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The Effect of Nanofertilizer in Grain Corn Germination and Growth Rate

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Nanofertilizers are now a cutting-edge tool in agricultural research. Sustainable and efficient agricultural practices are critical to meet the world's food demand while having as little impact as feasible on the environment. Hence, this study applied calcium aluminum layered double hydroxide (Ca-Al LDH) to the grain corn (*Z. mays*) to raise the growth rate, chlorophyll, and carotenoid content. This study aimed to compare the impact of synthesized Ca-Al LDH nanoparticles with the conventional nitrogen, phosphorus, and potassium (NPK) fertilizer, aluminium nitrate $(AlNO_3)_3$, and the control plant. Note that six weeks after the application of the nanofertilizer, the plants experienced a positive morphological growth rate, but when compared with NPK fertilizer, the nanofertilizer fell into the second position. The critical concentration that satisfactorily increases the growth parameter of plants was achieved at the dose concentration of 50 mg/L for NPK fertilizer and $Al(NO_3)_3$ as well as 100 mg/L for Ca-Al LDH nanofertilizer. Furthermore, the chlorophyll *b* and carotenoid content for plants treated with 50 mg/L Ca-Al LDH were revealed to be higher than 50 mg/L of NPK fertilizer, resulting in longer length measurements of the plant leaf. To emphasize, Ca-AL LDH nanoparticles have a remarkable potential to boost soil fertility and crop productivity while minimizing ecological issues.

Key words: Layered double hydroxide; fertilizer; seed germination

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The agricultural product refers to any commodity of plant cultivation and animal husbandry marketed for human consumption and animal feed. While it can be broadly categorized into a few simple groups, such as foods, fibers, fuels, and raw materials, the large population of human beings has put intensified pressure on this industry to meet the demand for specific products. Fertilizers, which are viewed as critical resources for the development of agricultural goods, have now provided a nasty aspect to the extent that they are capable of impairing the ecosystem service in which we currently live, declining the nutritional rate of food produced, as well as deteriorating the soil quality [1]. Many researchers discovered the connection between the indiscriminate employment of fertilizer with the release of hazardous greenhouse gases (e.g., nitrous oxide, N_2O) and the eutrophication problem [2,3,4]. This issue has driving concern since it mangles the natural biogeochemical cycles, affecting aquatic life and human being. Fertilizers that were once regarded to promote soil fertility and boost crop yields are no longer safe to be used since their multifold applications prioritizing macronutrients such as nitrogen, phosphorus, and potassium have caused a shortfall in crucial micronutrients, forcing the soil's quality to become unbalanced [5]. Consequently, it will affect plant development and the food's nutritional value.

Present-day, revolutionary fertilizers utilizing nanotechnology are eagerly expected to minimize these distinct repercussions. Since its discovery, up until recently, more scientists have been working to develop a spectrum of metal and metal oxide nanoparticles for use in plant science and agriculture, believing it to be one of the paths to a sustainable and tolerable agricultural sector [6]. While conventional fertilizers have low plant uptake efficiencies and need large amounts to be applied, nanofertilizer based on nanotechnology are able to improve uptake efficiency and boost the target delivery of nutrients to plants [7]. Its high surface area to volume ratio, which stimulates chemical reactivity and physical responses, is a major contributor to its abilities in this regard. As a response, the nutrient loss through leaching will be reduced, the soil will be more fertile, crop output will increase, and

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ultimately lessen environmental issues [8]. A study on improving plant phosphorus uptake has shown a positive result through the application of foliar zinc oxide (ZnO) nanoparticles, whereby it has subsequently increased the activity of phosphatase and phytase enzymes, resulting in an increase in phosphorus uptake by the legumes and cereals of approximately 11%, and that is without the addition of any external phosphorus fertilizer yet [6].

Despite the fact that it was widely acknowledged that nanoparticles alone could have a substantial positive impact on an agricultural product due to their chemical compositions that are primarily based on fertilizer nutrients (e.g., Zn from ZnO), the fertilizer incorporated into the nanomaterial, however, has shown to show an outstanding result due to the combination of the nanomaterial itself and the fertilizer integrated into its layer. For instance, based on one study regarding the controlled release of nutrients on rice (*Oryza sativa*), it was reported that nitrate (NO_3^-) and ammonium (NH_4^+) in nitrogen form of fertilizer have successfully released from nanozeolite over a long period than conventional ammonium sulfate ($(NH_4)_2SO_4$), which is from 12 to 20 days [9]. In addition, the NPK fertilizer added to the chitosan poly-methacrylic acid (CH-PMMA) nanoscale host has shown an increase in all the measured variables on the wheat plant sample when compared to the control [10]. These variables include the number of spikelets, grains per main-spike, spike length, shoot length, and plant height. Undoubtedly, the controlled released investigation found that nanoparticles provide stability by reducing these nutrients' leaching losses and extending the availability of the nutrients [11]. The fact that they are 10 to 100 times larger than ions, nanoparticles can therefore fixate more readily in clay's lattice region, resulting in a minimal nutrient leaching problem. On the other hand, the growth development of the wheat plant has also successfully demonstrated a positive result due to the presence of nanomaterial that acts as a host to protect this nutrient from degradation [12].

Layered double hydroxide (LDH) has emerged as one of the most prominent promising nanomaterial agents in carrying and protecting fertilizer [13,14]. The emphasis has shifted away from just fertilizer and has instead broadly been utilized in many fields, including sunscreen formulation in cosmetics, drug carriers in the pharmaceutical sector, catalysts in the catalytic industry, and many others [15]. This nanomaterial's performance in a wide range of uses can be linked to the confined microenvironment of the guest species and the interactions between the host and guest. The LDH is a two-dimensional (2D) nanostructure with a positively charged host layer, with each positive layer comprises octahedral sheets containing metal cations in the center and hydroxyl anions set at their vertices. Note that the host layer structure was proposed to sandwich the interlamellar layer filled with anion and water molecules. The generic formula for this nanomaterial is $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}(A^{n-})_{x/n}\cdot mH_2O$, with M^{2+} and M^{3+} representing the divalent and trivalent metal cation, respectively, while A^{n-} denotes the anionic guest (e.g., NO_3^-, CO_3^{2-}, SO_4^{2-}) within the lamellar layer [16,17].

There are many edges of LDH that make it a potent carrier for many anion compounds spanning from organic, inorganic and biomolecule. One of these is its efficacy in having excellent ion exchange and adsorption power along with low toxicity and low environmental risk. Furthermore, its capability to swap the desired anion from its interlayer due to weak electrostatic forces has created an injunction for the intercalation of various compounds for a reason to safeguard this substance from direct contact with the environment [18,19]. Moreover, considering its efficacy in protecting the substances, it has been deemed to increase the bioavailability of the active ingredients it holds and control the release of these substances in a respective medium [20]. Besides, the power to adsorb various substances into its layer has made LDH a versatile sorbent material for environmental remedies. For instance, it has been applied to remove various pollutants from an aqueous environment, such as radioactive materials, metals, phosphates, and many more [21].

This study strives to develop calcium-aluminum layered double hydroxide (Ca-Al LDH) as a potential nanofertilizer since it is composed of the nutrient of interest for plant development. No intercalation of conventional fertilizer was made into Ca-Al LDH, concerning Ca-Al LDH itself can stand to have potential as a nanofertilizer. This is because the composition is synthesized by combining two substances, calcium nitrate hexahydrate ($Ca(NO_3)_2\cdot 6H_2O$) and aluminium nitrate nonahydrate ($Al(NO_3)_3\cdot 9H_2O$). Other than that, the efficacy of this nanofertilizer against grain corn (*Z. mays*) seed germination was explored based on its growth rate and composition of chlorophyll and carotenoid, and it was compared with the conventional fertilizer and $Al(NO_3)_3$, a constituent of Ca-Al LDH.

EXPERIMENTAL

Chemicals and Materials

Hybrid grain corn (*Z. mays*) seed variation labeled as GWG 111 was purchased from Green World Genetics (GWG) Sdn. Bhd. The nanofertilizer (Ca-Al LDH) was synthesized via the co-precipitation method utilizing $Ca(NO_3)_2\cdot 6H_2O$ and $Al(NO_3)_3\cdot 9H_2O$. Apart from that, sodium hypochlorite (NaOCl), conventional fertilizer (NPK), Ca-Al LDH, $Al(NO_3)_3$, and the *Z. mays* seed were all used in the seed priming process with 3% NaOCl acting as a disinfectant. Deionized water was used to wash the seeds and soak the control seeds; meanwhile, 80% acetone was utilized during the chlorophyll and carotenoid content measurement procedure.

Synthesis of Nanofertilizer

This method originated by Jadam *et al*. (2021) [22] was adopted with some modifications. To synthesize the nanofertilizer Ca-Al LDH, 0.1 M $Ca(NO_3)_2·6H_2O$ solution and 0.025 M $Al(NO_3)_3·9H_2O$ were mixed in a 250 mL conical flask. This solution was titrated with sodium hydroxide (NaOH) while being stirred until the pH reached 11. In accordance, it was left for one hour under a nitrogen atmosphere, followed by ageing for 18 h in the oil bath shaker at 70°C. The resulting slurry was centrifuged, and the pellet was collected, washed, and dried in an oven for 72 h at 80°C. The sample is kept for further use.

Priming Process

Hybrid *Z. mays* seed underwent imbibition and sterilizing as part of the seed priming procedure. This process was carried out since it is an efficient hydration method to stimulate fast seed germination [23]. For this step, the seeds of *Z. mays* of similar size were immersed in 3% NaOCl for 2 minutes to sterilize. Subsequently, these seeds were washed with deionized water before being soaked in distilled water for 8 h in a dark room at ambient temperature.

Preparation of Plant Growth Medium

After 8 h of soaking the seeds in distilled water, all the seeds were placed in a tiny pot filled with soil to grow the corn into seedlings. The seeds were moved into a large container once their roots and shoots had started to emerge and had grown into seedlings for 7 days. Ultimately, 11 pots with soil content and a 20 cm diameter were prepared, and each of these containers were planted with three seedlings that have a comparable height.

Preparation of Foliar Fertilizer

NPK fertilizers, $Al(NO_3)_3$, and nanofertilizer were used to make foliar fertilizers in 20, 50, and 100 mg/L concentrations. As a solvent for nanofertilizer, dimethyl sulfoxide (DMSO) was utilized, whereas distilled water was used to dissolve NPK fertilizer and $Al(NO_3)_3$. The measurement of each solute and solvent based on concentration are summarized and tabulated in Table 1. As soon as they were prepared, the foliar technique was employed on the plant sample since it is the most effective way to treat nutrient deficiencies, reduce pollution, and boost nutrient usage efficacy by reducing the amount of fertilizer given to the soil. Consequently, this process involves feeding the plants by applying it directly to the leaves [24].

Measurement for Plant Growth Parameter

This practice was carried out once a week until six weeks when the plant had already developed into seedlings and was in an active growth state. Then, the plants' height and the leaves' sizes, including their length and width, were measured using a graduated meter rod before the mean values of the data gathered were calculated.

Table 1. Measurement of each solute and solvent based on concentration

Treatment	Concentration (mg/L)	Measurement
Conventional fertilizer (NPK)	20	10 mg of NPK solute 0.5 L of distilled water
	50	25 mg of NPK solute 0.5 L of distilled water
	100	50 mg of NPK solute 0.5 L of distilled water
Nanofertilizer (Ca-Al LDH)	20	10 mg of NPK solute 0.5 L of DMSO
	50	25 mg of NPK solute 0.5 L of DMSO
	100	50 mg of NPK solute 0.5 L of DMSO
Aluminium nitrate ($Al(NO_3)_3$)	20	10 mg of NPK solute 0.5 L of distilled water
	50	25 mg of NPK solute 0.5 L of distilled water
	100	50 mg of NPK solute 0.5 L of distilled water

Determination of Chlorophyll Content

After six weeks, the chlorophyll content of *Z. mays* plants was measured by grinding 100 mg of fresh leaves prior transferred it into 11 different test tubes. Subsequently, each test tube was filled with 10 mL of 80% acetone and kept at 4°C for 24 h. Within 10 minutes, the resultant mixture was centrifuged at 1250 rpm to separate the supernatant from the pellet [23]. Next, the supernatant was filled with 80% acetone until it reached 10 mL of volume in the test tube. Finally, this extract was made ready to be analyzed by UV-vis Spectrophotometer to which the chlorophyll absorbance was measured at 645 nm and 663 nm of wavelengths to calculate the contents of chlorophyll *a* and *b*, respectively, using the formula below:

$$C_a\left(\frac{mg}{g} fresh\ leaf\right) = 0.0127 \cdot D_{663} - 0.00269 \cdot D_{645}, \quad \text{Equation 1}$$

$$C_b\left(\frac{mg}{g} fresh\ leaf\right) = 0.0299 \cdot D_{645} - 0.00468 \cdot D_{663}, \quad \text{Equation 2}$$

where $C_a\ \left(\frac{mg}{g}\right)\ \ and\ C_b\ \left(\frac{mg}{g}\right)$ are the contents of chlorophyll *a* and *b*, while $D_{645}\ and\ D_{663}$ are the absorbance values based on UV-vis Spectrophotometer reading.

Determination of Carotenoid Content

The carotenoid content in the leaves sample was conducted in the same manner as the chlorophyll content analysis but with a different wavelength used to measure the absorbance value at 440.5 nm. The content of the carotenoid was then computed using the formula below:

$$C_{car}\left(\frac{mg}{g} fresh\ leaf\right) = 0.004695 \cdot D_{440.5} - 0.000268\,(C_a + C_b), \quad \text{Equation 3}$$

where $C_{car}\ \left(\frac{mg}{g}\right)$ is carotenoid content, and $D_{440.5}$ is the absorbance value of carotenoid according to the UV-vis Spectrophotometer reading.

Statistical Analysis

The data obtained from the plant evaluation were subjected to a one-way analysis of variance (ANOVA) in a completely randomized design with Data Analysis ToolPak in Microsoft Excel. Means were compared using Tukey's test at a 5% level. Note that the p-values <0.05 were considered significant [23].

RESULTS AND DISCUSSION

Plants' Growth Rate Analysis

The height of grain corn (*Z. mays*) was measured from the ground level to the highest leaf using a graduated meter rod, and the result is shown in Figure 1. The outcome illustrates the growth rate for *Z. mays* plants utilizing nanotechnology fertilizer from the beginning (week 0) up to week six. Besides that, the bar graph shows that until week six, *Z. mays* plants employing nanofertilizer experienced increasing growth rates in height, leaf length, and leaf width. These results demonstrate that nanofertilizer indeed provides the nutrients required for plant growth.

Even though it substantially enhanced the rate of plant development, evaluating this nanomaterial's effectiveness compared to other treatments, such as NPK fertilizer and $Al(NO_3)_3$, was also necessary. The rate of growth parameter means values for *Z. mays* up until week six between these three treatments are shown in Figure 2. The conventional fertilizer recorded the highest growth rate compared to nanofertilizer, $Al(NO_3)_3$, and control. The most apparent explanation for the acceleration of *Z. mays*' growth after applying conventional fertilizer was believed to be its chemical properties, which provide crucial inorganic macronutrients for plant development like nitrogen,

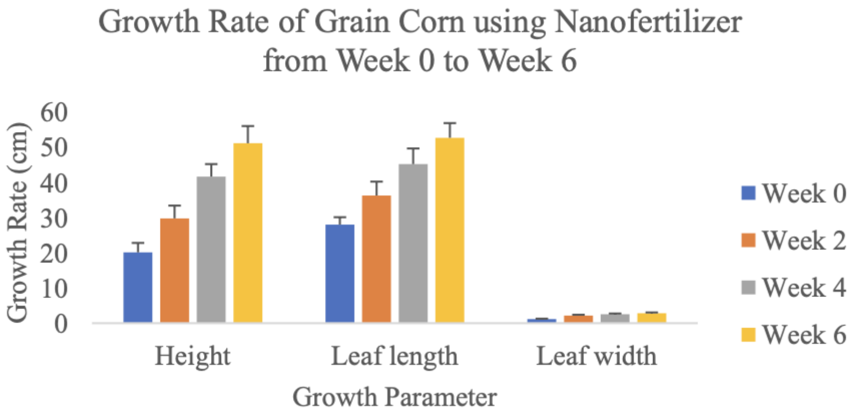

Figure 1. Grain corn growth rate using nanofertilizer from week 0 until week six

phosphorous, and potassium. Apart from that, *Z. mays* have long been renowned for its high nitrogen fertilizer needs, as nitrogen is a constituent of many significant molecules, including proteins, nucleic acids, and chlorophyll [25,26]. Potassium and phosphorus have also been vital for photosynthesis in plants. Here, phosphorus is mainly involved in intermediary metabolism, while potassium, is a precursor of respiration and acts as an osmoregulator, regulating the osmotic potential of cells [27].

In contrast, the nanofertilizer with the second-highest growth rate only contained calcium nitrate. This composition supplies the plant sample with fewer essential nutrients than standard NPK fertilizers, which offered the plant all necessary macronutrients. Note that nitrogen in nitrate plays a significant role in this situation as a critical component required for plant growth. However, it also carried calcium, a secondary macronutrient needed by plants. This explains why the *Z. mays* plant utilizing nanofertilizer grew slower than the plant receiving conventional fertilizers. Even though the nanofertilizer ranked second in elevating the plant growth rate, it has been recommended as a good alternative to NPK fertilizers since they can ramp up nutrient uptake by the plants and reduce the leaching issues. That is driven by the fact that it is larger than the ions, which enables it to be held in the soil and consequently leads to a minimal environmental issue. Ultimately, $Al(NO_3)_3$ had the lowest growth rate compared to control, nanofertilizer, and NPK fertilizer. This happens because the aluminium in $Al(NO_3)_3$ does not constitute one of the crucial macronutrients required for plant growth.

The statistical analysis was performed to see what further commercial fertilizers and nanofertilizer differed based on their effectiveness in enhancing plants' growth. The percentage differences between these three treatments were calculated based on the formula below, and the results are displayed in Table 2.

$$Percentage\ differences = \frac{treatment - control}{control}\ x\ 100. \quad \text{Equation 4}$$

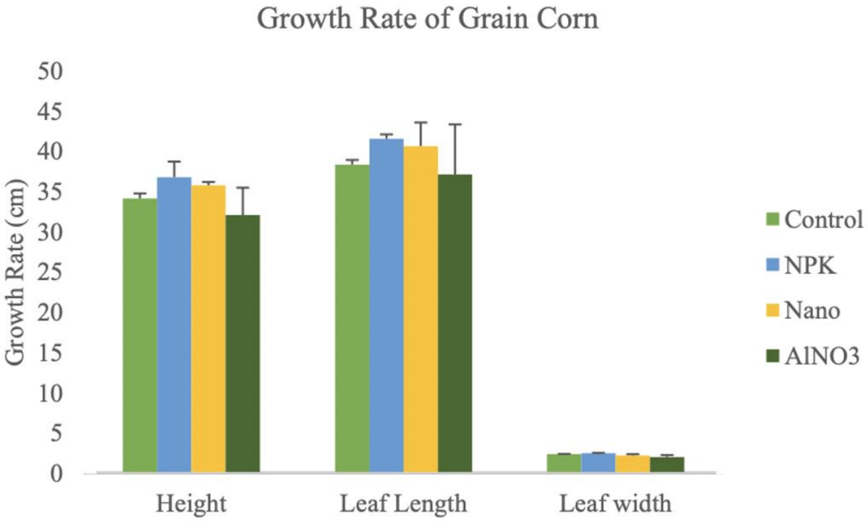

Figure 2. Mean values for grain corn's growth rate after week six

Table 2. Percentage differences in growth rate between control and treatment

Treatment	Growth Parameter		
	Height	Leaf length	Leaf width
Conventional fertilizer (NPK)	7.76	8.36	4.29
Nanofertilizer (Ca-Al LDH)	4.79	6.00	-15.81
Chemical fertilizer ($Al(NO_3)_3$)	-6.00	-3.21	-7.47

The result portrays no significant difference between conventional fertilizer and nanofertilizer as the p-value is more than 0.05, and the percentage difference is less than 3%. This demonstrated that nanofertilizer has the potential to improve its effectiveness, that is, through the incorporation of NPK fertilizer inside its host (Ca-Al LDH). Once that is done, it will significantly assist in enhancing the *Z. mays* growth rate since its host alone has improved the growth comparable to that of conventional fertilizer.

Plants' Critical Concentration

The optimum concentration of fertilizer used was examined to determine the successful nutrient uptake across the plant. Hence, this theory is supported by the fact that low nutrient concentrations can lead to nutrient deficiencies. In contrast, nutrient concentrations higher than the ideal concentration have no beneficial effects on plant growth. The difference in growth parameters based on the concentration of 20, 50, and 100 mg/L of each fertilizer is shown in Figures 3 to 5.

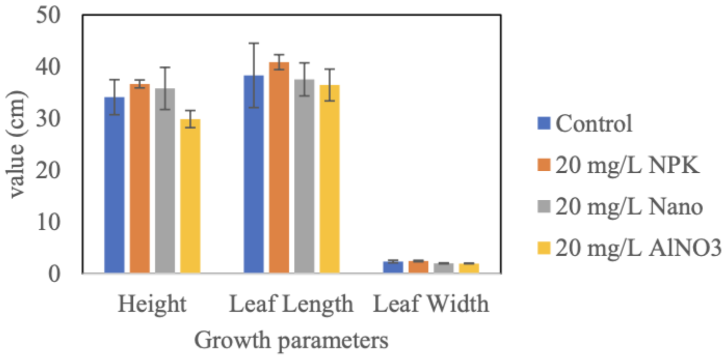

Figure 3. The growth rate of grain corn for the concentration of 20 mg/L

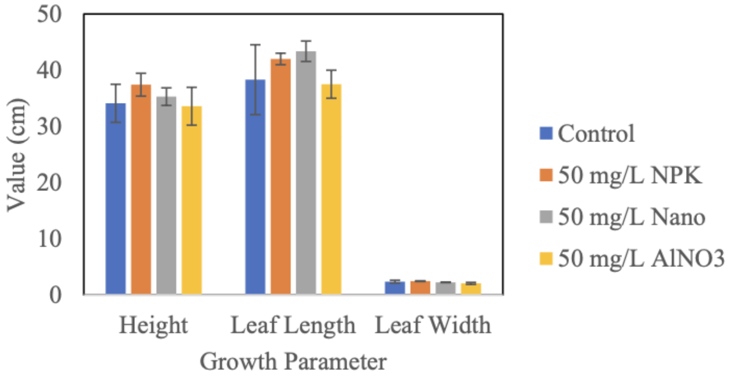

Figure 4. The growth rate of grain corn for the concentration of 50 mg/L

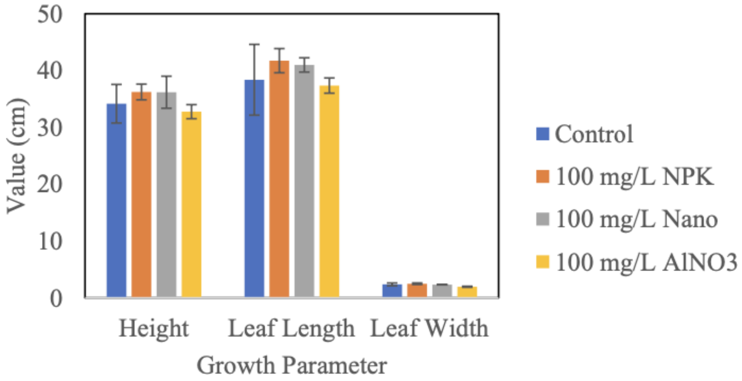

Figure 5. The growth rate of grain corn for the concentration of 100 mg/L

The result reveals that a 50 mg/L dosage of conventional fertilizer yields the highest growth measurements for height, leaf length, and width. Conversely, the maximum growth measurement for nanofertilizer was thought to occur at 100 mg/L. Meanwhile, for $Al(NO_3)_3$, the highest growth parameter measurement was recorded at 50 mg/L. These data extrapolate the optimum concentration for each fertilizer upon applying it to the plant. In contrast to nanofertilizer, conventional fertilizer simply necessitates a small dosage because it contains all the nutrients required for plant growth.

Even though NPK fertilizer recorded the maximum growth parameter measurement of 50 mg/L, the leaf length measurement for nanofertilizer exceeded the value of the NPK fertilizer leaf length measurement, as shown in Figure 4. Note that these findings are linked to the chlorophyll *a* content in Table 3, which suggests that nanofertilizer records the greatest value for chlorophyll *a* content among concentrations of 50 mg/L. The measurement of the leaf length for nanofertilizer with a concentration of 50 mg/L can be justified since the higher amount of chlorophyll *a* shows the high presence of chloroplast in the leaf structure.

Chlorophyll and Carotenoid Content

The chlorophyll and carotenoid content of *Z. mays* after applying various fertilizers with different concentrations are shown in Table 3. Both of these content measurement was analyzed as it has been linked to the photosynthesis process, which affects the growth parameter for the tested plant. The chlorophyll is primarily a photo-receptor that processes the energy of light used in photosynthesis. This photosynthesis process is the source of the energy and nutrients required for plant growth. The carotenoid also functions in photosynthesis as a light harvester [28]. Even though it is not involved directly with the photosynthetic pathway, it can still contribute to photosynthesis by using singlet-singlet excitation transfer to transport its light to chlorophyll [29].

Based on the data, a nanofertilizer with 100 mg/L has recorded the highest chlorophyll *a* content, whereas the high chlorophyll *b* content belongs to NPK fertilizer with a 20 mg/L concentration. As calcium elements are present in nanofertilizer, this can be used to explain why the chlorophyll concentration is rising [30]. Other than that, 100 mg/L nanofertilizer can subsequently be justified as having the highest chlorophyll *a* content since it is the most concentrated and have a high percentage of calcium compared with 20 mg/L and 50 mg/L concentration. On the other hand, all plant samples' physiological characteristics based on carotenoid content showed remarkably comparable values, suggesting that nanofertilizers are ineffective at promoting the development of carotenoids. Perhaps this is due to the composition of the carotenoid itself, which requires a longer time to be developed since it is comprised of eight isoprene units with a 40-carbon skeleton [29].

Table 3. Chlorophyll and carotenoid content of grain corn

Treatment	**Concentration (mg/L)**	**Physiological attributes**		
		Chlorophyll *a*	**Chlorophyll *b***	**Carotenoid**
Control		0.004214	0.009114	0.001644
Conventional fertilizer (NPK)	20	0.007749	0.015819	0.002224
	50	0.005235	0.010878	0.002160
	100	0.005988	0.011785	0.001728
Nanofertilizer (Ca-Al LDH)	20	0.004427	0.013170	0.002160
	50	0.006989	0.011301	0.002413
	100	0.017068	0.006141	0.001482
Chemical fertilizer ($Al(NO_3)_3$)	20	0.001804	0.012835	0.001719
	50	0.006741	0.012693	0.002178
	100	0.008649	0.006497	0.001592

CONCLUSION

The nanotechnology fertilizer successfully shows a stable growth rate from the germination phase until week six of growth through the measurements of plants' height, leaf length, and leaf width. It also recorded the second-highest growth rate compared to conventional fertilizer and $Al(NO_3)_3$. The critical concentration for nanofertilizer is 100 mg/L. In contrast, a concentration of 50 mg/L shows the maximum chlorophyll *a* content, indicating that at this point, the nanofertilizer has successfully increased the plant sample's measurement of its leaf length. Apart from that, this finding suggests that nanofertilizer can potentially increase the growth rate of grain corn (*Z. mays*) plants more than commercial fertilizers with the aid of intercalating the commercial fertilizer inside its host.

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