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# Physiological and biochemical traits, antioxidant compounds and some physico-chemical factors of *Spirulina platensis* cultivation as influenced by *Moringa oleifera* leaves extract culture medium enriched with sodium bicarbonate and kanwa

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**The prospects for using *Moringa oleifera* leaves extract (MLE) supplemented with different concentrations of kanwa or sodium bicarbonate ($NaHCO_3$) as a low-cost alternative growth medium of *Spirulina platensis* were evaluated in a small-scale outdoor cultivation system. The present study was aimed to evaluate the potential of MLE growth medium enriched with different concentrations (4 or 8 g $L^{-1}$) of kanwa and $NaHCO_3$ on growth, chlorophyll content, biochemical characteristics, antioxidant compounds and some physico-chemical factors. Jourdan's standard medium was taken as control. The results showed that the growth parameters such as cell population, biomass dry weight, cell productivity and specific growth rate were positively affected in MLE cultivation medium enriched with kanwa or $NaHCO_3$ at different concentration levels. The addition of urea, kanwa or $NaHCO_3$ in MLE cultivation medium at different concentration levels increased significantly ($p< 0.05$) the protein content, the peroxidase and polyphenol oxidase activity, the conductivity, pH, total dissolved solids and salinity from 20 to 25 days of cultivation whereas a decrease in carbohydrate and phenol content was recorded during all the period of the experimentation. The highest values of growth parameters were notably in MLE medium supplemented with urea and kanwa at 8 g $L^{-1}$. The MLE medium enriched with urea and kanwa at 8 g $L^{-1}$ was shown to be an appropriate growth medium that can be used as a low-cost alternative growth medium for commercial cultivation of *S. platensis*.**

**Key words:** Antioxidant compounds, biochemical traits, growth, *Moringa oleifera* leaves extract, physico-chemical factors, *Spirulina platensis*.

## INTRODUCTION

Microalgae is in high demand in many biotechnology sectors such as bioremediation, biofuels, biofertilizers (Kosamia et al., 2020; Markou et al., 2021), cosmetics, biomedicals (Mesquita et al., 2017; Mellou et al., 2019; Wen et al., 2020), aquaculture (Shah et al., 2018) and animal and human nutrition (Molino et al., 2018) because of the biological and commercial value of its products. In this respect, *Spirulina platensis* is one of the most promising microalgae (Lupatini et al., 2017). It represents the most abundant and common photosynthetic, filamentous, multicellular and microscopic microalgae in the tropics and subtropics (Nyabuto et al., 2015). *S. platensis* is commercially produced because of its high digestibility and interesting protein content (46-71%) of the dry weight of the algae, as well as high amounts of essential fatty acids and amino acids, vitamins, pigments (phycobiliproteins and carotenoids) and polysaccharides (Zhu et al., 2018; Corrêa and Teixeira, 2021). This cyanobacterium has gained in importance and international demand not only for its nutritional and therapeutic properties but also for its applications in human and animal nutrition, therapeutics and diagnostics (Panjiar et al., 2017; Ama Moor et al., 2020). It is generally recognized as safe from the US Food and Drug Administration and considered as the most complete food for the future by the Food and Agriculture Organization of the United Nations (Goulamabasse, 2018; Branyikova and Lucakova, 2020).

The growth and the biochemical composition of the biomass produced by S. platensis depend on many factors, the most important of which are temperature, nutrient availability and light (Madkour et al., 2012; Soni et al., 2019). The temperature of the culture medium of *S. platensis* was positively influenced by the addition of $FeSO_4.7H_2O$ and NaCl to Jourdan's medium, while $MgSO_4.7H_2O$, $CO(NH_2)_2$ and $NaHCO_3$ lead to a decrease of the temperature from 31.66 to 25.90°C (Ndjouondo et al., 2017). Production of spirulina with reduced costs is necessary when considering large-scale cultivation for industrial purposes. The cost of nutrients and availability are considered the second major factors influencing the cost of spirulina biomass production after harvesting (Vonshak, 1997). Zarrouk's medium has successfully served as the standard medium for *S. platensis* culture for many years (Zarrouk, 1966). Consequently, many media synthetic have been developed such as CFTIR medium (Venkataraman et al., 1995), OFERR medium (Singh, 2006) and Jourdan medium (Jourdan, 2013). However, they are expensive, require rapidly depleting minerals, and not readily available. The change of the nutrients in Jourdan's medium has the potential to produce a large scale biomass of *S. platensis* and could be suitable for its optimal growth culture conditions that could be beneficial for human's health. According to Ndjouondo et al. (2017), the dry weight, the specific growth rate and the cell productivity of *S. platensis* were positively influenced by the addition of $FeSO_4.7H_2O$, NaCl, $MgSO_4.7H_2O$, $CO(NH_2)_2$ and $NaHCO_3$ to Jourdan's medium at 0.01, 2.5, 0.1, 0.02 and 4 g $L^{-1}$, respectively. Nutrients such as phosphorus, nitrogen, calcium, potassium and iron present in agro-industrial effluents and vegetables can be used to increase microalgae growth. A number of green algal species have been shown to be able to utilize carbonates such as $Na_2CO_3$ and $NaHCO_3$ for cell growth (Emma et al., 2000).

Kanwa, a type of salt, is formed when salt water from a sea or lake evaporates and leaves behind colorful crystals of sodium chloride. It is also called halite or rock salt. Rock salts offer numerous health benefits, such as treating colds and coughs, as well as aiding digestion and contain various levels of trace minerals, such as manganese, copper, iron, and zinc (Nafee et al., 2013). Rock salt contains natural impurities having calcium sulfate ($CaSO_4$) and potassium chloride (KCl) as impurities. It is found in deposits of rock salt, brines, saline lakes, marshes, seawater and saline earth (Nafee et al., 2013).

Plants subject to stress conditions produce cytotoxic activated oxygen that can seriously disrupt normal metabolism, through oxidative damage of lipids, proteins, and nucleic acids (Abbaspour, 2012). In response to stress, plants activate powerful antioxidant systems, both enzymatic (e.g., SOD, POD, catalase, glutathione reductase) and non-enzymatic (flavonoid, PC, carotenoids, vitamins C and E) (Ashraf, 2009; Kahrizi et al., 2012). According to Kasote et al. (2015) and Mostafa et al. (2016), this increase of PPO and POD activity could be correlated to a decrease in oxidative stress and reactive oxygen derivatives produced during photosynthesis and to the high content of phenols which would act as antioxidant by producing an enzymatic substrate to alleviate the harmful effects of reactive oxygen species.

The increase of some physico-chemical parameters such as salinity and total dissolved solids in *S. spirulina* cultivation media had being previously explained by the presence of electrically charged atoms which increase with the evaporation of water in media and to the change

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of the other variables of the culture media due to uptake of nutrients brought by the different concentrations of $NaHCO_3$ in MLE media or by the increase in alkalinity and concentration of dissolved ionic salts resulting from MLE media (Mutanda et al., 2014; Rusydi, 2018). Soni et al. (2019) reported that for flourishing and optimal growth, temperature for *S. platensis* is between 30 and 35°C and pH value between 8.5 and 10.5.

The appropriate organic waste collected from digested sago starch (Miah et al., 2000), molasses (Andrade and Costa, 2007), rice mill effluents (Usharani et al., 2012), palm oil empty fruits bunches (Suharyanto et al., 2014), and digested rotten apple (Mia et al., 2019) were also used as growth media for *S. platensis* culture. Thus, for the mass production of *S. platensis* particularly in developing countries as Cameroon, there is a need to find an effective, cheaper and readily available alternative cultivation media.

Moringa *(Moringa oleifera* Lam.) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has a high nutritional value and an impressive range of medicinal and industrial purposes (Khalafalla et al., 2010; Adebayo et al., 2011; Moyo et al., 2011). Moreover, Moringa leaves extract (MLE) has received enormous attention from the scientific community because of its rich source in hormones, antioxidants, vitamins and minerals such as iron, calcium and potassium as well as vitamins and macronutrients which have plant growth-promoting capabilities and often applied as exogenous plant growth enhancers (Rady et al., 2013; Yasmeen et al., 2013; Khan et al., 2017a, b). Thus, MLE contains appreciable amounts of macro and micronutrients and readily available and cost-effective feed to substitute inorganic fertilizers and support good *S. platensis* growth. To the best of our knowledge, few information is so far available for the use of MLE as *S. platensis* growth media.

The present study was aimed to evaluate the effect of MLE medium supplemented with different concentrations of kanwa or bicarbonate on the growth, chlorophyll content, biochemical characteristics, antioxidant compounds and some physico-chemical factors of *S. platensis* in order to define the optimal growth cultivation conditions.

## MATERIALS AND METHODS

### Plant material and growth medium

The cyanobacterium *S. platensis* strain used in this study was obtained from the culture pond of SAGRIC Common Initiative Group Farm, Douala-Cameroon. *S. platensis* was grown on Jourdan's modified medium consisting of (per liter): 8 g $NaHCO_3$, 5 g NaCl, 3 g $Na_2CO_3.10H_2O$, 2 g $KNO_3$, 0.15 g $MgSO_4.7H_2O$, 0.12 g $(NH_4)_2HPO_4$, 0.05 g $CO(CH_2)_2$, 0.02 g $FeSO_4.7H_2O$ and 0.02 g $CaCl_2$ and maintained at pH 8 by use of dilute 0.2 M NaOH solution. Jourdan's medium was taken as control. For the five experimental groups, the *M. oleifera* leaves extracts (MLE) used as growth medium, was supplemented with 0.05 g $L^{-1}$ of urea (U) and 4 or 8 g $L^{-1}$ of kanwa (NaCl: K) or sodium bicarbonate ($NaHCO_3$: B), respectively as follow: MLE+U; MLE+U+B4; MLE+U+K4, MLE+U+B8 or MLE+U+K8. The depth of the culture in the open concrete tanks was 15 cm with 15 L of MLE. For preparation of *M. oleifera* leaves extracts (MLE), the young moringa leaves were collected from a mature moringa tree from SAGRIC Common Initiative Group Farm. An amount of 40.0 g of young moringa leaves was suspended in 1.0 L of distilled water for 7 days. The suspension was stirred using a homogenizer to help maximize the amount of the extract. The solution was then sieved and filtered through a 30-mesh Tylor net. The extract diluted with distilled water at a 1:8 ratio (v/v) was used as MLE medium. The algae *S. platensis* cells were inoculated at a concentration of 15% (V inoculation/V media). The initial pH of all culture media was adjusted to 8 with 0.2 M NaOH before addition of *S. platensis* cells. Cultures were carried out in 25-L open concrete tanks under daylight in a greenhouse for 25 days. Growth and maintenance of the culture was done at 30 ± 2°C under 12/12 h light-dark cycles. Cultures were agitated by aeration at a flux of 20 L/h provided by a diaphragm pump. Samples were collected every 5 days for assessment of the cyanobacteria growth as well as estimation of biochemical components status. All experiments were carried out with three replicates.

### Growth and productivity parameters determination

*S. platensis* cell populations were determined by direct microscopic counting method described by Usharani et al. (2012). The number of filaments was evaluated using a light microscope (Cyscope® HP, Sysmex-Partec, Japan).

For dry weight concentrations measurement, homogenous suspension of *S. platensis* sample *(*200 ml) was filtered through Whatman no. 1 filter paper and oven dried at 50°C for 48 h. The dry filter containing *S. platensis* dry weight was cooled and weighed. The difference between the initial and final weight was taken as dry weight of *S. platensis* biomass.

The cell productivity of *S. platensis* was calculated according to the formula described by Jarisoa (2005):

$$P = X_2 - X_1 / t_2 - t_1$$

Where $X_2$ and $X_1$ represent the biomass concentrations at the times $t_2$ and $t_1$.

The specific growth rate (μ) was calculated as follows (Göksan et al., 2007):

$$\mu = \ln X_2 - \ln X_1 / t_2 - t_1$$

where $X_2$ and $X_1$ represent the biomass concentrations at the times $t_2$ and $t_1$ respectively.

### Biochemical characteristics determination

Total soluble carbohydrates were estimated by phenol-sulphuric acid method (Dubois et al., 1956). 1 g of plant fresh materials was weighed and digested by hot ethanol 80% two times, each time by 5 mL ethanol and then filtered by Whatman No. 2. Filter paper and the extracts were diluted by distilled water to the volume of 50 mL. 1 ml for each sample was placed in the test tube and then 1 mL

**Table 1.** Variation of cell population, biomass dry weight, cell productivity and specific growth rate of *S. platensis* of Moringa leaf extract media under different concentrations of kanwa or $NaHCO_3$.

| Cultivation media | Treatment | Cell population (cp $mL^{-1}$) | Dry weight (mg) | Cell productivity (g $L^{-1}$ $day^{-1}$) | Specific growth rate (cell $day^{-1}$) |
|---|---|---|---|---|---|
| Jourdan medium | Control | 90037 ± 2363$^a$ | 1.01 ± 0.42$^a$ | 0.25 ± 0.002$^a$ | 0.028 ± 0.009$^a$ |
| | MLE+U | 20783 ± 1258$^e$ | 0.40 ± 0.26$^d$ | - 0.04 ± 0.003$^d$ | - 0.09 ± 0.004$^d$ |
| | MLE+U+B4 | 47848 ± 725$^d$ | 0.64 ± 0.62$^c$ | 0.053 ± 0.008$^c$ | 0.010 ± 0.005$^c$ |
| Moringa leaf extract | MLE+U+K4 | 61296 ± 793$^c$ | 0.74 ± 0.40$^b$ | 0.14 ± 0.002$^b$ | 0.015 ± 0.006$^b$ |
| | MLE+U+B8 | 63778 ± 2422$^c$ | 0.79 ± 0.74$^b$ | 0.15 ± 0.007$^b$ | 0.018 ± 0.006$^b$ |
| | MLE+U+K8 | 67825 ± 815$^b$ | 0.86 ± 0.02$^b$ | 0.16 ± 0.006$^b$ | 0.022 ± 0.005$^a$ |

Data are mean ± standard error (n = 5) . MLE: *Moringa oleifera* leaves extract, U: urea, K: kanwa (NaCl), B: $NaHCO_3$.. Means followed by the same letter in the same column are not significantly different ($p < 0.05$) as determined by Fisher LSD test.

phenol solution added. The procedure was followed by adding 5 mL of sulphuric acid by shaking well. The yellow-orange colour was pipetted off and the wavelength was read in 490 nm by spectrophotometer (Pharmaspec UV-1700 model). The amount of carbohydrates was presented from the glucose standard curve.

Total soluble protein content was measured according to the method described by Bradford (1976) using bovine serum albumin (BSA) as a protein standard. Fresh leaf samples (100 mg) were homogenized with 4 mL Na-Phosphate buffer (pH 7.2) and then centrifuged at 13000 g for 4.5 min at 4°C. 1 ml of supernatant is added to the Bradford reagent (5 mL) and the mixture was incubated thereafter in the dark for 15 min. Then, it was pipetted in spectrophotometer cuvettes and absorbance was measured at 595 nm using a UV spectrophotometer (PG instruments T60).

#### Chlorophyll content determination

Total leaf chlorophyll (CHL) of plants was extracted in 80% (v/v) aqueous acetone and absorption was measured in spectrophotometer (Thermospertronic Heλios β) at 645 and 663 nm (Arnon, 1949). CHL was calculated using the formula:

Total leaf chlorophyll = (20.2 x D645 + 8.02 x D663) x (50/1000) x 100/5) x ½

Where, D: Absorbance

#### Antioxidant compounds determination

The activity of peroxidase (POD) and polyphenol oxidase (PPO) were determined according to Thorpe and Gaspar (1978) and Van Kammen and Broumer (1964) methods, respectively. For the assay of POD and PPO, a fresh *S. platensis* sample was extracted in 10 mL potassium phosphate buffer (50 mM, pH 6.0). The homogenate was subsequently centrifuged (6000 g, 30 min at 4°C) and the supernatant was collected. The pellet was re-suspended in the same buffer centrifuged under the same conditions as previously. The second supernatant was added to the first to obtain extract which was used for PPO and peroxidase POD activity. POD activity was determined by measuring the oxidation of guaiacol and the increase in absorbance at 420 nm was recorded in 3 min. PPO activity was assayed by measuring the decomposition of $H_2O_2$ by following the decline in its absorbance at 330 nm for 30 s. The activity was defined as Unit/µg of proteins contents.

The phenol content (PC) was determined according to the method described by Singleton and Rossi (1965). Ethanol extracts (0.2 mL) were added to 1.6 mL of $H_2O$ and 0.5 mL of Folin-Ciocalteu reagent and incubated at 25°C for 10 min. Afterwards, 1 mL of a 7.5% solution of $Na_2CO_3$ was added to each sample and left at 40°C for 20 min in a water bath, with intermittent shaking. The absorbance of the sample was recorded at 760 nm. The calibration curve was performed with gallic acid and the results were expressed as mg of gallic acid equivalents per g of dry weight.

#### Physico-chemical parameters determination

The conductivity, temperature, pH, total dissolved solids and salinity of media were measured according to the methods described by Rodier et al. (2009). The physico-chemical parameters were recorded daily using multi-parameters (HI 98130, HANNA Instruments, Rhodes Island, USA).

#### Statistical analysis

The data obtained were represented as the mean ± standard error. All of the statistical analyses were conducted using SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). The one-way analysis of variance (ANOVA) with Duncan's Multiple Range Tests was used to compare differences between treatment means when significant F values were observed at $p < 0.05$.

## RESULTS AND DISCUSSION

### Growth parameters

Growth of *S. platensis* was expressed in terms of cell population (CP), biomass dry weight (DW), cell productivity (CPr) and specific growth rate (SGR) (Table 1). The highest values of CP (90037 ± 2363 cp $mL^{-1}$), DW (1.01 ± 0.42 mg/L), CPr (0.25 ± 0.002 g $L^{-1}$ $day^{-1}$) and SGR (0.028 ± 0.009 cell $day^{-1}$) were recorded in the Jourdan medium (control) compared to all other treatments comprising moringa leaf extract (MLE) during all the period of experimentation (Table 1). The highest values of CP (67825 ± 815 cp $mL^{-1}$), DW (0.86 ± 0.02 mg $L^{-1}$), CPr (0.16 ± 0.006) and SGR (0.022 ± 0.005) registered in MLE enriched with urea and kanwa at 8 g $L^{-}$

$^{1}$ were also significantly ($p < 0.05$) higher than those grown in other MLE media compared to control (Table 1). Kanwa, a type of salt, is formed when salt water from a sea or lake evaporates and leaves behind colorful crystals of sodium chloride. According to Nafee et al., (2013), it also contains natural impurities such as calcium sulfate, potassium chloride and various levels of trace minerals, such as manganese, copper, iron, and zinc. Moreover, MLE has received enormous attention from the scientific community because of its rich source in hormones, antioxidants, vitamins and minerals such as iron, calcium and potassium as well as vitamins and macronutrients which have plant growth-promoting capabilities and often applied as exogenous plant growth enhancers (Rady et al., 2013; Yasmeen et al., 2013; Khan et al., 2017a, b). In this study, the CP, DW, CPr and SGR were significantly ($p < 0.05$) increased when the MLE cultivation medium was enriched with $NaHCO_3$ or kanwa compared to those supplemented only with urea (Table 1). These results could be explained by the fact that the supply of MLE cultivation medium $NaHCO_3$ or kanwa can improve water quality and increase the quality of *S. platensis* growth and it will also cause good removal of turbidity in the cultivation media so that the light penetration increases will improve photosynthesis and production of *S. platensis* (Ogbonna and Chukukwu, 2018; Silva et al., 2020). This may be also due to uptake of nutrients (carbohydrates and ash) and minerals (Na, K, Ca, Mg) brought by MLE and supplemented $NaHCO_3$ or kanwa which increase cell growth and the metabolism of carbon in the photosynthetic activity of *S. platensis* (Nweze and Nwafor, 2014a,b). A number of green algal species have been shown to be able to utilize carbonates such as $Na_2CO_3$ and $NaHCO_3$ for cell growth (Emma et al., 2000). Large-scale production of *S. platensis* biomass is essentially a complex process involving a large number of variables and for their successful growth; the nutrient sources and the temperature needs to be conditioned to meet as many of the essential requirements of the organism (Ndjouondo et al., 2017). On the other hand, the values of CP (20783 ± 1258 cp $mL^{-1}$) and DW (0.40 ± 0.26 mg $L^{-1}$) and those of CPr (- 0.04 ± 0.003) and SGR (- 0.09 ± 0.004) of *S. platensis* were negatively affected by MLE supplemented only by urea (Table 1). These low values of CP, DW, CPr and SGR were influenced by nutrients found in *S. platensis* cultivation media (Magwell, 2017).

## Biochemical characteristics

In this study, the presence of kanwa or $NaHCO_3$ in MLE cultivation medium of *S. platensis* at different concentration levels resulted in a significant ($p < 0.05$) increase in proteins content compared to Jourdan standard medium (control) except in the MLE medium supplemented only with urea while a significant ($P < 0.05$) decrease in carbohydrate and phenol contents was recorded during all the period of experimentation (Figure 1b and c). The results showed that the MLE medium enriched with urea and kanwa (8 g $L^{-1}$) was significantly ($P < 0.05$) higher than other treatments. This could be related to the high amount of nutrients in the medium such as colorful crystals of sodium chloride and natural impurities having calcium sulfate and potassium chloride (Nafee et al., 2013). The effect of the MLE 'supplemented with urea and kanwa could be also explained by the increase of nitrogen assimilation due to the high amount of inorganic carbon provided by $NaHCO_3$ in the medium. A number of green algal species have been shown to be able to utilize carbonates such as $Na_2CO_3$ and $NaHCO_3$ for cell growth (Emma et al., 2000). In this study, the carbohydrate content remains very low compared to the protein content (Figure 1a and b). Depraetere et al., (2015) reports that when the amount of nitrogen is high or excessive, it would lead to carbohydrate hydrolysis. The present study also revealed significant ($P<0.05$) decrease in MLE medium supplemented only with urea for all the treatments compared to Jourdan standard medium (Figure 1a, b and c). This effect of urea could be attributed to the low concentration used for this experiment (50 mg $L^{-1}$). According to Rangel-Yagui et al. (2004), the best cellular growth for *S. platensis* was observed with 500 mg $L^{-1}$ of urea at a light intensity of 5600 lux.

## Chlorophyll content

The enrichment of MLE cultivation medium with urea, kanwa or $NaHCO_3$ at different concentration levels led to a significant decrease ($p< 0.05$) in chlorophyll content during all the cultivation period of *S. platensis* compared to Jourdan standard medium (Figure 2). This depressive effect may be attributed to salt-induced weakening of protein-pigment-lipid complex or increased chlorophyllase (Strogonov, 1970). The significant ($P< 0.05$) decrease of chlorophyll content could be also due to low (50 mg $L^{-1}$) concentration of urea supply. According to Rangel-Yagui et al. (2004), the highest concentration of chlorophyll in the biomass was observed with 500 mg $L^{-1}$ at a light intensity of 1400 lux.

## Antioxidant compounds

In the present study, antioxydant compounds of *S. platensis* were expressed in terms of polyphenol oxidase (PPO), peroxidase (POD) and phenol content (PC) (Table 2). The highest values of PPO (6.02 ± 0.044 UE µg-1) and POD (0.63 ± 0.03 UE µg-1) were recorded in the medium which contains MLE enriched with urea

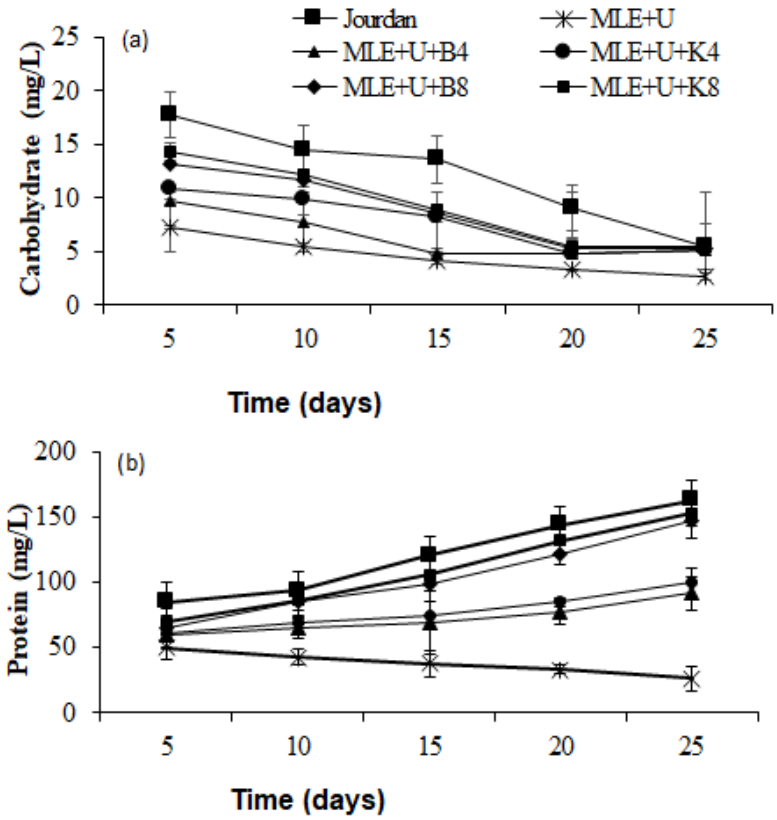


**Figure 1.** Variation of some biochemical characteristics in Moringa leaf extract (MLE) in response to kanwa or $NaHCO_3$ at different concentration levels. (a) Carbohydrate, (b) proteins. Data are Mean ± standard error (n = 5). Mean followed by the same letter are not significantly different ($p < 0.05$) as determined by Fisher LSD test. Bars indicate standard error.

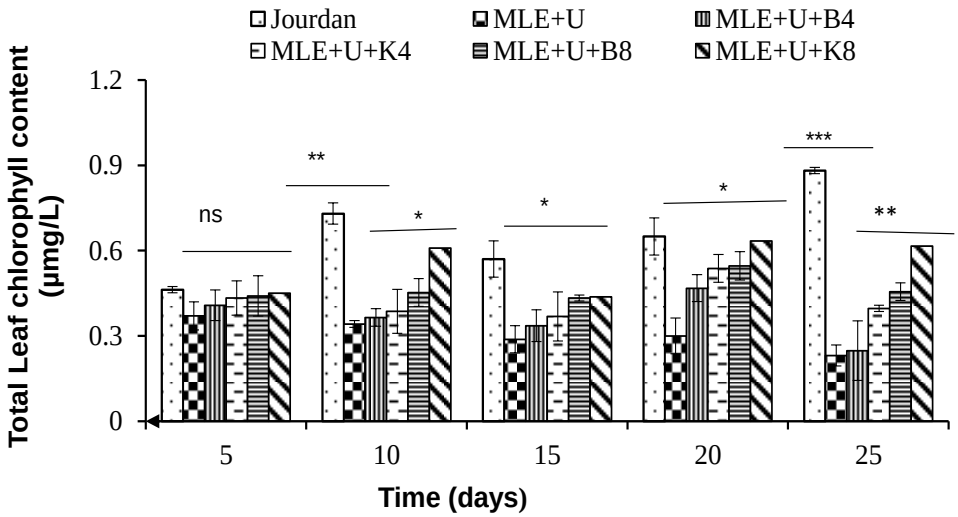


**Figure 2.** Variation of total leaf chlorophyll content in Moringa leaf extract (MLE) in response to kanwa or $NaHCO_3$ at different concentration levels. Data are Mean ± standard error (n = 5). Mean followed by the same letter are not significantly different ($p < 0.05$) as determined by Fisher LSD test. Bars indicate standard error.

**Table 2.** Variation of antioxidant compounds of *S. platensis* in response to kanwa or $NaHCO_3$ at different concentration levels.

| Media | Treatment | PPO ($10^{-3}$×UE µg$^{-1}$) | POD ($10^{-3}$× UE µg$^{-1}$) | PC (mg l$^{-1}$) |
|---|---|---|---|---|
| Jourdan medium | Control | 2.00 ± 0.024$^{d}$ | 0.03 ± 0.03$^{d}$ | 2.09 ± 0.07a |
| MLE | MLE+U | 6.02 ± 0.044$^{a}$ | 0.63 ± 0.03$^{a}$ | 1.00 ± 0.01e |
| | MLE+U+B4 | 4.07 ± 0.002$^{b}$ | 0.27 ± 0.02$^{b}$ | 1.55 ± 0.21d |
| | MLE+U+K4 | 3.33 ± 0.012$^{c}$ | 0.26 ± 0.01$^{bc}$ | 1.63 ± 0.25c |
| | MLE+U+B8 | 2.30 ± 0.018$^{cd}$ | 0.18 ± 0.02$^{cd}$ | 1.80 ± 0.21bc |
| | MLE+U+K8 | 2.23 ± 0.010$^{cd}$ | 0.17 ± 0.02$^{cd}$ | 1.88 ± 0.11b |

Data are mean ± standard error (n = 5). MLE: *Moringa oleifera* leaves extracts, U: urea, K: kanwa (NaCl), B: $NaHCO_3$, POD: peroxidase and PPO: polyphenol oxidase, PC: phenol content. Means followed by the same letter in the same column are not significantly different ($p < 0.05$) as determined by Fisher LSD test.

compared to Jourdan standard medium (control) which showed the lowest values (2.00 ± 0.024 UE µg$^{-1}$) and (0.03 ± 0.03 UE µg$^{-}$1), respectively (Table 2). On the other hand, the highest value of PC was noted in Jourdan standard medium (2.09 ± 0.07 mg L$^{-1}$) while the lowest value was observed in MLE cultivation medium supplemented only with urea (1.00 ± 0.010 mg L$^{-1}$) (Table 2). In response to stress, plants activate powerful antioxidant systems, both enzymatic (e.g., SOD, POD, catalase, glutathione reductase) and non-enzymatic (flavonoid, PC, carotenoids, vitamins C and E) (Ashraf, 2009; Kahrizi et al., 2012). The activity of antioxidant enzymes (PPO and POD) was notably increased with the cultivation period of *S. platensis* in all MLE medium supplemented with different concentrations of kanwa or $NaHCO_3$ compared to Jourdan standard medium (Table 2). The use of MLE based medium supplemented with a high concentration of kanwa or $NaHCO_3$ may cause the increasing trend of the amount of PPO and POD activity. According to Kasote et al. (2015) and Mostafa et al. (2016), this increase in enzymatic activity could be correlated to a decrease in oxidative stress and reactive oxygen derivatives produced during photosynthesis and to the high content of phenols which would act as antioxidant by producing an enzymatic substrate to alleviate the harmful effects of reactive oxygen species.

## Physico-chemical parameters

In this study, some physico-chemical parameters (conductivity, pH, total dissolved solids and salinity) of *S. spirulina* cultivation media led to a significant ($p < 0.05$) increase in MLE media enriched with different concentrations of urea, kanwa or $NaHCO_3$ from 20 to 25 days of cultivation compared to Jourdan medium and others treatments (Figure 3a, c, d and e). This increase of salinity and total dissolved solids could be explained by increase with the evaporation of water in media and to the change of the other variables of the culture media due to uptake of nutrients brought by the different concentrations of kanwa or $NaHCO_3$ in MLE media **(**Rusydi, 2018). This progressive increase in total dissolved solids and salinity could be explained by the increase in alkalinity and concentration of dissolved ionic salts resulting from MLE media with different concentration of kanwa or $NaHCO_3$ following the evaporation of water from the media as reported by Mutanda et al. (2014). The temperature of *S. platensis* cultivation media varied significantly ($p < 0.05$) between 29 and 31°C and the pH between 8 and 10 with the highest value of temperature (31°C ) recorded in MLE medium supplemented with urea and Kanwa at 8 g L$^{-1}$ and the lowest (29°C) was noted in Jourdan standard medium (Figure 3b). The results obtained are in agreement with those reported by Soni et al. (2019) which indicated that for flourishing and optimal growth, temperature for *S. platensis* is between 30 and 35°C and pH value between 8.5 and 10.5.

## Conclusion

In general, the results of this study showed that the growth parameters such as cell population, biomass dry weight, cell productivity and specific growth rate were positively affected in MLE cultivation medium enriched with kanwa or $NaHCO_3$ at different concentration levels. This result may be due to uptake of nutrients (carbohydrates and ash) and minerals (Na, K, Ca, Mg) brought by MLE and supplemented $NaHCO_3$ or kanwa which increase cell growth and the metabolism of carbon in the photosynthetic activity of *S. platensis*. The addition of kanwa or $NaHCO_3$ in MLE cultivation medium at different concentration levels increased significantly the presence of electrically charged atoms which physico-chemical parameters (conductivity, pH, total dissolved solids and salinity) from 20 to 25 days of cultivation, the protein content and antioxidant enzymes (PPO and POD) activity whereas a decrease in carbohydrate content was

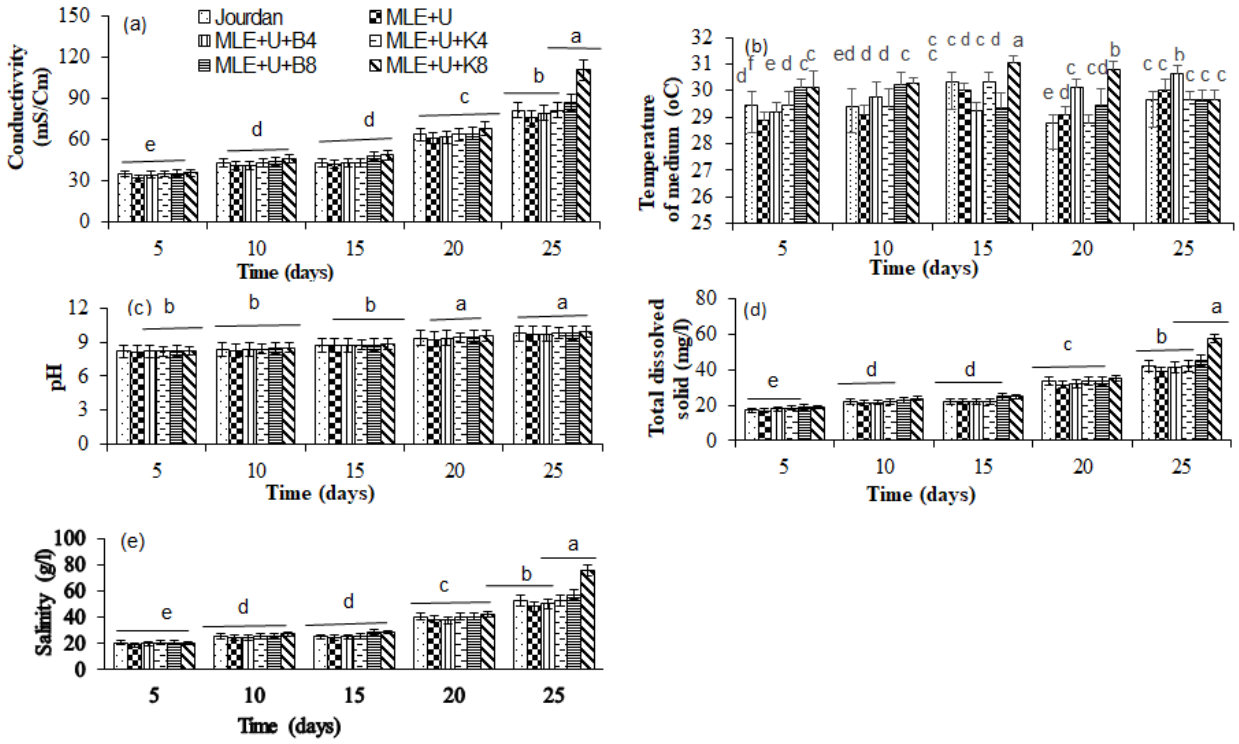


**Figure 3.** Variation of some physico-chemical parameters in Moringa leaf extract (MLE) in response to kanwa or $NaHCO_3$ at different concentration levels. (a) Conductivity, (b) Temperature, (c) pH, (d) Total dissolved solid, (e) Salinity. Data are Mean ± standard error (n = 5). Mean followed by the same letter are not significantly different ($p < 0.05$) as determined by Fisher LSD test. Bars indicate standard error.

recorded during all the period of the experimentation. This increase in enzymatic activity could be correlated to a decrease in oxidative stress and reactive oxygen derivatives produced during photosynthesis and to the high content of phenols which would act as antioxidant by producing an enzymatic substrate to alleviate the harmful effects of reactive oxygen species. This study draws attention to a good view on MLE as a cheaper and easily available organic fertilizer that need to be enriched for the culture of *S. platensis*. Thus, further investigations should be done in the commercial cultivation of *S. platensis* using MLE in agriculture, food industry, cosmetics, pharmaceutics and medicine.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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