



Uptake of Nutrients N, P, K Seaweed of *Kappaphycus alvarezii* (Doty) Were Cultivated in Media with Different Concentrations of BAP

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Abstract

The results showed that in the first period the cultivation, the highest uptake of T-N ($0.132 \pm 0.06 \text{ ppm g}^{-1}\text{d}^{-1}$) was observed in concentration of 3.0 ppm and not significantly different from other treatments. Highest uptake of T-P ($0.143 \pm 0.04 \text{ ppm g}^{-1}\text{d}^{-1}$) was detected in a concentration of 2.0 ppm and significantly different from the control treatment and a concentration of 4.0 ppm. The highest K^+ ($3.05 \pm 0.91 \text{ ppm g}^{-1}\text{d}^{-1}$) uptake was observed in the treatment of 3.0 ppm which significantly different from the control treatment and a concentration of 1.0 ppm. In the second period the cultivation, the highest uptake of T-N ($0.19 \pm 0.07 \text{ ppm g}^{-1}\text{d}^{-1}$) was obtained in the treatment concentration of 2.0 ppm and not significantly different from other treatments. The highest uptake of T-P ($0.18 \pm 0.05 \text{ ppm g}^{-1}\text{d}^{-1}$) was observed at a concentration of 2.0 ppm and significantly different from the control treatment and a concentration of 4.0 ppm. Meanwhile, the highest uptake of K^+ ($3.08 \pm 1.14 \text{ ppm g}^{-1}\text{d}^{-1}$) was found in the treatment of 3.0 ppm which significantly different from other treatments.

Keywords: Concentration; 6-benzylaminopurine; *K. alvarezii*; nitrogen uptake Phosphor and potassium.

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1. Introduction

Often, essential nutrients for seaweed growth are low in the water thus affecting growth. Therefore, these elements are found to be low in the seaweed tissue. Nutrient requirements of seaweed are divided into three categories, namely: macro nutrients such as: C, N, P, K, Mg, Ca, S, H, O and micronutrient Fe, Mn, Zn, Cu, B, Mo, Cl and vitamins such as cyanocobalamine (B12), thiamin and biotin. The most important macro nutrients to support the growth of seaweed are C: N: P and the concentration must be in a balanced ratio [1].

Nitrogen (N) is the building blocks of amino acids (proteins), enzymes, nucleic acid, nucleoprotein and alkaloids. N deficiency will limit cell division and enlargement. Phosphorus (P) plays an important role in the process of photosynthesis, respiration, transfer and storage of chemical energy in the form of ATP, cell division and enlargement, as well as carbohydrate metabolism and acts as an activator of enzymes in the cycle as Calvin [2]. While potassium (K) plays a role in regulating the rate of photosynthesis, activates an enzyme involved in the production of ATP. K^+ deficiency resulted in low production of ATP, thus all processes associated with the ATP to be slow [3].

In red algae as *K. alvarezii*, potassium is indispensable in the formation of gel [4]. The gel strength kappa carrageenan types for their potassium ions [5] and produces a gel structure that is stronger and stiffer than iota carrageenan [6]. Kappa carrageenan extracted from *K. alvarezii* containing potassium ranged from 7.12 to 17.33% [7].

Very difficult to visualize a microscopic layer of water that come into contact with the surface of the thallus in which cells interact with water. Movement of water and the form of nutrients concentration gradient on the thallus surface become factors that inhibit nutrients uptake by the seaweed. However, the concentration gradient of nutrients and water movement should be sufficient for seaweed to occurrence of a nutrient uptake of water into the cells of the seaweed [8].

Cytokinin is a group of specific hormones that play an important role as a regulator of growth and stimulate protein synthesis and cell cycle [9], cell division (cytokinesis), differentiation and cell development and growth of plants [10,11]. Controlling settings biological processes associated with metabolism, activity growth, nutrient uptake and translocation of nutrients in plant tissue so as to prevent the plant from environmental stress, improve water use efficiency and photosynthesis [12,13,14] and at high concentrations, cytokines inhibit the biological and physiological processes associated with plant growth when used in high concentrations [15].

One of a class of aromatic cytokinins that have high activity, are stable and resistant to oxidation is 6-benzylaminopurine (BAP). BAP cytokinin type is very effective to stimulate a variety of physiological and metabolic processes of plants [12,13]. Therefore, the objective of the research was to determine the effect of growth hormone 6-benzylaminopurine (BAP) varied concentrations on the uptake of nutrients T-N, T-P and K^+ of seaweed *K. alvarezii*.

2. Materials and Methods

2.1. Seaweed Preparation

Type of seaweed used as seed is *K. alvarezii* collected from the cultivation by seaweed farmers. Healthy seaweed with a uniform size with a diameter of thallus (± 0.6 cm) was selected. Initial weight of each clump of seeds is 50 g. All thallus used as seed is washed with sea water and then sterilized with antibiotics (tetracycline) and 0.25 ppm (chloramphenicol) 0.25 ppm [16].

2.2. Laboratory Culture

The sea water is used first disinfected with 60% chlorine concentration of 30 ppm and aerated for 48 hours. Once it is added thiosulfate 10 ppm to ensure free of residual chlorine. Fifteen of 15 L capacity containers were used for the trial. Designed specifically for recirculation system thereby create a continuous water turbulence as where the natural conditions at sea. Each container was filled with maximum water are 15 L and added Conway fertilizer each 2.0 ppm as additional nutrients to *K. alvarezii*. Then, the growth hormone 6-benzylaminopurine (BAP) was added to the media in accordance with the treatment being tested in this study (see the experimental design).

Cultivation is carried out for six days and was divided into two periods of each of the three days of the first period and three days of the second period. Before cultivation performed water sampling to determine the initial concentration (X_i) nutrients in the cultivation media and after three days to do more water sampling to determine the final concentration (X_t) nutrients in the cultivation media. Furthermore, water changes were made in total on cultivation media and do the same thing in the second period. During cultivation of seaweed in the laboratory used a light source lamp tube (TL) capacity of 20 watts as much as 6 pieces with an intensity between 1800-2100 lux meter and the lighting period is divided into 12 h L : 12 h D. Temperature of media was controlled in the range of 28-30°C and salinity in the range of 30-33 ppt.

2.3. Nutrient uptake measurement

Variable uptake observed: Total nitrogen (T-N), total phosphate (T-P) and potassium (K^+). T-N concentration measurements and T-P in water samples is done by using a spectrophotometer in the laboratory of water quality FIKP Hasanuddin University. While K^+ by using atomic absorption spectrometry (AAS) and was conducted in the Central Research Brackish Water Aquaculture (CRBWA) Maros, South Sulawesi. Total uptake was calculated based on the amount of nutrients in the media before and after the maintenance of seaweed in a specific time by using the formula of Tyler and his colleagues [17] as follows:

$$T = \frac{X_i - X_t}{t \times B}$$

Where: T = Total uptake of nutrients ($\text{ppm g}^{-1} \text{d}^{-1}$); X_i = initial concentration of nutrients in the medium (ppm); X_t = final concentration of nutrients in the medium (ppm); t = time (days) and B = dry weight thallus (g).

2.4. Experimental design and Data Analysis

The experimental design used was a completely randomized design (CRD) comprised of 5 treatments (BAP concentration) as follows: Treatment A (control), B (1.0 ppm), C (2.0 ppm), D (3.0 ppm) and E (4.0 ppm). Each treatment was given three replications so that there are 15 experimental unit. The data were analyzed using ANOVA with 5% confidence level[18]. The results showed the treatment effect (F count larger than F table), then proceed with the least significant difference test (LSD).

3. Results and Discussion

3.1. Nitrogen uptake

Average total nitrogen uptake (T-N) seaweed *K. alvarezii* that in the period of the first three days and the second three days both cultivated shown in (Figure 1). Analysis of variance (ANOVA) observation first and second periods of treatment showed no effect ($P > 0.05$) on the nitrogen uptake.

Although the treatment had no effect, but the amount of nitrogen uptake increased with increasing concentrations of BAP treatment and increasing the cultivation period as shown in (Figure 1). The highest T-N uptake in the first three days period on the concentration treatment of 3,0 ppm with the uptake value of 0.13 ± 0.06 ppm g⁻¹d⁻¹ of dry weight then declined at concentrations treatment of 3.0 ppm and lowest on the control treatment (concentration of 0.0 ppm) with uptake value of 0.04 ± 0.02 ppm g⁻¹d⁻¹ of dry weight. While on the second three days period, the highest T-N uptake at 2.0 ppm treatment with uptake value of 0.19 ± 0.07 ppm g⁻¹d⁻¹ of dry weight then declined at concentrations treatment of 3.0 ppm and 4.0 ppm and lowest on the concentration treatment of 0.0 ppm with uptake value of 0.09 ± 0.03 ppm g⁻¹d⁻¹ of dry weight.

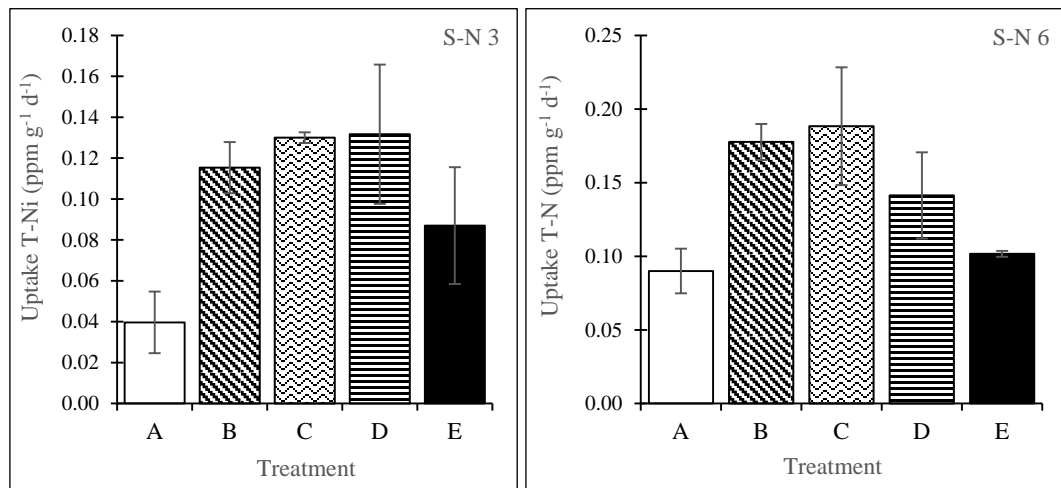


Figure 1: Uptake T-N on each treatment, the first three days period (S-N 3) and a second three days period (S-N 6)

The average range of T-N uptake on the second period between ($0.19 \pm 0.07 - 0.09 \pm 0.03$ ppm g⁻¹d⁻¹) higher than T-N uptake on the first period between ($0.04 \pm 0.02 - 0.13 \pm 0.06$ ppm g⁻¹d⁻¹) on all treatments BAP concentration. An increased uptake of T-N with increasing cultivation time, even though the treatment was not significantly different. T-N uptake value obtained in this study is lower than the uptake NH₄⁺ *K. alvarezii* cultivated in medium with high concentrations of NH₄⁺ (30 ppm) range between 11,90-12,10 ppm g⁻¹d⁻¹ during

the first 1-6 hours and higher than the value of the uptake at medium to concentrations of NH_4^+ 10 and 20 ppm, increased uptake even in dark conditions [19].

Research conducted by Pereira and his colleagues [20] that treatment with a concentration of NO_3^- and NH_4^+ are different, the results are not significantly different from the uptake of these two elements in *Porphyra dioica* during the first period of four days to three days of the second period. Similarly, the cultivation of seaweed *Gracilaria vermiculophylla* conducted in a short time. The level of NH_4^+ and NO_3^- uptake increased with increasing concentration of nitrogen in the cultivation media and then decreased with increasing nitrogen in the tissues [21]. *K. alvarezii* can accumulate nitrogen (NH_4^+ and NO_3^-) in the tissues when high concentrations of these elements in water [22,23] and used again as a nutrient for metabolism needs water deficit when nitrogen [23].

If the uptake value T-N by *K. alvarezii* assumed as the number of T-N were transferred from cultivation medium by calculating the percentage difference in T-N concentration of dissolved at the initial and the end of each cultivation period, the number of T-N is transferred can be known. In the first period the number of T-N were transferred from the cultivation media during the period of the first three days of 21.86% d^{-1} of the initial concentration (5.44 ppm) in the treatment of BAP concentration of 3.0 ppm or the highest uptake of T-N. While on treatment of BAP concentration of 0.0 ppm (control) is only 7.68% d^{-1} of the initial concentration (4.57 ppm) or the T-N uptake is lowest. Furthermore, during the second three days period, the T-N dissolved transferred from cultivation media reaches 30.30% d^{-1} of the initial concentration (5.61 ppm) in the treatment of BAP concentration of 2.0 ppm or the T-N uptake highest. While in the control treatment the uptake was only 17.37% d^{-1} from initial concentration (4.95 ppm) or lowest T-N uptake.

An increased number of T-N were removed from cultivation media with increasing cultivation time up to six days. Number T-N were moved more in the treatment of BAP concentration of 1.0 ppm - 3.0 ppm compared to control treatment and a concentration of 4.0 ppm. This suggests that the growth hormone BAP increase the uptake of T-N (NH_4^+ and NO_3^-) in *K. alvarezii* during the first 6 days of cultivation. According to Calvo and his colleagues [24] that the application of plant growth regulators (PGR) significantly increasing N uptake in plants and can be done through the cultivation medium and spraying the leaves. Cultivation of *K. alvarezii* on medium with concentrations of NH_4^+ and NO_3^- 100 times higher than the existing concentration in sea water can nitrate uptake 18.2% and ammonium 70.5% [22].

Some species of seaweed are reported to have a high ability to nitrogen uptake, among others: *Gracilaria chilensis* and *Ulva lactuca* can uptake NH_4^+ and NO_3^- 100% of the waste water aquaculture abalone (*Haliotis rufescens*) [25]. *Porphyra dioica* can uptake NH_4^+ during periods of light and 35% NO_3^- during the dark period if both sources of nitrogen are available in sufficient quantities [20].

3.2. Phosphate uptake

Average total phosphate uptake (T-P) seaweed *K. alvarezii* that in the period of the first three days and the second three days both cultivated shown in (Figure 2). Analysis of variance (ANOVA) observations of the first

period and the second showed that the treatment effect ($P < 0.05$) on the T-P uptake.

In the first three days of the cultivation period T-P highest uptake $0.143 \pm 0.04 \text{ ppm g}^{-1}\text{d}^{-1}$ of dry weight in the treatment concentration of 2.0 ppm and significantly different by treatment concentration of 0.0 ppm (control), 1.0 ppm and 4.0 ppm, but did not differ significantly by treatment concentration of 3.0 ppm. While TP lowest uptake of $0.022 \pm 0.02 \text{ ppm g}^{-1}\text{d}^{-1}$ of dry weight in the control treatment and did not differ significantly by treatment concentration of 4.0 ppm

In the first three days of the cultivation period T-P highest uptake $0.183 \pm 0.05 \text{ ppm g}^{-1}\text{d}^{-1}$ of dry weight in the treatment concentration of 2.0 ppm and significantly different from the control treatment and a concentration of 4.0 ppm but not significantly different by treatment concentration of 1.0 ppm and 3.0 ppm. While T-P lowest uptake of $0.079 \pm 0.02 \text{ ppm g}^{-1}\text{d}^{-1}$ of dry weight in the control treatment and a concentration of 4.0 ppm.

T-P uptake increased with increasing concentrations of BAP treatment and cultivation periods. In the first three days of the cultivation period showed that the increased uptake of T-P was highest in treatment of 2.0 ppm concentrations then decreased at treatment concentrations of 3.0 ppm and 4.0 ppm. The same thing happened in the second three days cultivation period as shown in (Figure 2). But the value of the average uptake of T-P in the second period is higher than the first cultivation period. This suggests that the growth hormone BAP applied to the cultivation medium could increase the uptake of T-P on *K. alvarezii*. As it was explained that application of PGR on cultivation medium significantly accelerate P uptake in plants [24].

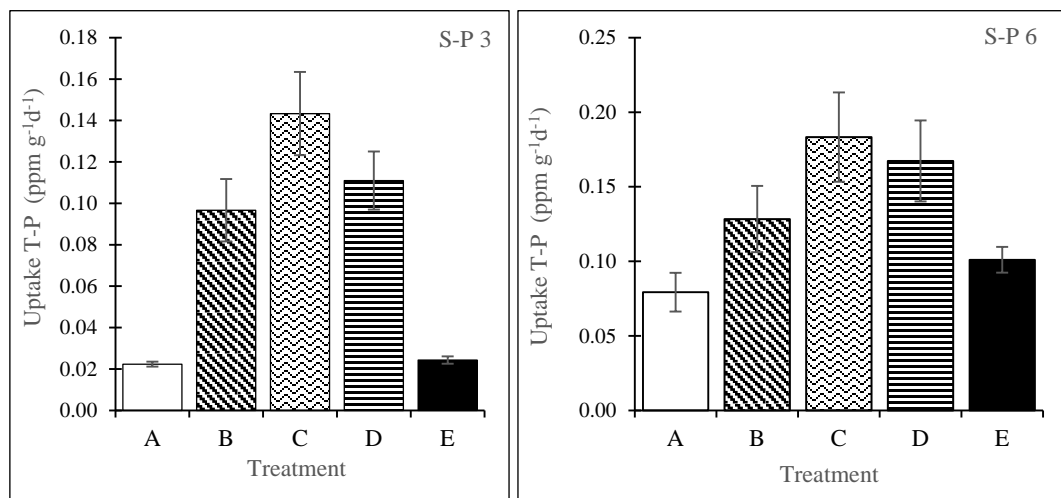


Figure 2: Uptake T-P on each treatment, the first three days period (S-P 3) and a second three days period (S-P 6)

Increased uptake value T-P in the second maintenance period shows that phosphorus is needed seaweed *K. alvarezii* the growth process as well as other crops. Phosphorus is one of the macronutrient as the limiting factor of productivity and growth of macro algae [26] because his role is very important in photosynthesis and energy storage in plants [27]. He also explained that most of the absorbed solar energy will be converted into high-energy chemical substances in the presence of phosphorus in the form of adenosine triphosphate (ATP).

Furthermore, ATP is used for photolysis of water (H_2O) to produce H^+ and O_2 and various processes in the dark reactions including the preparation of sugars [28].

If the uptake value T-P by *K. alvarezii* assumed as the number of T-P were removed from cultivation medium by calculating the percentage difference in T-P concentration of dissolved at the initial and end of each cultivation period. Thus, to determine these assumptions especially in the treatment with the highest T-P uptake value (concentration of 2.0 ppm) and treatment with the lowest uptake value (controls) on each maintenance period as follows: In the first period the number of T-P were removed from cultivation medium on the treatment of 2.0 ppm is 14.09% d^{-1} from an initial concentration of dissolved phosphate (9.72 ppm) and the control treatment was 2.12% d^{-1} from an initial concentration of dissolved phosphate (9.92 ppm). While in the second period the number of T-P were removed from cultivation medium on the treatment of 2.0 ppm is 17.49% d^{-1} from an initial concentration of dissolved phosphate (9.72 ppm) and the control treatment was 7.78% per day from an initial concentration of dissolved phosphate (9.92 ppm). According to Hayashi and his colleagues [22] that *K. alvarezii* able to uptake or remove 27% of phosphate d^{-1} from cultivation medium the taken of the fish farming waste *Trachinotus carolinus* with nutrient concentrations 100 times higher than the nutrients contained in seawater.

Nevertheless, an increasing number of T-P absorbed or transferred from the media with increasing cultivation time. The average amount of T-P was remove to the second cultivation period is higher than the first period in all treatments BAP concentration. The pattern of uptake or remove of T-P obtained in this study is different from the pattern of phosphate uptake obtained by Pereira and his colleagues [20] in *Porphyra dioica* where the first four days of the cultivation period is higher phosphate uptake (61%) and then decreased in the second three days period to 44% d^{-1} . While on *Ulva reticulata* able to uptake or remove phosphate as much as 33% d^{-1} of waste water fish cultivation [29].

The results obtained in this study, is much lower when compared to the amount of phosphate to remove *K. alvarezii* from cultivation medium as reported by Hayashi and his colleagues [22]. his is caused by differences in the concentration of phosphate in the media. In this study, the concentration of phosphate in cultivation medium ranged from 9.72 - 9.92 ppm after the addition of fertilizer Conway 2.0 ppm as a source of nutrients in each treatment. The concentration range of only 5.78-5.90 times higher than seawater phosphate concentrations (1.68 ppm) were used as a source of water in this study.

3.3. Potassium uptake

Average total potassium uptake (K) seaweed *K. alvarezii* that in the period of the first three days and the second three days both cultivated shown in (Figure 3). Analysis of variance (ANOVA) observations of the first cultivation period showed of treatment effect ($P < 0.05$) and in the second period of cultivation treatment showed no effect ($P > 0.05$) on the K uptake.

In the first three days of the cultivation period K highest uptake 3.05 ± 0.91 ppm $g^{-1}d^{-1}$ of dry weight in the treatment concentration of 3.0 ppm and significantly different by treatment concentration of 0.0 ppm (control)

and 2.0 ppm, but did not differ significantly by treatment concentration of 2.0 ppm and 4.0 ppm. Though lowest K uptake 1.32 ± 0.20 ppm $g^{-1}d^{-1}$ of dry weight, but it is not significantly different with treatment concentration of 2.0 ppm. Treatment concentration of 1.0 ppm, 2.0 ppm and 4.0 ppm showed no significant different on the K uptake.

In the second three days cultivation period, although the treatment had no significant effect but the average uptake of K in all treatments was higher than the first cultivation period (Figure 3). In general, applications of growth hormone BAP through cultivation media showed the increase the uptake of potassium in *K. alvarezii* higher than the control treatment. These hormones also increase the uptake of N and P as described at the initial of this article [24] explains that the PGR that are applied either through the cultivation media or by the spraying the leaves served to increase the uptake of N, P and K in plants.

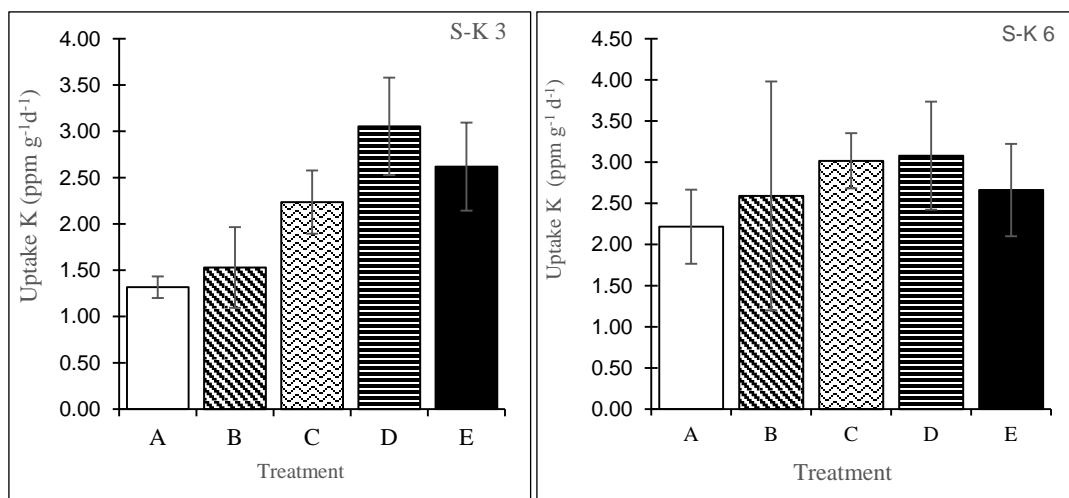


Figure 3: Uptake K on each treatment, the first three days period (S-K 3) and a second three days period (S-K 6)

Potassium is the same as N and P is a macronutrient that is the primary and indispensable for the growth of plants [3]. He also explained that one of the functions of K in plant growth is activating an enzyme involved in the production of ATP during photosynthesis. When the plant K deficiency, the rate of photosynthesis and the production of ATP is reduced so that all physiological processes that depend on chemicals this high-energy becomes blocked [30]. Potassium deficiency caused many plants vulnerable to biotic and abiotic stress thus reducing the quantity and quality of production [31].

Cases of K deficiency that occurs in many agricultural crops will not occur in the cultivation of macroalgae in the sea. According Bardi [32] that the K concentration of sea water is very high ranging from 392.0 - 400.0 ppm and is the fourth largest metal ions after Na^{+} , Mg^{2+} and Ca^{2+} . Thus K never be the limiting factor of growth and metabolism of marine algae.

The importance of potassium in *K. alvarezii*, other than regulate various physiological processes as in land plants [30]. Especially the enzymes synthesized high molecular weight organic compounds such as starch,

cellulose and proteins [31]. Potassium is crucial in the formation of the gel strength of kappa carrageenan types [33] and carrageenan extracted from *K. alvarezii* is types kappa [34]. Kappa carrageenan gel has a structure that is coarser and not soluble in cold water. Instead iota carrageenan having a smooth gel structure and is soluble in cold water because their calcium ion [33].

4. Conclusion

BAP growth hormone significantly increases the uptake of nutrients (T-N, T-P and K) in seaweed *K. alvarezii* and the value of uptake increased with increasing time cultivation. BAP concentration effectively increasing nutrient uptake in seaweed *K. alvarezii* is 2.0 – 3.0 ppm for nitrogen, 2.0 ppm for phosphorus and 3.0 ppm for potassium. The real effect of growth hormone BAP in increasing the uptake of nitrogen and potassium only seen in the first period during the three days of cultivation, while phosphorus until the second period during the six days of cultivation.

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