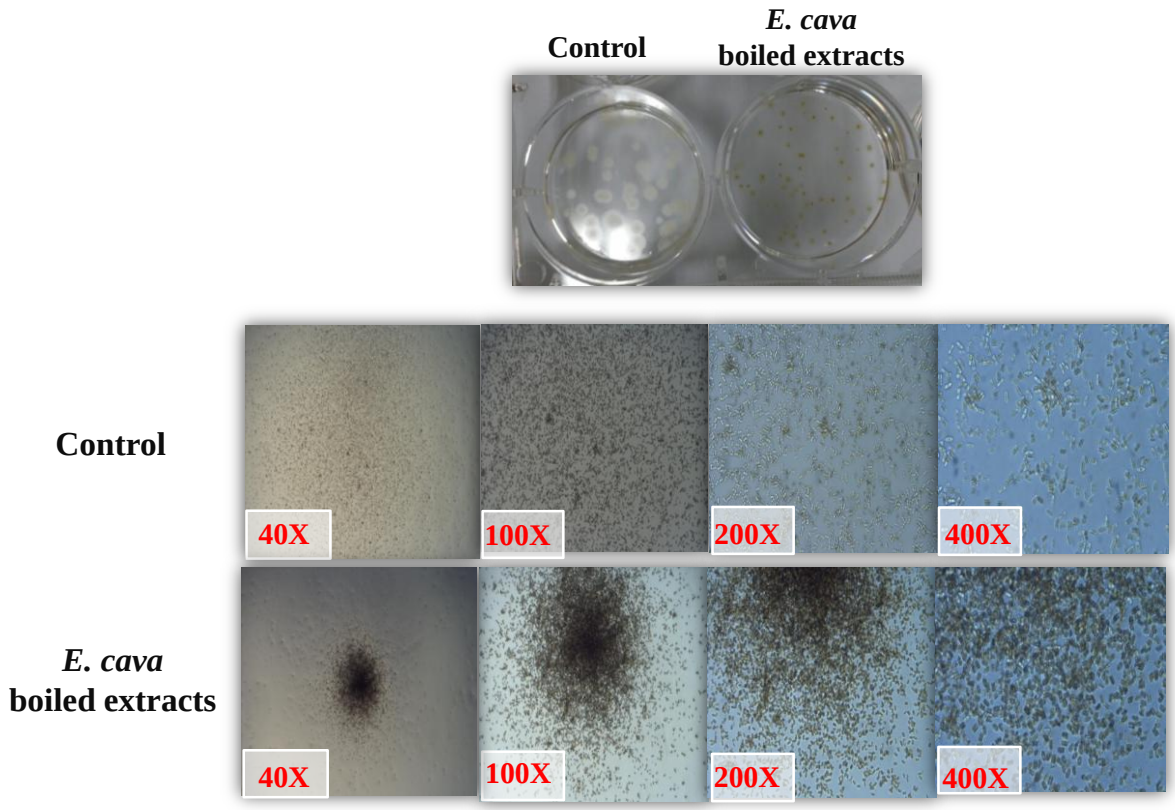

Fig. 3. An increased growth was observed for EBE treated-diatom compared to the growth of non-treated (control) diatom under microscopic magnification levels of 40, 100, 200, and 400x.

3.2 Development of growth-promoting substance of benthic diatom

Results for the growth promoting activity of benthic diatom with the treatment of growth-promoting substance EBE and MP 1 are shown in Fig. 4. EBE was treated into Petri dishes with concentrations of 0.1 mg/ml, 0.5 mg/ml, 1 mg/ml. These results indicates a growth of 101 %, 114 % and 165 % respectively for the concentrations of 0.1mg/ml, 0.5 mg/ml and 1 mg/ml. EBE shows the highest growth rate of 184% compared with the MP 1 under the same concentration. Additionally the growth of the diatoms increase with increasing concentrations of EBE. These data shows that the identified EBE affects the growth of diatom. In addition, development of growth-promoting substance with added phosphate, nitrogen and silica is found to be an effective growth promoter for diatom (Daume et al., 2003). Use of this growth promotory substance replaces the use of expensive phosphate, nitrogen, silica market products with PF, NM, SS. Therefore use of this novel growth promotory substance benefit the mass cultivation of abalone in an industrial scale.

PF, NM, to determine the appropriate concentration of the mixture of the SS and the EBE Experimental results for each concentration as follows (Fig. 5). Results confirmed for each concentration, all the graphs 1mg/ml is the most active were excellent.

As shown Fig. 6, EBE and MP1 indicated an increased growth of 145% and 117%respectively at concentrations of Growth of mixtures of EBE, PF and NM indicated a growth of 244%, whereas mixture of PF and NM indicated an increase growth of 172.9%. In addition, the growth of diatoms was high in EBE mixed with PF, NM and SS (306.5%). When the concentration of SS was increased up to 2mg/ml, we observed that the growth of diatoms increased slightly (310.2%). Also, the growth of diatoms in MP1 mixed with NM, PF

and SS indicated an increase in growth (267.0%). SS at the concentration of 2mg/ml indicated a slight increase in growth (270.2%). Although SS is considered as a necessary growth factor for diatoms, no differences was observed for the increase of growth with increasing concentrations of SS. The highest level of growth was observed for the EBE mixed with PF, NM and SS each at the concentration of 2mg/ml. But we confirmed that the most efficient concentration of SS was 1mg/ml according commercial specifications as only a little difference of growth was observed between 1mg/ml and 2mg/ml concentrations of SS.

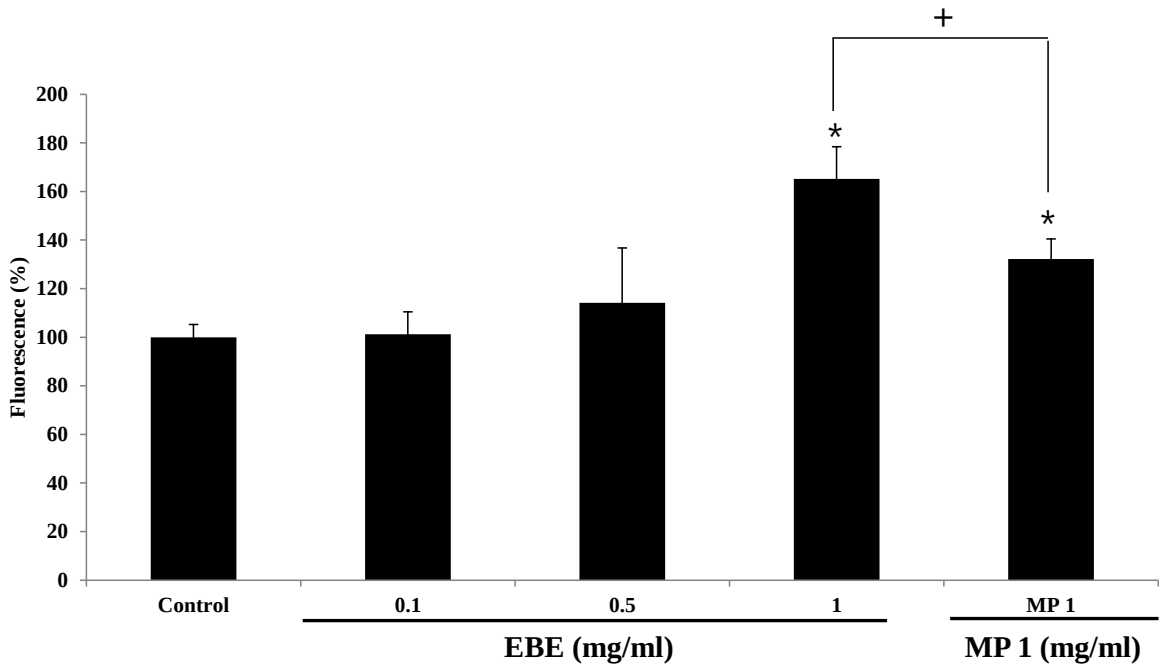

Fig. 4. Effect on growth of *Navicula* sp. with various concentrations of EBE and MP at the concentration of 1 mg/ml fluoresence intensity of chlorophyll-a at the wavelength of was analyzed as a measurement of the amount of diatom. Values marked by * denote a significant difference at $P< 0.05$.

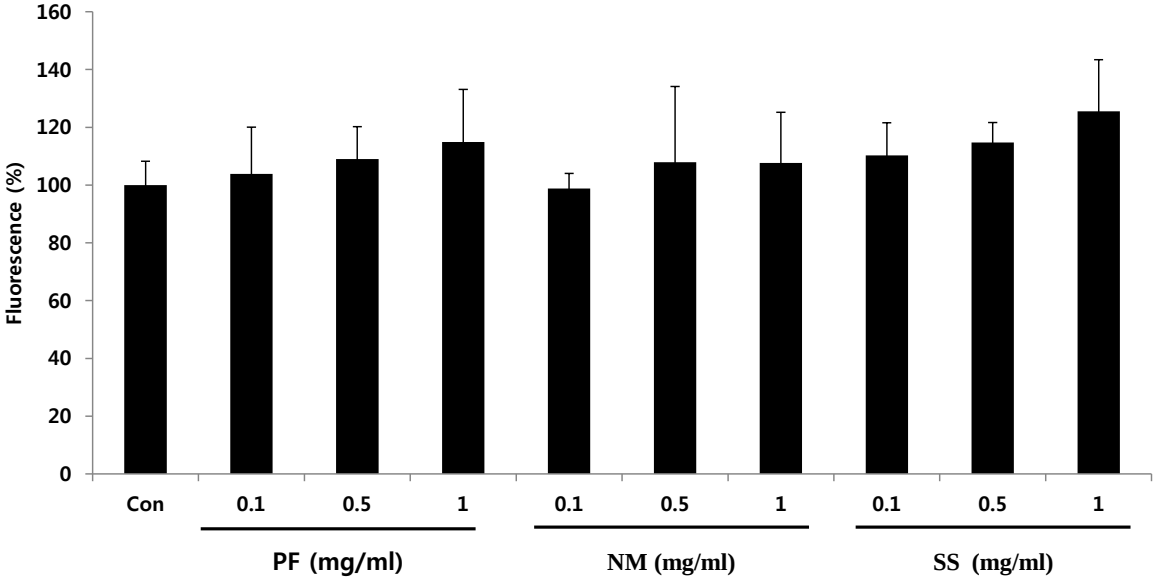

Fig. 5. Effect on growth of *Navicula* sp. with various concentrations of Phosphate-fertilizer(PF), Nitrogenous manure(NM) and sodium silicate(SS). Fluoresence intensity of chlorophyll-a at the wavelength of was analyzed as a measurement of the amount of diatom. Standard error is represented by the error bars.

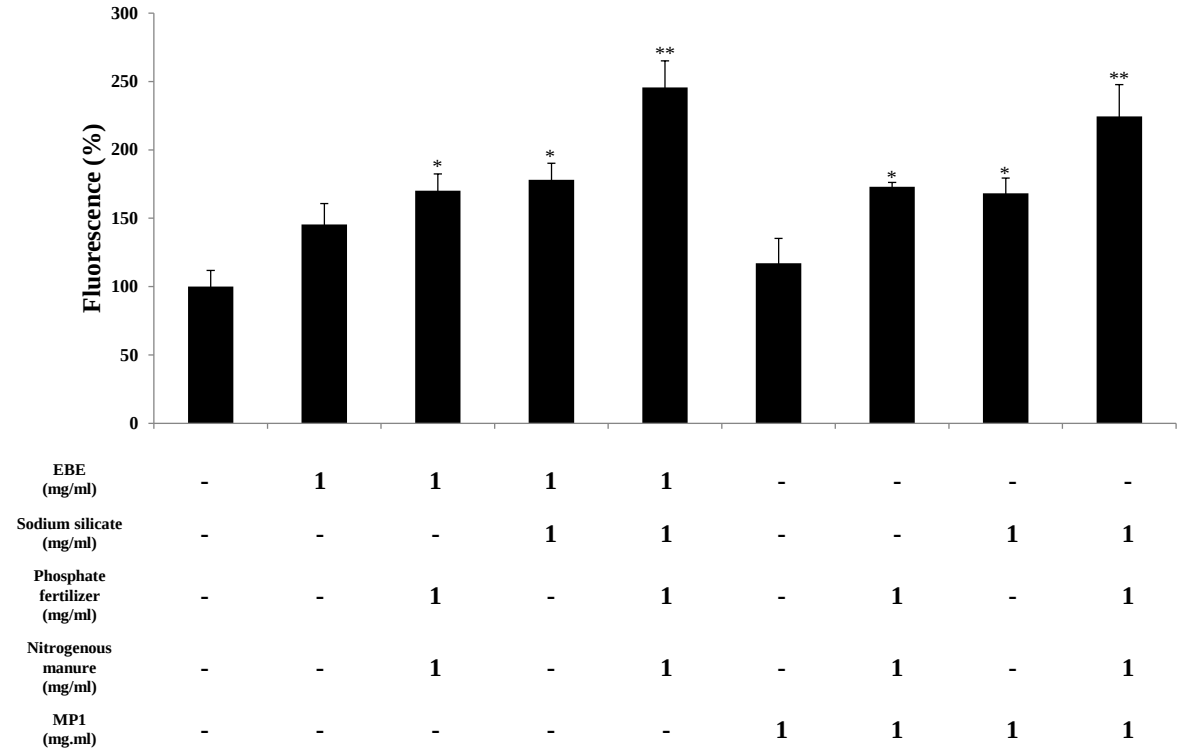

Fig. 6. In order to confirm growth of diatom, various concentrations of Mixture each samples were add. Standard error bars are shown.

3.3 Confirmation of the Growth effectof NPGS

The results for the growth of diatoms treated with NPGS are indicated in Fig .7. MP1 and MP2 are two of the marketed products recently used in commercial abalone farms in Wando. According these results the growth of diatoms with treated MP1 (133.88%) and MP2 (151.79%), indicates a relatively low growth compared to EBE (164.62%) and NGPS (231.21%) treated group. In other words, EBE indicates higher efficiency of growth compared to existing market products. Especially, the activities in NGPS mixed material was the most.

3.4 Separation of the active ingredient from EBE

Figure 8 indicates the growth of diatoms treated with carbohydrate, protein, polyphenol and minerals purified from EBE. The growth of diatoms respectively indicates values of 188.55%, 112.21%, 102.41% and 101.11% for EBE, carbohydrate, protein, polyphenol and minerals. These results suggests that the growth of diatom in minerals indicates similar level as in EBE.

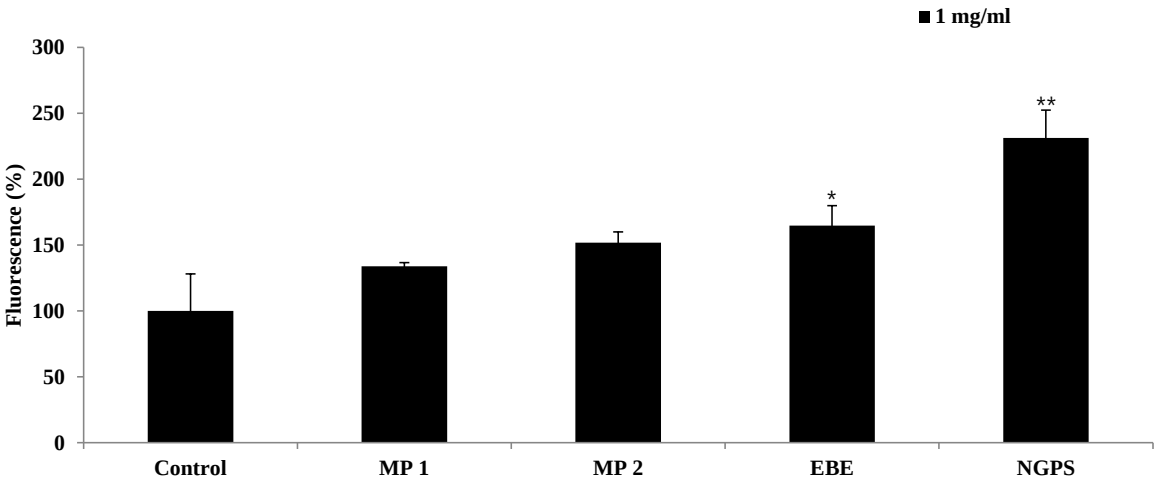

Fig. 7. The growth of *Navicula* sp. treated with MP 1, MP 2 EBE and NGPS. NGPS indicates an increased growth compared with the control (non-treated).

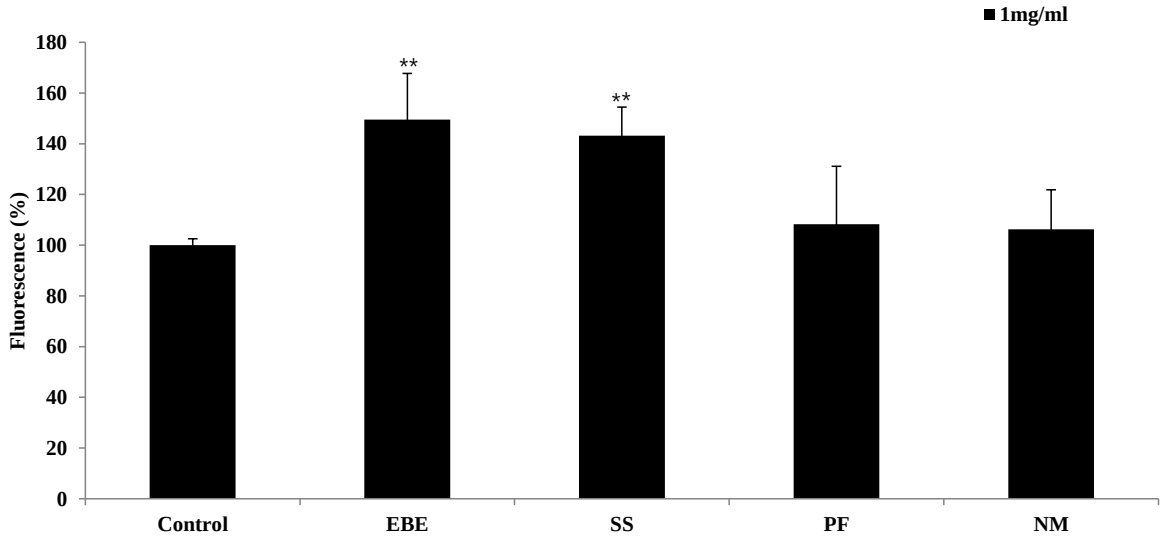

Fig. 8. The growth of diatoms treated with carbohydrate, protein, polyphenol and minerals purified from EBE. The growth effects of *Navicula* sp. of EBE comparing with control (non-treated), carbohydrate, protein, polyphenol and minerals.

3.5 Confirm of functional components of EBE

We have identified at growth of diatom by component in EBE. Therefore, separate of proteins, carbohydrates, minerals and polyphenols from EBE. This result of confirmed at after separate, there is an effect on growth in minerals.

3.6 UPLC analysis of EBE, treated EBE diatom and non-treated EBE diatom

A comparison of UPLC data between diatoms treated with EBE (B), non-treated EBE (C) and EBE (A) are shown in Fig. 9. EBE is treated in diatom for growth comparison at treated EBE and non-treated EBE. According to UPLC data one of the active ingredient in diatoms treated with EBE was estimated as 6,6 – bieckol. EBE treated at diatom UPLC was confirmed in the substance to be estimated 6,6 – bieckol. Additionally the UPLC peak corresponding to bieckol confirmed the presence of the compound in *E.cava* (Fig. 10). This result, comfirmed while diatom growth condense as 6,6 – bieckol.

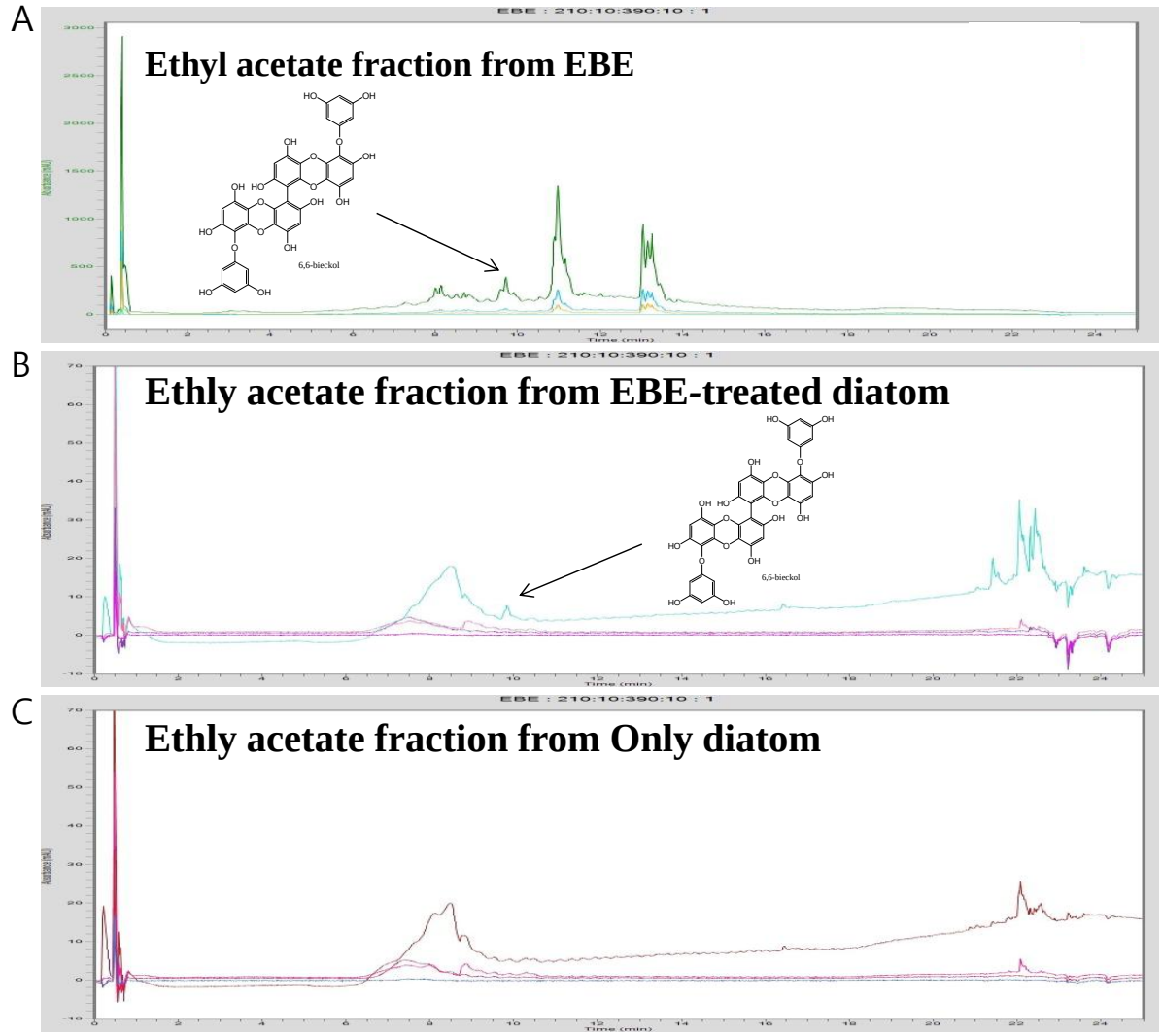

Fig. 9. Comparison between the UPLC (Ultra Performance Liquid Chromatography) data for ethyl acetate fractions of diatom treated with EBE, non-treated EBE and EBE.

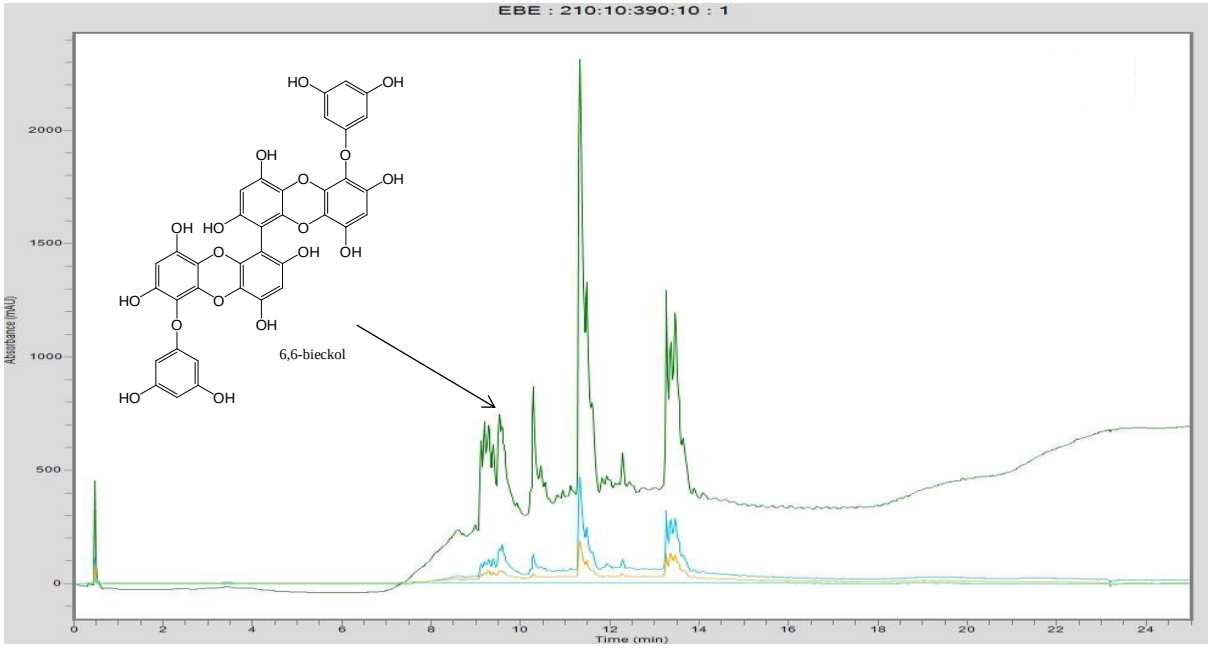

Fig. 10. *Ecklonia cava* analyzed by UPLC for the confirmation for the presence of 6,6 - bieckol.

4. Discussion

Abalone farms in Korea is maintaining their demand at a high level as National Fisheries Research & Development Institute (NFRDI) succeeded in producing the artificial seed since 1970 (Han et al., 2006). Although abalone farms in Korea have the best techniques around the world, the major problem of spat mortality haven't been solved. To sustainably, Adhesive diatoms are well known as a essential growth factor for the production of artificial seeds for the abalone (NA, 1996).

The juvenile abalones are known to feed on benthic diatoms until they reach 5mm in size. Recent evidence suggests that abalone mortality have increased up to 40% due to the lack of sufficient diet. Therefore, the growth of diatom that affects the settlement, survival and growth rate of abalone is of utmost importance for the severability of juvenile abalones (Kin et al,.). In this regard we investigated on finding growth accelerators of diatoms to decrease the mortality of juvenile abalones. Evidently, EBE was found to be a suitable material for the growth acceleration of diatoms in terms of commercial aspect. Although the EBE extracted from *Ecklonia cava* is the remaining water solution for desalination and rinse using steam, that contains a lot of nutrients in the *Ecklonia cava*. Thus the EBE has a lot of application possibilities despite of by-product.

According to the findings of this study EBE had a pronounced effect on the growth of diatoms. It was undoughtily proved that treatment of EBE, increased the number of diatoms as well as it’s growth rate.

It was assumed that the prey for the abalone is more plentiful higher the density of diatoms. Therefore the abalone can feed on more quantity of diatoms compared to non-EBE treatmed

group. Therefore, this method allows to decrease the mortality of juvenile abalones. Also, these findings suggests that the growth of diatoms in EBE increased compared with existing market products. A much faster growth was observed after introducing number of minerals to the EBE.

The EBE was found to contain 66.2% carbohydrate and minerals. Additionally, fucose that is the principle ingredient of Fucoidan in these carbohydrates was estimated as 35.68 %. Generally, the use of market products such as cheap fertilizers which contain essential minerals affects the growth of diatoms. But these market products have relatively low concentration of Fucose in comparison to EBE mixture. As a result, EBE can be employed as an effective mixture to increases the growth rate of diatoms. In this regard EBE can provide diatoms with growth factors through biomagnification and the resulting juvenile abalones that feed on those diatoms may become high-quality spats.

The biomagnification effects of PF, NM and SS have already been reported in several other studies. During this study a method was optimized to use a mixture of PF, NM and SS with EBE to identify the growth increase of abalone. The diatoms treated with NGPS mixture had an increased growth rate compared with existing market products.

The presence of eckol in diatoms was confirmed of through UPLC. 6,6 - bieckol is has previouslly been reported as an effective antibacterial (Fukuyama et al,. 1989), antioxidant (Kang et al,. 2004), anti-inflammatory (Jung et al,. 2009) and anticancer (Hwang et al,. 2006) natural product. Abalone spat fed with these diatoms, have benefitial effects on human.

Although these experiments were carried out under the controlled conditions in the laboratory it is thought that these novel findings are applicable to reduce the mortality of abalone in

farms. Further studies are required to determine the essential minerals that directly affects the growth of these diatoms and the active component of EBE that enhance the growth of abalone spat.

References

Hyoung-Kyun Han and Sung Bum Hur. (2000). Dietary value of benthic diatoms for newly settled abalone, *Haliotis discus hannai* Ino. J aquaculture, 13, 153-161

Jae Min Back, Chul Won Kim, Sang Goo Lim and Chan Sun Park. (2003). Effect of benthic diatoms on the Settlement rate of larvae and surval and growth of juvenile abalone (*Haliotis discus gannai*). J. Kro. Fish. Soc. 36, 591-595

Gui-hwan Na. (1996). Annual Variation of chlorophyll a and pheopigments of the benthic microalgae on the corrugated PVC sheets for abalone seedlings. J. Aquaculture, 9. 65-71

Joon-back Lee, Jong-Hun Choa and Il-Soo Kim. (1991). Distribution and species composition of periphytic diatom around the coast of cheju island. Cheju Nat. Univ., 15. 61-72

Mi-Hee Chung and Chung-Il Choi. (2001). The epiphytic forms of diatoms on seagrass. J. Natural Science and Technology. 3. 145-152

Miao Yang, Wen Zhao and Xi Xie. (2014). Effects of nitrogen, phosphorus, iron and silicon

on growth of five species of marine benthic diatoms. J. Acta Ecologica Sinica. 34. 311-319

Soon-Kyung Kin and Ae-Jung Kim. (1997). The study on the Amount of Trace Elements in some fermented fish products(jeot-gal) from some areas of the west xoast in korea. J. Korean Soc Food Sci Nutr. 26. 1063-1067

D.Watson, S. Daume, J. Prince, L. Veazley, B. Knott. (2004). The influence of light intensity on the density of different diatoms as feed for juvenile greenlip abalone (*Haliotis laevigata*). J. Aquaculture. 235. 345-359

Seung-Hyern Kim, Mi Jeong Kim, Se Jin Park and Sung Bum Hur. (2013). Effect of the concentrated-diatom of *Caloneis schroederi* and *Rhaphoneis* sp. for seedling production of *Haliotis discus hannai*. J. Korean Malacol. 29. 197-205

D. Watson, S. Daume, J. Prince, L. Beazley, B. Knott. (2004). The influence of light intensity on the density of different diatoms as feed for juvenile greenlip abalone (Haliotis laevigata). J. Aquaculture. 235. 345-359

Iker Uriarte a, Rodney Roberts, Ana Farías. (2006). The effect of nitrate supplementation on the biochemical composition of benthic diatoms and the growth and survival of post-larval abalone. J. Aquaculture. 261. 423-429

Tomohiko Kawamuraa, Takahiro Saidob, Hideki Takami, Yoh Yamashita. (1995). Dietary value of benthic diatoms for the growth of post-larval abalone *Haliotis discus hannai*. J. Experimental Marine Biology and Ecology. 194. 189-199

Nurit Gordon a, Amir Neori a, Muki Shpigel a, John Lee b, Sheenan Harpaz. (2006). Effect of diatom diets on growth and survival of the abalone Haliotis discus hannai postlarvae. J. Aquaculture. 252. 225-233

Fukuyama Y, Kodama M,Miura I, Kinzyo Z, Kido M,Mori H, et al (1989). Structure of an antiplasmin inhibitor, eckol, isolated fromthe brown alga *Ecklonia kurome* Okamura and inhibitory activities of its derivatives on plasma plasmin inhibitors. Chem Pharm Bull(Tokyo) 37. 349–53.

Hye Sook Kang, Hae Young Chung, Ji Young Kim, Byeng Wha Son, Hyun Ah Jung and Jae Sue Choi (2004). Inhibitory phlorotannins from the edible brown alga *Ecklonia stolonifera* on total reactive oxygen species (ROS) generation. Arch Pharm Res 27.194–8.

Won-Kyo Jung,Young-Wook Ahn, Sang-Hoon Lee, Yung Hyun Choi, Se-Kwon Kim, Sung SuYea, et al (2009). *Ecklonia cava* ethanolic extracts inhibit lipopolysaccharide-induced cyclooxygenase-2 and inducible nitric oxide synthase expression in BV2 microglia via the MAP kinase and NF-kappaB pathways. Food Chem Toxicol. 47.410–7.

Hyejeong Hwang, Tong Chen, Ronald G. Nines, Hyeon-Cheol Shin, Gary D. Stoner (2006). Photochemoprevention of UVBinduced skin carcinogenesis in SKH-1 mice by brown algae polyphenols. Int J Cancer 119.2742–9.