

Short Communication: Phytohormone Quantification of *Kappaphycus alvarezii* at Different Cultivated Ages

Short Communication: Kuantifikasi Fitohormon *Kappaphycus alvarezii* pada Umur Tanam yang berbeda

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Abstract

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Kappaphycus alvarezii is used in industry as a food ingredient, a source of hydrocolloids, cosmetics, food additives, animal feed, fertilizers, biofuels, and medicinal ingredients. *K.alvarezii* is cultivated in several areas in Indonesia, including on the island of Lombok. The production of *K.alvarezii* is related to its growth and it is related to its phytohormones. *K.alvarezii* cultivated in Lombok is currently not known for its composition and amount of phytohormones. The content of phytohormones at different planting ages is of course different. The purpose of this study was to determine the number of phytohormones in seaweed *K. alvarezii* at different planting ages. This study conducted in Ekas Bay, Lombok, Nusa Tenggara Barat from June to December 2020. The study used experimental methods and descriptive data analysis. The results of this study explain that currently cultivated *K. alvarezii* contains cytokinins, auxins and gibberellins. Cytokinins obtained on the 9th day of planting were 11.62 ppm and increased on the 18th day to 12.34 ppm. At the next planting age, it continued to decrease on the 27th day to 11.43 ppm and at harvest time, the 45th day of 10.69 ppm. Auxin detected in the seeds used was 644.93 ppm, on the 27th day of 14.27 ppm and the 36th day of 1078.25 ppm. Giberilin was detected on the 9th day at 0.49 ppm. This study concluded that *K. alvarezii* at different planting ages had different phytohormones content.

Keywords: Seaweed, aquaculture, growth, auxin, cytokinin, gibberellins

Abstrak

Kappaphycus alvarezii digunakan dalam industri sebagai bahan makanan, sumber hidrokoloid, kosmetik, bahan tambahan makanan, pakan ternak, pupuk, biofuel, dan bahan obat. *K. alvarezii* dibudidayakan di beberapa daerah di Indonesia, termasuk di Pulau Lombok. Produksi *K. alvarezii* terkait dengan pertumbuhan dan fitohormonnya. *K. alvarezii* yang dibudidayakan di Lombok saat ini belum diketahui komposisi dan jumlah fitohormonnya. Kandungan fitohormon pada umur tanam yang berbeda tentu saja berbeda. Tujuan dari penelitian ini adalah untuk mengetahui jumlah fitohormon pada rumput laut *K. alvarezii* pada umur tanam yang berbeda. Penelitian ini dilakukan di Teluk Ekas, Lombok, Nusa Tenggara Barat pada bulan Juni s/d Desember 2020. Penelitian ini menggunakan metode eksperimen dan analisis data deskriptif. Hasil penelitian ini menjelaskan bahwa *K. alvarezii* yang dibudidayakan saat ini mengandung sitokinin, auksin, dan giberelin. Sitokinin yang diperoleh pada hari ke-9 penanaman sebesar 11,62 ppm dan meningkat pada hari ke-18 menjadi 12,34 ppm. Pada umur tanam

berikutnya terus menurun pada hari ke-27 menjadi 11,43 ppm, dan pada saat panen hari ke-45 sebesar 10,69 ppm. Auksin yang terdeteksi pada benih yang digunakan sebesar 644,93 ppm, pada hari ke-27 sebesar 14,27 ppm, dan pada hari ke-36 sebesar 1078,25 ppm. Giberilin terdeteksi pada hari ke-9 sebesar 0,49 ppm. Penelitian ini menyimpulkan bahwa *K. alvarezii* pada umur tanam yang berbeda memiliki kandungan fitohormon yang berbeda.

Kata Kunci : Rumput laut, akuakultur, pertumbuhan, auksin, sitokinin, giberelin

1. Introduction

Kappaphycus alvarezii seaweed is a fishery commodity that is cultivated in the waters of the province of West Nusa Tenggara (NTB), namely on the islands of Lombok and Sumbawa. Production of *Kappaphycus alvarezii* on Lombok according to the Department of Marine Affairs and Fisheries (2020) in 2015 production was 311,451.15 tons, while in 2016 production was 227,082.39 tons. Production in 2019 was 212,928.76 tons. Simatupang *et al.*, (2021), Lombok is a producer of *K. alvarezii* that supplies the global market. The carrageenan content of *K. alvarezii* cultivated in Lombok was 8.6-11.7%. Cokrowati *et al.* (2020a) *K. alvarezii* cultivated in Lombok had a dry carrageenan yield of 10.02 g with an absolute growth of 169.29 g. Naseri *et al.* (2020) carrageenan is a polysaccharide extracted from *K. alvarezii*, and carrageenan is used as a texturizer in food and non-food products. Varderama *et al.* (2015), *K. alvarezii* is used in industry as a food ingredient, a source of hydrocolloids, cosmetics, food additives, animal feed, fertilizers, biofuels, and medicinal ingredients. Reshna *et al.*, (2021) carrageenan from *K. alvarezii* is a natural polysaccharide that can function in drug delivery, tissue engineering, and wound healing. Carrageenan has a strong water-absorbing ability and unique gel-forming characteristics, making it a good hydrogel for medicine.

Internal factors that play a role in the growth of *K. alvarezii* is the condition of the seaweed itself. This condition is the phytohormones present in cultivated seaweed. If the phytohormones possessed are in sufficient quantities to support the growth of the thallus, optimal growth can occur. The results of research by Suryati *et al.* (2015); Parab & Shankhdarwar (2021) explained that seaweed contains growth-regulating hormones such as auxins, cytokinins, gibberellins, and various minerals that can increase the growth of *K. alvarezii* micropropagules. Fadilah *et al.* (2016) explained that kinetin and indole-3-acetic acid have an important role in the growth of *K. alvarezii*. Gracia *et al.*, (2020) seaweed extract has phytohormones that can be used to increase crop production. *Padina durvillaei* and *Ulva lactuca* extracts contain sulfates, flavonoids, and phenolic compounds. Phytohormones that are owned are gibberellin, abscisic acid, indoleacetic acid, cytokinins, jasmonic acid, and salicylic acid. Mori *et al.* (2017) red algae have indole-3-acetic acid (IAA), N6-(2-isopentenyl) adenine (IP), abscisic acid, and salicylic acid. Chaturvedi *et al.*, (2022) phytohormones in seaweed play a role in physiological processes in the body, especially as growth stimulants, abiotic stress relievers from the environment, and antimicrobials.

K. alvarezii cultivated in Lombok is currently unknown for its composition and amount of phytohormones. The content of phytohormones at different planting ages is of course different. The age of seaweed seeds with good quality can be determined by knowing the number and types of phytohormones at each planting age. Basmal (2009) on hormones in *K. alvarezii* explained that seaweed contains trace minerals (Fe, B, Ca, Cu, Cl, K, Mg, and Mn) as well as growth regulators, are auxins, cytokinins, and gibberlines. The content of growth regulators is widely found in the thallus (stem) of seaweed. Cokrowati *et al.*, (2020b) explained the results of their research that *K. alvarezii* cultivated in Gerupuk Lombok waters contained different chlorophyll and phycoerythrin contents. It also affects the growth of *K. alvarezii*. Based on the description above, it is necessary to conduct research on the phytohormonal quantification of *K. alvarezii* seaweed at different planting ages. The purpose of this study was to determine the number of phytohormones in seaweed *K. alvarezii* at different cultivated ages.

2. Material and Method

2.1. Place of Research

K. alvarezii taken from farmers in Ekas Bay, Ekas Buana Village, Jerowaru District, East Lombok Regency, Nusa Tenggara Barat (Figure 1). The research location is as shown in the picture. *K. alvarezii* cultivated is *K. alvarezii* green morphotype, as shown in figure 2. Cultivation is carried out using a floating raft, cultivation time is 45 days. *K. alvarezii* was taken every 9 days for phytohormone analysis. Phytohormone analysis was carried out at Analytical Chemistry Laboratory, Mataram University, Lombok.

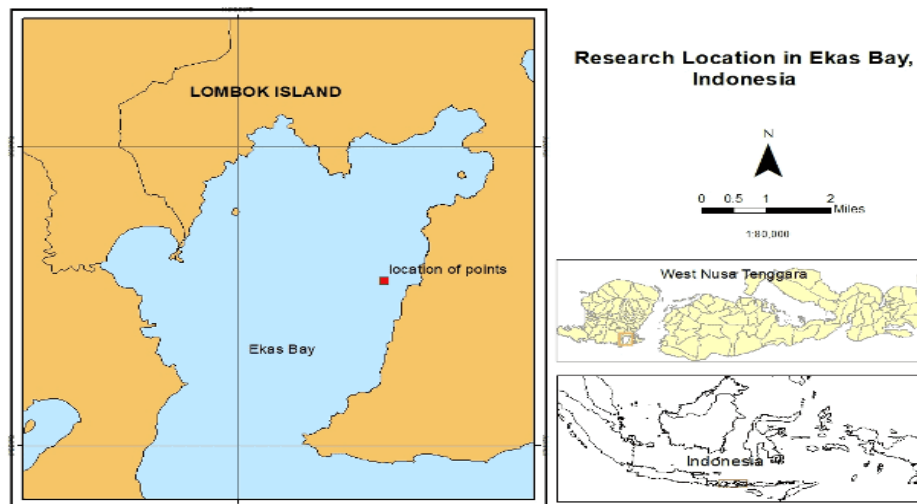


Figure 1. Research Location, Ekas Bay, Lombok Timur, Nusa Tenggara Barat; 8°50'27"S 116°27'24"E



Figure 2. *K. alvarezii* green morphotype cultivated in Ekas Bay

2.2. Research Materials

2.2.1. Extraction of *K. alvarezii* and quantification of phytohormone

Extraction of *K. alvarezii* was prepared by pulverizing 30 g of *K. alvarezii* thallus using a mortar and mixed with 99% methanol as solvent as much as 30 ml. The extract was then filtered using whiteman paper and accommodated in a test tube. The extract was taken as much as 5 mL using a syringe and filtered again using a sterile 0.45 μ L milipore filter and then accommodated in a tube. The extract was diluted to a concentration of 1000 ppm by taking 2 mL of methanol and pouring it into a tube then adding 2 μ L of *K. alvarezii* extract. The extract was ready to be injected into the HPLC machine for characterization of auxins, cytokinins and gibberellin. The extract was quantified for its phytohormones using HPLC. The analysis was carried out using HPLC Shimadzu LC-10 AT VP, equipped with a pump model LC-10AT, UV-Vis detector SPD-10AT, Rheodyne injector equipped with a 20 L loop and an automatic injector SIL-10AT. The data on the quantity of *K. alvarezii* seaweed phytohormones are tabled and graphed using Excel. Furthermore, the number of phytohormones was analyzed descriptively and reviewed based on the literature.

2.2.2. Observation of morphology and cell development

Morphological and cell development observations were observed every 9 days. Observation of morphological development was done visually. Cell observations were made by making tissue slices and staining and then observing with a microscope.

3. Result and Discussion

The results of the phytohormonal analysis in the thallus of *K. alvarezii* are as shown in table 1 below. The results of the analysis explained that currently cultivated *K. alvarezii* contains cytokinins, auxins and gibberellins. Cytokinins and auxins were more dominantly detected compared to gibberellins. The following is a chromatogram of *K. alvarezii* phytohormones at different planting ages (Figure 3). Morphology and cell of *K. alvarezii* thallus based on the age of cultivation, different shape as shown in the figure 4.

Table 1. Phytohormones in *K. alvarezii* at different planting ages

Phytohormone (ppm)	Days to-					
	0	9	18	27	36	45
Cytokinin	0	11,62	12,34	11,43	11,66	10,69
Auxin	644,93	0	0	14,27	1078,25	0
Gibberellin	0	0,49	0	0	0	0

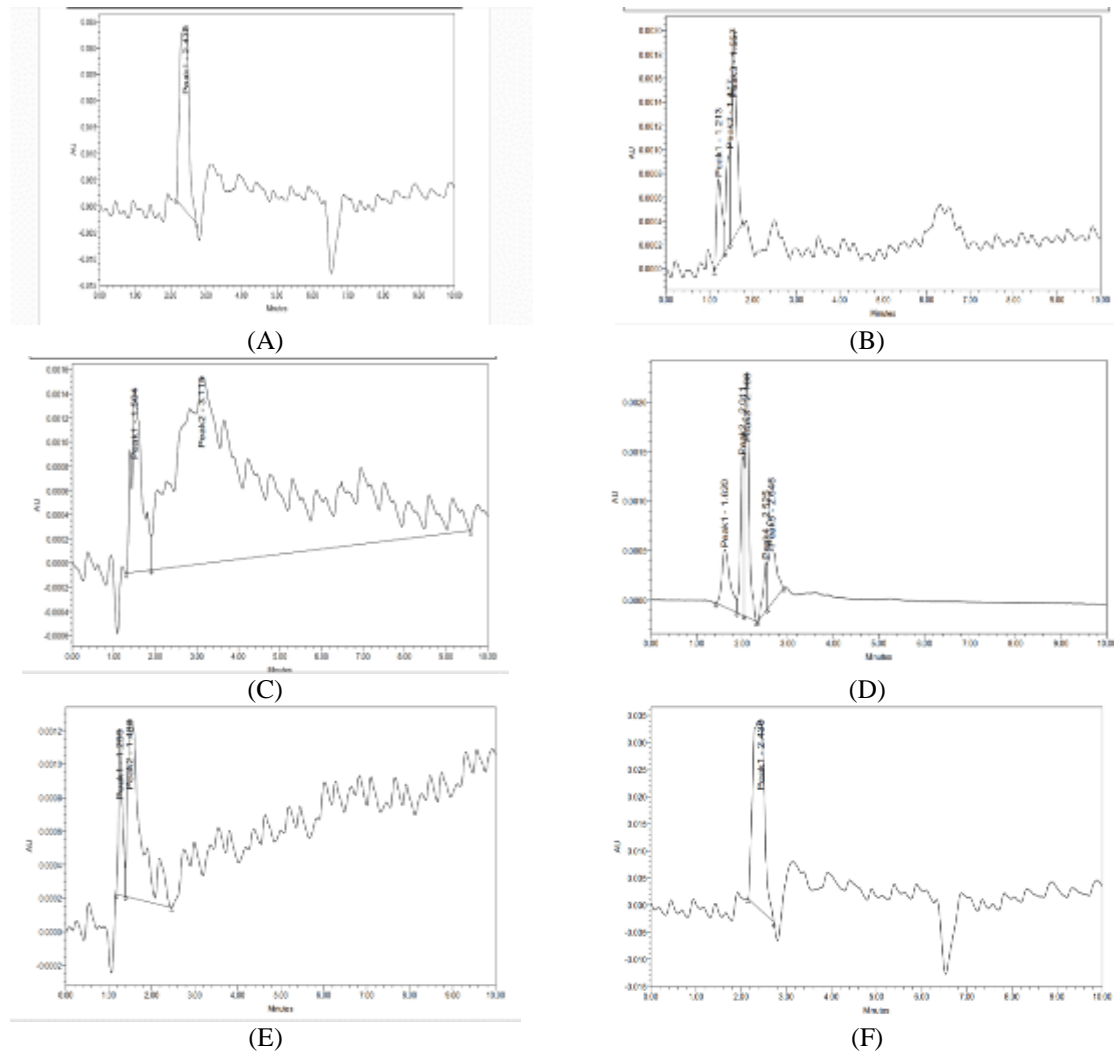
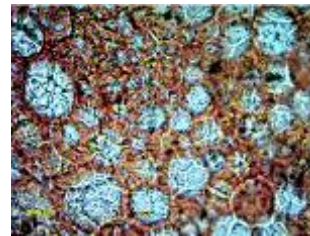
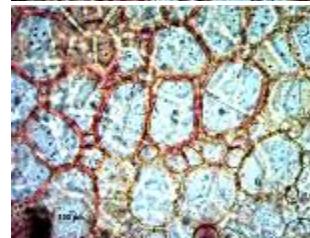


Figure 3. Chromatogram of phytohormones of *K. alvarezii* at different planting ages: (A) Day-0; (B) Day-9; (C) Day-18; (D) Day-27; (E) Day-36; (F) Day-45.

Day-0



Day-9



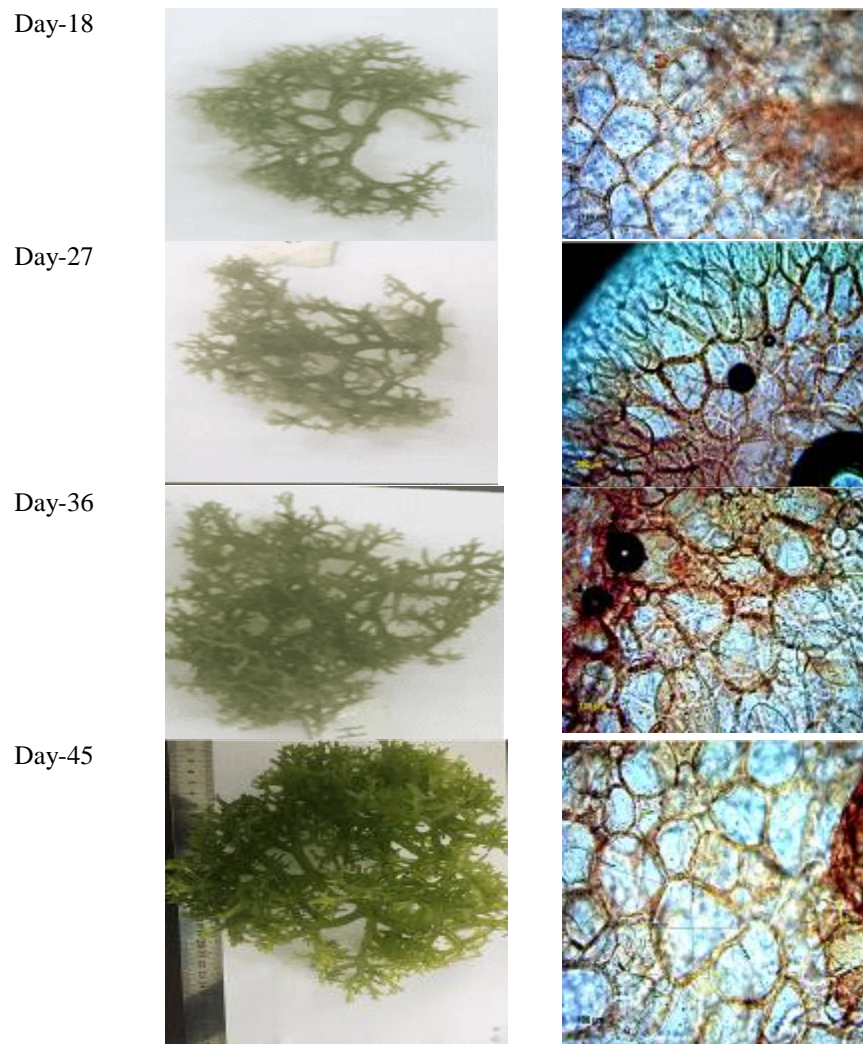


Figure 4. *K. alvarezii* and its cells at each age of cultivation

Cytokinins were detected from the 9th day to the 45th day of planting with different amounts. The 9th day was 11.62 ppm and increased on the 18th day to 12.34 ppm. At the next planting age, it continued to decrease on the 27th day to 11.43 ppm. The number of cytokinins decreases on the 27th day because *K. alvarezii* began forming carrageenan. At harvest age, namely the 45th day, the amount was 10.69 ppm. More cytokinins were formed on the 9th and 18th days because during that time *K. alvarezii* grew optimally to form new thallus branches and enlarge the thallus. So that cytokinin is needed in greater quantities and is formed according to these needs. The period of time after that, the growth of seaweed is more concentrated for carrageenan formation. So the need and synthesis of cytokinins also decrease.

Le Bris (2017), cytokinins are compounds with a structure close to adenine and linked to the isopentenyl side chain. The most common form of cytokinin that occurs naturally in plants is zeatin. Cytokinins are present in plant tissues at growing points, namely the root zone, young leaves, fruit and seeds. Cytokinins stimulate cell division, regulate shoot meristem size, leaf number, and leaf and shoot growth. Cytokinins can stimulate the differentiation and growth of shoots. Cytokinins control root vascular morphogenesis, mineral uptake and nutrient movement. Chloroplast biogenesis and rate of photosynthesis depend on cytokinins. Kasim *et al.*, (2021) cytokinins stimulate the growth of prospective thallus. Cytokinins control cell division and the development of thallus branches. The thallus can respond to biotic and abiotic stress through cytokinins. Such stress can inhibit the growth of *K.alvarezii* thallus. Moubayidin *et al.* (2009) cytokinins have the ability to promote cell division. Isopentenyla-adenine, trans-zeatin and dihydrozeatin are cytokinins found in higher plants. Cytokinin activity is controlled by a fine balance between synthesis and catabolism.

Li *et al.* (2017), auxin is an organic acid that affects cell expansion and division, cell elongation and differentiation, and various physiological responses. Significantly affects the final shape and function of cells and tissues in plants. Auxins are produced mainly in shoot and root meristems and are transported into plant vessels and to other parts of the plant. Vanneste & Jir'I (2009), plant growth is determined by the distribution of auxin in the tissue. The timing and pattern of auxin distribution is mediated by auxin biosynthesis and auxin transport between cells. In cells where auxin accumulates, it triggers developmental changes in certain cell

types. In other words, instead of instructing cells what to do, auxin accumulation selects cells at specific positions and at specific times for changes in the developmental program.

Auxin in *K. alvarezii* seedlings at the beginning of planting was 644.93 ppm and its levels decreased until the 27th day of 14.27 ppm. On that age, *K. alvarezii* began forming carrageenan. Auxin levels increased again on the 36th day, which was 1078.25. These conditions can describe the growth that occurs in *K. alvarezii*. Cokrowati *et al.* (2021), explained that most of the phytohormones in *K. alvarezii* are in the form of auxins. Ljung *et al.* (2005), auxin plays a role in root development, including the initiation and emergence of lateral roots, apical meristem pattern of roots, gravitropism, and root elongation. Auxin biosynthesis occurs at the tips of plant parts and roots. with Auxin needed for root development can come from one source or both. Mulyaningrum *et al.* (2013), auxin plays a role in initiating cell division, and the organization of meristems into tissues. Garcia *et al.* (2020) algae extract contains growth regulators including auxin. These substances have the function of inducing physiological processes in plant medicine, germination, root growth, nutrient mobilization, maturation, stress tolerance, and disease resistance.

Gibberellin in *K. alvarezii* was detected on the 9th day at 0.49 ppm. Possibly at other planting ages, the levels are so small that they are not detected. Garcia *et al.* (2020) The number of gibberellins in algae is very small and identified only on their biological activity. Gao & Xiangdong (2017), gibberellins are phytohormones produced by plants and fungi that have a role in modulating various processes of plant growth and development. Gibberellins triggers plant growth and development through protein degradation. Gibberellins act as mobile molecules that can pass through the plasma membrane for cell-to-cell transport. Dalero *et al.* (2019), explained that gibberellins in seaweed are a small part of growth regulators that have a role in seaweed growth. Mori *et al.* (2017) gibberellins were not detected in red algae, but their activity was seen in growth. Quijano-Alives *et al.* (2018) red algae are a source of gibberellins which play a role in plant growth and development. Kalivanan *et al.* (2012) gibberellins synthesized amylase to break down starch into simple sugars for growth.

The morphological development of *K. alvarezii* thallus was seen at each cultivation age as well as the development of the thallus cell profile. On day-0, the cell profile looks denser with a smaller cell size. Most of the thallus is still young and will still multiply the thallus at that age. By the number of cytokinins on day-9 of 11.62 ppm, it increased to day-18 at 12.34 ppm. This means that the cells in the thallus are trying to multiply and form a new thallus. The cell size increased with the increasing age of *K. alvarezii* and the thallus. *K. alvarezii* green morphotype growth tends to form many new thallus and lush. This growth of *K. alvarezii* did not lead to thallus elongation. The profile picture of *K. alvarezii* cells shows that the vacuole increases in size with increasing cultivation age. Ginneken (2018) explains that in red seaweeds the cell wall contains carrageenans, agars, xylans, floridean starch (amylopectin-like glucan), water-soluble sulphated galactan. Baweeja (2016) The growth of the thallus of *K. alvarezii* is multiaxial and differentiated into a single-layered epidermis, larger vacuole cortex and densely fibrous medulla. The epidermis is covered by a thick mucilaginous layer. The medulla consists of filamentous, long cells. these cells are small, which are sometimes interspersed with large cells and show an irregular outline. these cells are connected via pith connections..

4. Conclusions

The conclusion of this study was that *K. alvarezii* at different planting ages had different phytohormones content. Cytokinins were detected on the 9th day of planting until the 45th day of harvest with a range of 11.62 ppm to 12.34 ppm. Auxin was identified at the beginning of planting, 644.93 ppm, on the 27th day of 14.27 ppm and the 36th day of 1078.25 ppm. Giberilin was identified on the 9th day which was 0.49 ppm.

5. Suggestion

The suggestion from this research is improvement growth of *K. alvarezii* can be done based on planting age, because the phytohormone content varies at each age.

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