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Exploring The Potency of Microalgae-Based Biofertilizer and Its Impact on Oil Palm Seedlings Growth

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ABSTRACT

Indonesia is a major producer of palm oil. Consequently, the use of chemical fertilizers has become more extensive. Microalgae represent a potential alternative for enhancing and protecting crops based on their cell elements. This study applies dry biomass or liquid culture formulation of the green microalgae *Haematococcus pluvialis* to the rhizosphere of oil palm pre-nursery as a biofertilizer. Soil application of microalgae biomass of 0.5 g/l (MA) or liquid culture of 10% (v/v) (BCMA) is carried out to assess its effects on 4-months-old oil palm at the nursery stage. The compatibility test between microalgae and bio fungicide agents in agricultural practices, *Trichoderma* spp., is also tested on both microalgae formulations. The result shows that both microalgae biomass and liquid culture, alone or combined with *Trichoderma* spp., give a better growth performance to the oil palm. The application of MA and BCMA result in a maximum increment of plant height, leaves count, and chlorophyll content. Furthermore, the application of BCMA gives better oil palm growth performance, which may probably be influenced by the accessibility of nutrients for microalgae growth. The study reveals that soil application of microalgae as biofertilizers can improve oil palm growth performance.

INTRODUCTION

Oil palm is one of the primary strategic commodities in Indonesia by contributing between 1.5 - 2.5 percent of its gross domestic product (GDP) (CPOPC, 2020). Indonesia is currently leading as the largest palm oil producer globally (Ajeng et al., 2020). To provide nutrient availability in an oil palm plantation, the use of chemical fertilizer is a common standard practice to gain higher productivity. Extensive use of chemical fertilizers in plantations can potentially cause a diminishment of ecosystem status and a decrement of soil biological characteristics (Garcia-Gonzalez & Sommerfeld, 2016). On the other hand, soil quality conservation is essential for commercial plantations like oil palm sustainability. Therefore, optimizing and substituting chemical fertilizer with biological-based organic fertilizers, also known as biofertilizers, is a wiser alternative. Biofertilizers are living microorganisms or natural compounds derived from microorganisms (bacteria, fungi, and microalgae). Due to their eco-friendly characteristics, biofertilizers are considered a sustainable alternative to substitute synthetic or chemical fertilizers. Harnessing biofertilizers may improve the biological and chemical properties of the soil, such as restoring soil fertility, stimulating plant growth, affecting plant production, and diminishing environmental pollution (Abdel-Raouf et al., 2012).

In the last decade, the introduction of beneficial microbes in cell biomass or microbial phytohormones and metabolites in fertilizers is considered an effective way to promote plant growth. The use of microalgae-based biofertilizers is similar to compost application in reducing the application of chemical fertilizer in plantations due to their capacity

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to increase soil fertility and crop yields (Abinandan et al., 2019; Alvarez et al., 2021; Chia et al., 2020) landfills. The secondary metabolites and bioactive compounds of microalgae are effective and promising tools used as fertilizer agents that might contribute to high crop productivity (Kang et al., 2021). By producing metabolites such as phytohormones, and polysaccharides, green microalgae might contribute to agricultural production by several modes such as (1) nutrient fertilization agent through the release of insoluble or immobile nutrients in the soil, (2) carbon organic matter enricher due to biomass photosynthetic capacity of microalgae, and (3) promoter for mineralization processes of macro and micronutrient that implied improvement of soil organic matter and enhance the utilization of macro and micronutrients in soil and rhizosphere, and (4) phytohormones (gibberellins, auxin, and cytokinin) producer that are known to play crucial roles in plant development (Gonçalves, 2021; Kholssi et al., 2019; Ortiz-Moreno et al., 2019).

Some studies have proven that the utilization of algae-based fertilizer can improve the growth of rice, maize, onion, mungbean, cereals, date palm, and tomato (Dineshkumar et al., 2019; Pereira et al., 2009; Saadaoui et al., 2019; Tripathi et al., 2008). Amelioration of the nitrogen demand, increment of acetylene reduction activity (ARA), and diminishing stress impacting the growth and yield are found in the application of blue-green algal-based biofertilizer to the rice plants (*Oryza sativa* L.) (Dash et al., 2016; Pereira et al., 2009). Garcia-Gonzalez & Sommerfeld (2016) find the rapid germination rate and increment of plant growth and floral production in tomato plants after applying the green algae *Acutodesmus dimorphus* culture in the form of cellular extract or dry biomass. Coppens et al. (2016) demonstrated similar result in the use of microalgae *Nannochloropsis* into tomato crop which also improved the quality of the fruits with an increment in the sugar and carotenoid content of tomato fruits.

Microalgae can also be considered an option for synthetic fertilizers since they can be cultivated in dry seasons and by using a bioreactor system. Microalgae cultivation is more time effective, consistent, and reliable (Jochum et al., 2018; Saadaoui et al., 2016). The typical process of microalgae-based fertilizer consists of harvesting the microalgal dry biomass, its cell extract, or its cell suspension from the cultivation media and can immediately be applied onto the soil or through foliar spraying (Behera et al., 2021; Chiaiese et al., 2018; de Souza et al., 2019). Some use carriers or certain media to maintain viability (Chiaiese et al., 2018). Although potential benefits of microalgae biofertilizer in many monocotyledon plants have been recorded, there is no report on the utilization of microalgae in oil palm. This study focuses on applying chlorophyte microalgae *Haematococcus pluvialis*, unicellular green microalgae which grow in fresh and marine water, as biofertilizers in oil palm. The richness of micronutrients (Ca, Cu, Fe, Mg, Mn, P, and Zn) in *H. pluvialis* biomass allows plants to uptake and accumulate these elements in their cells, enhancing plant growth (Serwotka-Suszczak et al., 2019).

The oil palm indigenous beneficial fungi, *Trichoderma* spp., are commonly employed in many crop plants as a plant-growth promoting bio inoculant and biocontrol for various plant pathogens (Contreras-Cornejo et al., 2013; Situmorang et al., 2013). In this research, *Trichoderma* spp. isolated from oil palm basal stem rot endemic area (Situmorang et al., 2013) are used to assess compatibility between *H. pluvialis* and this root endophytic fungus. This study aims to observe the effects of the application of microalgae-based biofertilizer on oil palm (*Elaeis guineensis*) seedlings in a pre-nursery stage.

MATERIALS AND METHODS

Study Site and Experimental Design

The experiment was conducted from March to October 2020 in an oil palm nursery located in Sentul, Bogor, West Java, Indonesia. A randomized complete block design was performed to evaluate various microalgae treatments' impact on oil palm plants. Experiments were conducted with three replicates per treatment with 45 days of cultivation time (under control conditions in the nursery).

In Planta Application

The selected isolates were formulated as consortia (Table 1) and tested in oil palm seedlings pre-nursery stage. In total, 300 ramets with an age of one-month-old from Tenera (Dura x Pisifera) oil palm ortets were used. The development of palm height (IH), the average leaf number (AL), the shoot's dry mass per plant (SM), and the concentrations of photosynthetic pigments (CP) were recorded. The trial consisted of 50 seedlings for each treatment. Five seedlings representing each treatment were sampled for dry shoot weight and destructive root's nutrient content to measure the effect of applied treatment.

Table 1. The term of in-planta trial

Treatment	Description
MA	0.5 g/l microalgal biomass
BCMA	10% (v/v) microalgae liquid culture
MATr	MA + *Trichoderma* spp.
BCMATr	BCMA + *Trichoderma* spp.
C	No treatment (control)
F	Commercial fertilizer NPKMg (15-15-6-4)

Source and Preparation of Isolates Used

Microalgae *Haematococcus pluvialis* (UTEX 2505) from the UTEX culture collection of algae and *Trichoderma* spp. from Sinar Mas Culture Collection (SMCC) were used in this study.

For inoculum preparation, *H. pluvialis* was cultured in Bold's Basal Medium. The stock culture was inoculated (10^4 cell/ml) into the filtered Palm Oil Mill Effluent (POME) medium with 0.30 g/l NPK fertilizer (15:15:6.4). The pH level of all media was adjusted to 7.2 – 7.4. All cultures were maintained at a standard temperature of 25 ± 2°C, applied aeration, under light intensity 2-3 Klux (photoperiod 24:0 h L/D cycle) for 15 days. The microalgae biomass was harvested by centrifugation at 4,000 rpm for 5 minutes, and the collected biomasses were air-dried at 50°C for five days.

Trichoderma spp. were cultured in Potato Dextrose Agar (PDA) medium and incubated for 5-7 days. Spores were harvested using sterile distilled water to a Petri dish containing fungal spores. Their suspension was poured into a 40 ml conical tube. The number of spores was quantified using a hemocytometer. Spores were then inoculated into rice bran. Every ten grams of bran were applied with microalgal biomass and microalgae liquid culture.

Determination of Macronutrient and Micronutrient Content

Both nutrient content of MA and BCMA were analyzed using the inductively coupled plasma (ICP) method. The concentration of macronutrients (N, P, K, and Mg) and micronutrients (Ca, B, Cu, Fe, Mg, Mn, and Zn) in root samples after 45 days of treatment was measured using the ICP method. The selected nutrients were the essential nutrients required for oil palm seedling growth.

Measurement of the Vegetative Growth Parameters and Leaf Pigments

Root and shoot lengths were manually measured, among other vegetative growth characteristics. The pigment contents were measured using a spectrophotometric approach and calculated using the method described by Lichtenthaler & Buschmann (2001). Briefly, each plant's oil palm leaves (1 g) were homogenized in 4 ml of 80% acetone and 0.1% (w/v) $CaCO_3$ and incubated overnight at 4°C. The measuring absorption was set up at 663 nm and 645 nm.

Data Analysis

All recorded plant growth parameters were compared and statistically assessed using One-way ANOVA and Duncan's Multiple Range Test (DMRT) tests. The data was generated using RStudio, a visualization of ggplot programs built into the R software. The data provided are the means ± standard errors of three replicates at a significant threshold of $p<0.05$. The data had to be centered and scaled before the analysis.

RESULTS AND DISCUSSION

Macronutrient Characteristics of Microalgae Biomass and Liquid Culture

Microalgae are known for their cell's carbohydrates, protein, and hormones. The possible mechanism of action of microalgae-based biofertilizers in improving nutrient availability for plant growth depends on microalgae nutrient content (Toribio et al., 2020). The inductively coupled plasma (ICP) analysis is performed and reveal that all recorded macronutrient (N, P, K, Mg) in microalgae with liquid culture (BCMA) are higher than without liquid culture (MA). The total N, P, K, and Mg of BCMA were 9.61, 5.92, 2.18, and 2.44 fold higher than MA, respectively (Fig. 1). Several environmental factors such as light, pH, temperature, and nutrition mainly affect microalgae growth, biomass yields, and the micro and macro metabolites (Rasdi & Qin, 2015). We assumed that the higher BCMA/MA ratio was due to the accumulation of nutrient content from microalgae biomass and liquid media. Such a similar result compared with the previous report argued that the liquid culture of *Dictyosphaerium chlorelloides* in Zehnder medium showed the nitrogen, phosphorus, potassium, and magnesium content were 8.68, 5.76, 2.88, and 0.46 fold higher than its biomass content, respectively (Babiak & Krzemińska, 2021; Kumar et al., 2017).

The basal medium influenced the high nutrient content found in BCMA, while MA only consisted of microalgal biomass. The microalgae cell contained more nitrogen and phosphorus that can be used as biofertilizer (Fig. 1). The macro elements (potassium and magnesium) might be essential for redox reactions and plants' metabolism. Nutrient microalgal biomass and microalgae liquid culture might play a vital role in improving plant growth performance.

The study assessed the impact of microalgae-based biofertilizer from the growth characteristics, chlorophyll content, and nutrient content on the palm's root biomass. In general, the findings show that microalgae affected the growth of treated plants (Table 2), except for dry shoot weight (Fig. 2).

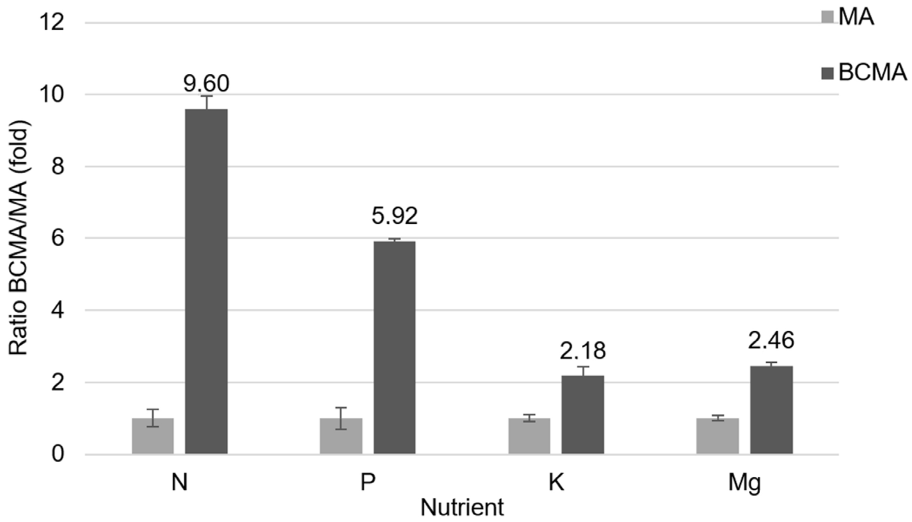

Fig. 1. Nutrient content of MA and BCMA obtained from ICP analysis. Data represent the mean ± the standard error (n=3)

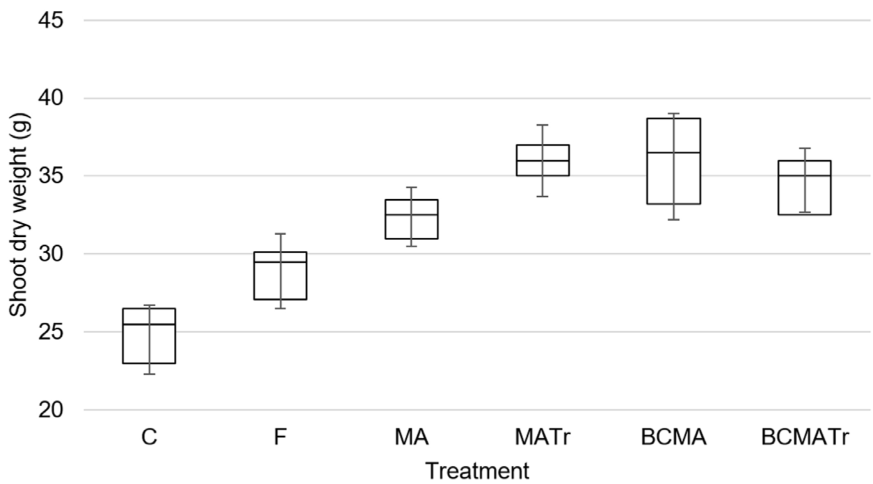

Fig. 2. Effects of several treatments on shoot dry weight of oil palm seedlings in the pre-nursery stage. There was no significant difference among all treatments (p>0.05) with One-way ANOVA

Table 2. Vegetative growth parameters of oil palm seedlings in various treatments at 45 days old seedlings

Treatment	Description	Vegetative Growth Parameter	
		∆ Height (cm) H_{t45}-H_{t0}	Average of Leaf Number
C	No treatment (control)	18.04±0.70[d]	6.28±0.18[c]
F	Commercial fertilizer NPKMg (15-15-6-4)	18.98±1.61[d]	6.43±0.61[c]
MA	0.5 g/L microalgal biomass	22.09±0.48[c]	8.28±0.29[b]
MATr	MA + *Trichoderma* spp.	22.90±0.84[c]	8.72±0.21[b]
BCMA	10% (v/v) microalgae liquid culture	25.23±1.63[a]	8.78±0.65[a]
BCMATr	BCMA + *Trichoderma* spp.	23.74±0.84[b]	8.84±0.62[a]
	F	34.32	42.22
	p-value	0.03	0.04

Remarks: The growth parameter is represented as the mean ± the standard error (n=25) followed by different case letters indicating significant differences (at p ≤ 0.05) using One-way ANOVA and Duncan's Multiple Range Test (DMRT) test

Growth Response of Oil Palm Seedling in Pre Nursery Stage

General observations of the treated plants indicated that microalgae treatment applied 45 days after transplanting to the pre-nursery stage enhanced plant growth. The height of palms treated with BCMA or BCMATr tends to have a higher increment than those treated with MA, MATr, or control (Table 2). The variation of palms height in each measurement was about 15 to 25 cm. Among the treatments, BCMA had a significant effect on height increment ($p<0.05$), followed by BCMATr, which have significantly higher values than MA, MATr, F, and C (Table 2).

The average leaf number (AL) was positively affected by all microalgae-based treatments and is significantly higher compared to both negative (C) or positive (F) controls ($p<0.05$) (Table 2). Consecutively, the highest to the lowest average of leaf numbers are found at the treatment of BCMA, BCMATr, MATr, MA, F, and C. Moreover, the BCMA and BCMATr treatments influenced better plant performances than those applied with MA and MATr, suggesting that supplementation with 10% (v/v) microalgae liquid culture is a suitable formulation. The total shoot dry weight (SDW) is not significantly different among all treated plants (Fig. 2). It is implied that the treatment of both microalgal biomass and microalgae liquid culture exhibited relatively lower effects on shoot dry weight. Nonetheless, these are slightly higher than those treated with chemical fertilizer (F). The 45 days old oil palm seedlings treated with BCMA, MATr, BCMATr, MATr, and F exhibited root lengths of 33.40, 32.50, 30.80, 30.54, 28.60 cm, respectively, compared to control plants with 26.80 cm length.

The study indicates that microalgae in liquid culture (BCMA and BCMATr) affected a better growth of oil palm seedlings. The addition of microalgal cells and cultivation medium improved microalgae's condition in terms of stability and viability in plant rhizosphere are, in fact, not a proper habitat for microalgae (Alvarez et al., 2021). Suspension medium can maintain the microalgal cells before directly contacting the soil, and thus, the living microalgae cells increase the utilization of nutrients in the plant growth medium, and the quality of beneficial microorganisms in the soil ecosystem also increase (Agwa et al., 2017; Mahanty et al., 2017; Maqubela et al., 2010). Therefore, by producing growth-promoting compounds, releasing many nutrients, and fixing atmospheric nitrogen in a form that plant roots can absorb, their efficiency in augmenting plant growth can be improved. In addition, the role of microalgae biomass, which contains sufficient nutrients and growth regulators, can enhance cell division and expansion, as reflected in the improvement of plant vegetative growth. Elarroussi et al., (2016) found that polysaccharides extracted from microalgae *Spirulina platensis* significantly promoted the development of *Capsicum annum* and *Solanum lycopersicum*, as demonstrated by the increment of plant weight and leaf size/number. Similarly, seaweed polysaccharides have been shown to have various biological effects on plant growth (López-Arredondo et al., 2013).

Our results revealed a positive effect of MATr or BCMATr on the increment of oil palm height and the number of leaves per plant (Table 2), the total SDW (Fig. 2). Similar results were reported by Elshahawy & El-Sayed (2018) that the combination of *Trichoderma* spp., and microalgae *Chlorella vulgaris* extracts applied in maize were more efficacious in improving the plant height and plant dry weight biomass. Besides improving plant growth performance, the efficacy of *Trichoderma* spp. was enhanced with *C. vulgaris* extract in improving the anti-aging ability of maize, thereby reducing the incidence of corn late wilt (Elshahawy & El-Sayed, 2018). This research results suggest that the addition of *Trichoderma* to the microalgae-based biofertilizer in treatment with MATr and BCMATr was not hindering microalgae's potency. Instead, it should improve the product's power to benefit biocontrol. However, further study on the relation of microalgae-*Trichoderma* influence to the oil palm-pathogen interaction is required for confirmation.

Leaf Concentration of Photosynthetic Pigments

Combined data analysis for the pre-nursery stage shows a significant interaction between microalgae biomass or liquid culture treatments with or without combination with *Trichoderma* spp., in particular, chlorophyll a, b, and total chlorophyll content, which evaluated on 45 days after treatment. The results show significant increments in all microalgae-based treatments compared to C and F (Fig. 3).

H. pluvialis is widely known for its high carotenoid content (Casella et al., 2020; El-Baz et al., 2018; Serwotka-Suszczak et al., 2019). Microalgae utilize accessorial pigments known as carotenoids throughout their photoautotrophic development phase. They have the functional benefit of light-harvesting, protein assembly in photosystems, and defense against photo-induced free radical exposure (Guedes et al., 2011). In agricultural practice, the carotenoid content of microalgae which are also recognized as antioxidants and fertilizers may affect soil improvement by promoting mineralization processes of macro-and micronutrients (Gonçalves, 2021). Additionally, the significant increments of chlorophylls a, chlorophylls b, and total chlorophyll content in all treated plants are due to the positive effects of microalgae biomass and microalgae liquid culture application. These applications have a role in supplying N and P nutrients as exhibited by ICP analysis (Fig. 1), where N is responsible for enhancing the chlorophyll content. Thus, microalgae-based biofertilizer in the form of 0.5 g/l microalgae biomass or a 10% (v/v) microalgae liquid culture is a potential substitution for nitrogen fertilizers needed by the seedlings to enhance the chlorophyll content in the leaves.

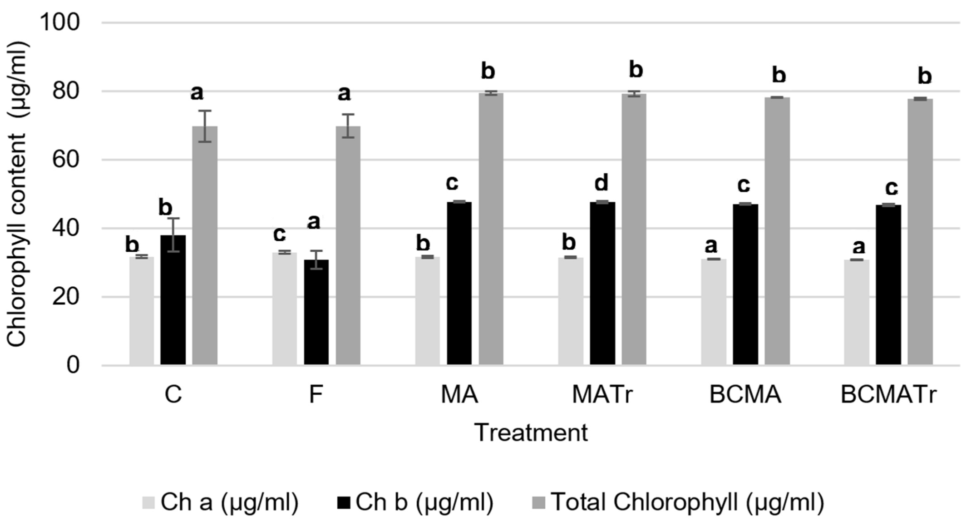

Fig. 3. Concentration of photosynthetic pigments in leaves; chlorophyll a (µg/ml), chlorophyll b (µg/ml) in pre-nursery stage. Data represent the mean ± the standard error (n=3). Different letters indicate a significant difference ($p<0.05$), using One-way ANOVA and Duncan's Multiple Range Test (DMRT) test

The Concentration of Nutrient in the Oil Palm Root

N, P, and K are essential macronutrients extensively utilized as fertilizers in modern agricultural methods. The NPK concentrations in root biomass are evaluated to assess the effect of microalgae on oil palm nutrient uptake. In this study, the four macro elements, i.e., nitrogen, phosphorus, potassium, and magnesium, are enhanced in all treated plants compared to control. Among the four macroscopic elements, the nitrogen in all the treated plants is notably highly increased compared to the control. The highest N concentrations are recorded in oil palm seedlings treated with BCMA, MA, MATr, BCMATr, and F, which are increased by 90.23%, 79.82%, 72.74%, 72.41%, and 15.93%, respectively compared to control (Fig. 4).

The plant N profile is described in the palms root concentration and the chlorophyll readings during the oil palm cultivation. It indicates that N absorbed by roots is partly invested in photosynthetic organelles. Moreover, the nitrogen absorbed by plants treated with microalgae is higher than plants treated with chemical fertilizer (F). It indicated the added-value benefit of microalgae as a prospective N provider to plant-based on their nutrient availability from microalgae biomass and liquid medium content (Fig. 4). However, further analysis and researches are still required.

A similar effect of microalgae treatment is found for P uptake. All microalgae treatments, individually or in combination with *Trichoderma* spp., significantly increase the P contents of oil palm roots compared to both F and C (Fig. 4). Oil palm seedlings treated with MA, MATr, BCMA, BCMATr, and F exhibited the P content increment of 35.71%, 28.57%, 28.50%, 21.43%, and 12.79%, respectively, compared to the untreated plants. The high P compound in the treated plants may be attributed to the expression of the phytase gene characterized in microalgae. Phytase plays a role in solubilizing inorganic P (Rivera-Solís et al., 2014)no gene coding for PAPs have so far been characterized. In this study, six PAP homologue genes were identified and characterized in silico in C. reinhardtii (CrPAP1 to CrPAP6. When microalgae are applied to the soil, biomass decomposition is also considered one of the possible mechanisms for supplying organic P compounds or polyphosphates to increase the available P of plants (Alvarez et al., 2021). The high content of N and P in plant roots treated with microalgae biomass and liquid culture attributes to the positive role of microalgae which improved plant nutrient level due to its richness of macronutrients, polyamines, and vitamins. This study shows that microalgae-based biofertilizers contained high essential plant nutrients such as N and P.

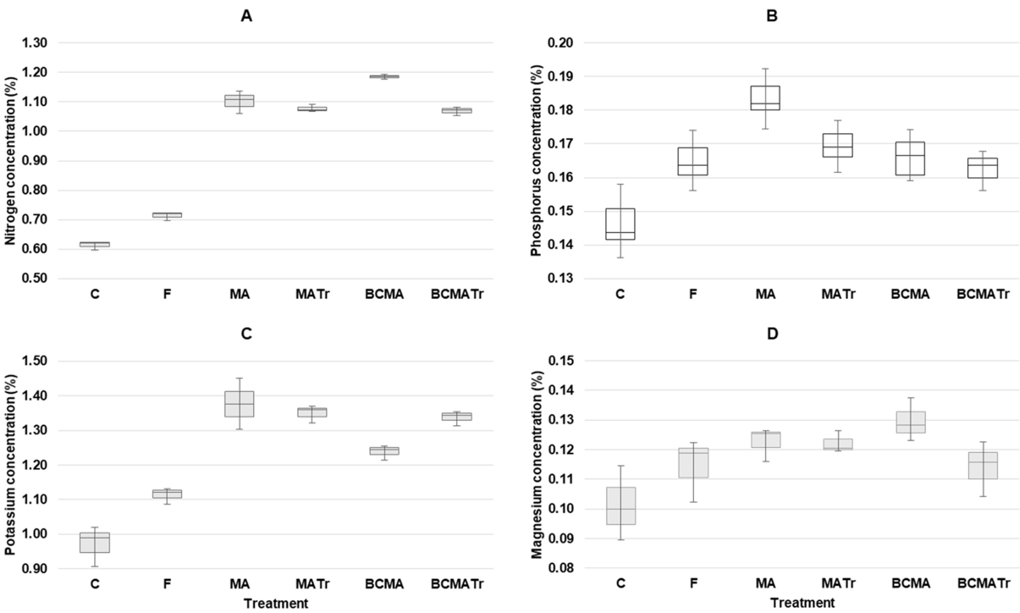

Fig. 4. Nutrient content of: (a) Nitrogen total, (b) Phosphorus, (c) Potassium, and (d) Magnesium, in oil palm seedling's root in the pre-nursery stage

Microalgae-based biofertilizers also affected potassium concentration in roots. Potassium levels observed at treatments of MA, MATr, BCMATr, BCMA, and F were only slightly increased by 39.41%, 35.22%, 33.55%, 23.71%, and 11.63%, respectively, in comparison with control (Fig. 4). The K concentration is found in the treated plants may be caused by the potassium solubilization process of *Trichoderma* spp. (Chen et al., 2021; Halifu et al., 2019). The potassium solubilization process by *Trichoderma* spp. provides nutrient content to the plant and improves microalgae's growth-promoting effect on the oil palm seedlings (Table 2).

Magnesium (Mg) is another essential macronutrient to oil palm seedlings. It activates enzymes related to respiration, photosynthesis, and nucleic acid synthesis. Furthermore, it aids in phosphate metabolism by acting as a carrier of phosphate compounds throughout the plant (Ayanda et al., 2020)namely kieserite, ground magnesium limestone (GML. All treated oil palm seedlings with MA, MATr, BCMA, BCMATr, and F exhibited a level of Mg in the range of 0.11-0.16 ppm (Fig. 4).

Heat-map represents a unique relationship between various treatments to micronutrient concentration of root biomass. Micronutrient elements like boron, iron, manganese, copper, sodium, and zinc are observed in oil palm root samples after 45 days of treatment. The clustering analysis describes all the micronutrient contents' normalized average mean values in both treated and control plants. Boron is found in low concentrations in treated and untreated palms (Fig. 5). Boron is essential for plant pollen germination and cellular activity, such as nucleic acids synthesis, cellular division, and integrity of cellular membranes (Shireen et al., 2018)ion fluxes (H^+, K^+, PO_3^{-4}, Rb^+, Ca^{2+}. The micronutrient heat-map analysis revealed two dominant groups between six treatments based on the range of their nutrient content in the root. The treatment BCMA and BCMATr are clustered together. It might be caused by high nutrient availability in the microalgae growth medium (Fig. 1).

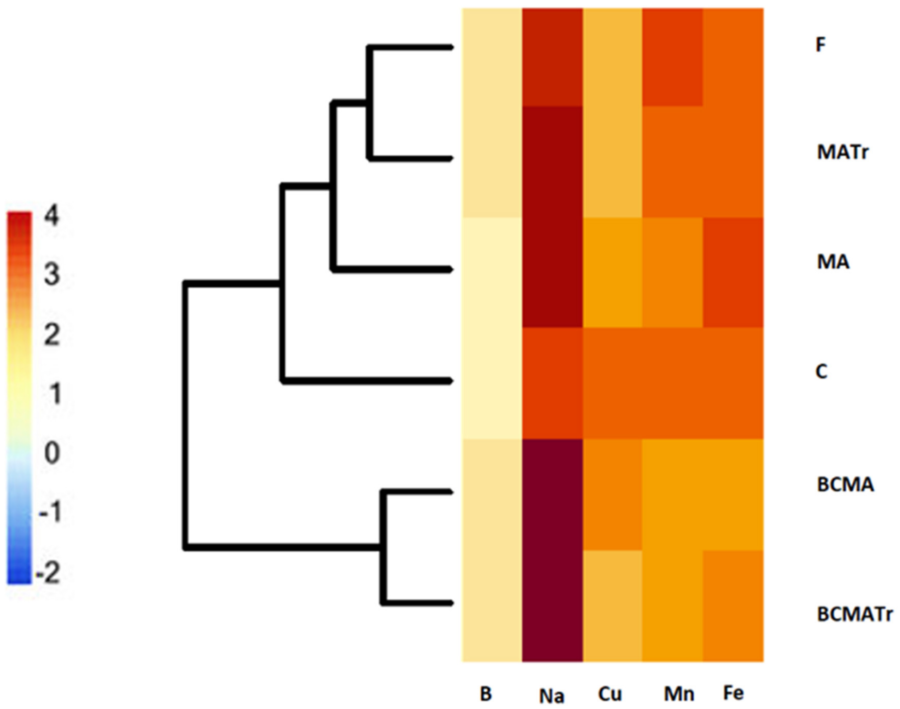

Fig. 5. Heat-map illustrates the normalized mean values (centered and scaled) of micronutrient content from the root sample with three replications

Meanwhile, treatment control, MA, MATr, and F are grouped in different clusters. MATr and F treatment have the same Cu and Fe concentration (Fig. 5), probably caused by siderophore production from *Trichoderma* spp (Zhao et al., 2020). Micronutrients such as zinc, sodium, copper, manganese and iron are found in MA, MATr, BCMA, and BCMATr than the untreated plants. It is possibly due to natural micronutrients available in *H. pluvialis* biomass (Cu, Fe, Mg, Mn, and Zn), which allowed plants to uptake and accumulate these elements in their roots and support plant growth (Serwotka-Suszczak et al., 2019).

The application of microalgae as a biofertilizer leads to better plant growth by improving nutrient absorption, increasing soil's water-holding capacity, and increasing the intermolecular space between soil molecules. The root system receives adequate aeration (Uysal et al., 2015). Green microalgae, such as *H. pluvialis*, incorporate organic C into their biomass through photosynthesis and release polysaccharides that afterward serve as a carbon source and increase soil aggregation and stabilization (Alvarez et al., 2021; Costa et al., 2018). With the high content of sulfated polysaccharide type oligo-carrageenan (OCs) in microalgae *H. pluvialis*, the interaction of oligo-carrageenan with plant plasma membrane receptors may activate plant growth simultaneously (González et al., 2013; Liu et al., 2018). Additionally, It has been found that polysaccharides include a high concentration of functional groups that enable them to bind certain microelements with significant nutritional values, hence increasing the roots' nutrient availability (Kumar & Singh, 2020). This study reveals that Cu, Fe, and Mn micronutrients are more concentrated in the roots when seedlings are treated with microalgae (Fig. 5), which also implies that the microalgal polysaccharides might be more focused act as metal ion chelating agents.

This study reveals that soil application of microalgae cell biomass or formulated in liquid culture as commonly used in oil palm agricultural practices is a potential option for effective fertilizer use. Compared with untreated plants, the dry weight of shoot and the number of leaves per plant of oil palm seedlings applied with microalgae biomass and liquid culture are the highest (Table 2). Several studies have shown that using microalgae in rice plants increases their productivity (Abinandan et al., 2019; Kaushik, 2014; Paudel & Pradhan, 2012). Soil application with blue-green algae is considered as a possible alternative source of N to boost rice productivity (Abinandan et al., 2019). The soil inoculation of *Nostoc* spp., *Hapalosiphon* spp., and *Aurosira fertilissima* have demonstrated improvements in rice seed germination, vegetative rice growth, the weight of rice grains, and rice protein content (Dash et al., 2016; Kaushik, 2014). These improvements are attributed to the growth-promoting hormones of blue-green algae biomass such as auxins, cytokinin, and gibberellic acid (Lu & Xu, 2015). Morais (2013) clearly shows that chlorophyte microalgae, *H. pluvialis* can excrete amino acids and growth-promoting compounds. The presence of these nutrients can promote the growth of soil microbial populations and crop growth

CONCLUSION

This study demonstrates the positive effects of microalgae applied as biofertilizers for oil palm growth. In addition, the application of the oil palm indigenous bio fungicide agent *Trichoderma* spp. to the microalgae-based biofertilizer does not suppress the microalgae potency. It should instead empower the future bio-products in a matter of plant growth-promoting and protecting simultaneously, although further study on the relation of microalgae-*Trichoderma* influence to the oil palm-pathogen interaction is required for confirmation. In conclusion, using a microalgae-based biofertilizer in the oil palm is a prospective idea. Future investigation on biochemical fractionation and agronomical evaluations are beneficial for a future in-depth investigation into microalgae mode of action as biofertilizers. To our knowledge, this is the first report of microalgae used for oil palm, which opens more comprehensive access for future study on its potential as a sustainable alternative for chemical fertilizers.

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