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Promoting increased *Chlorella sorokiniana* Shih. et Krauss (Chlorophyta) biomass production using *Moringa oleifera* Lam. leaf extracts

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***Chlorella sorokiniana* Shih. et Krauss, a unicellular green alga was assayed to assess its to promotion potentials response of aqueous and ethanolic leaf extracts of *Moringa oleifera* Lam. *C. sorokiniana* grown in 200 ml aliquots of modified basal medium for two weeks: was treated with the aqueous and ethanolic extracts at 10, 20, 30, 40 and 50% concentrations, respectively. A control was set up without *Moringa* extract and each treatment replicated thrice. The increases in biomass (individuals/mL) were monitored at two - day intervals. Both aqueous and ethanolic leaf extracts had appreciable promoter effects (61.49 and 95.55%, increases respectively) over the control. The ethanolic exhibited more growth activity (21.09%) than the aqueous extract. The promoter effects of both extracts increased proportionally with the increase in levels of concentration with time. There were significant differences in the interactive effects of the extracts, concentration and day (duration) ($p<0.05$). The findings of this study indicate the potential use of the aqueous and ethanolic leaf extracts of *M. oleifera* as possible enrichments for *C. sorokiniana* growth medium.**

Key words: *Moringa*, leaf extracts, *Chlorella*, medium enrichment, food supplement.

INTRODUCTION

In recent decades, the world energy crisis has triggered off the race for development of efficient as well as cheap sources of biofuels. The demand for algae as natural sources of food supplements, biofuels and efficient carbon sequestrates has necessitated the need for their efficient mass production at minimal cost. The concept of algae culture or algae farming has been derived as a result, and many forms of algae fuels such as cooking oil, bio-diesel, bioethanol and biogasoline are in the process of development. It has been reported that oil productivity of *Chlorella* exceeds the yield of the best oil seed crops and its biodiesel yield is 12 000 L/ha compared with 1190 L/ha for rapeseed (Schenk et al., 2008; Sharif - Hossain et al., 2008).

C. sorokiniana Shih. et Krauss is a unique single-celled fresh water micro-alga with grass-like odor caused by high chlorophyll content. In perfect growing conditions *Chlorella* converts inorganic chemical elements to organic matter through photosynthesis (Vashishta et al., 2000). *Chlorella* is rich in fats (85% unsaturated fats), complex

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carbohydrates, amino acids, vitamins and minerals. Moreover, it contains RNA (up to 10%) and DNA (up to 3%); chlorophyll, and carotenoids; an array of phytonutrients and enzymes (including pepsin for digestion); polysaccharides as well as the unique *Chlorella* Growth Factor (CGF). Also, it has one of the highest amounts of beta-carotene among all green products (Chinnasamy et al., 2009), hence its use as a health food supplement. Furthermore, it helps in aeration of water by removing carbon dioxide and restoring oxygen in the process of photosynthesis. The growth of this alga is, therefore, encouraged in sewage disposal plants where it outgrows and suppresses harmful bacteria by its rapid rate of multiplication (Akpor and Muchie, 2010). Mohan et al. (2009) reported that *Chlorella* is used to keep the air in space vehicles pure, supply food in space stations and prolonged space flight trips. The stale air rich in carbon dioxide can be fed into a floodlit container, containing water, mineral nutrients and *Chlorella*; and the alga restores oxygen by photosynthesis.

Moringa oleifera Lam., “Miracle tree”, has recently attracted great attention in every field of scientific research. *M. oleifera* (Moringaceae) is cultivated across the tropics and used for a variety of purposes (Jahn, 1984). The seed powder, a good water purifier contains polyelectrolytes, which constitute active ingredients in water treatment (Muyibi and Evison, 1995). Also, the extract obtained from the leaves of *Moringa* in 80% ethanol contains growth enhancing principles for higher plants (Makkar and Becker, 1996). In view of the fact that *Chlorella* biomass is much sought after for use as health food supplement and for biofuels among other uses, enhanced production using non-toxic media enhancers is desirable. Hence, *Moringa* leaves which are cheap, readily available and affordable are very handy.

The objectives of this study were to investigate the promoting effect of the leaf extracts of this multi-purpose plant (*M. oleifera*) on *C. sorokiniana* and its potential use as nutrient enrichment for culture media in commercial production of *Chlorella.*

MATERIALS AND METHODS

Collection and identification of plant materials

Axenic culture of *C. sorokiniana* was collected from the Department of Microbiology, University of Nigeria, Nsukka. Young leaves of *M. oleifera* were collected from Ajuona Obukpa, in Nsukka Local Government Area of Enugu State, Nigeria. Both plant materials were identified in the Department of Plant Science and Biotechnology of the, University of Nigeria, Nsukka using morphological characteritics and taxonomic keys (Shishira and Krauss, 1963; Hutchinson and Dalziel, 1963; Roloff et al., 2009).

Preparation of plant extract (*Moringa oleifera*)

Drying process

Harvested young fresh leaves of *M. oleifera* were washed and dried for seven days under the shed, at room temperature, to avoid loss of the active compounds. The dried leaves were milled at the National Centre for Energy Research Development, University of Nigeria with a local hand milling machine and weighed on a Mettler balance. The powdered sample was stored in an air-tight glass bottle for further use.

Extraction processes

Fifty grams of the powdered leaf sample were used for the production of aqueous and ethanolic extracts following the procedure of Handa (2008). Ten grams of each of the dried extracts were dissolved in one litre of distilled water for use in the experiment (10 mg of aqueous extract in 1 ml solution).

Sub-culturing of *C. sorokiniana* cells

The axenic culture from the Department of Microbiology was sub-cultured in a modified Basal Medium, following the procedures of Ogbonna et al. (1997). Two litres of *Chlorella* medium were produced by sub-culturing at the ratio of 1:5 inoculum to the growth medium in glass jars plugged with non-absorbent cotton wool. The jars were left on a sterilized work bench in a screen house in the Botanic Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, for seven days. Each of the bottles containing the organisms was agitated at least three times every day to avoid clumping of the cells (Ukeles, 1973; Ogbonna et al., 1997).

Experimental design

Completely Randomized Design (CRD) of two different plant extracts (aqueous and ethanolic extracts), a microalga (*C. sorokiniana*) and six concentrations including a control was used. The concentrations were replicated three times.

Application of *M. oleifera* leaf extract

Two hundred millilitre aliquots of basal medium prepared following the methods of Ogbonna et al. (1997) were placed in 33 transparent glass jars and 40 ml aliquots of *Chlorella* culture were added. The setup was allowed to stand on a sterilized bench in the screen house for two weeks, and shaken three times daily to prevent clumping of cells.

The *C. sorokiniana* cultures were treated with aqueous and ethanolic leaf extracts *of M. oleifera* after two weeks of culture. Five levels of concentrations of the diluted aqueous and ethanolic extracts and a control were set up with 200 ml of *Chlorella* culture as follows: Level 1: 2 ml extract + 200 ml *Chlorella* culture = 100 mg extract/Litre = 10%; Level 2: 4 ml extract + 200 ml *Chlorella* culture = 200 mg extract/Litre = 20%; Level 3: 6 ml extract + 200 ml *Chlorella* culture = 300 mg extract/Litre = 30%; Level 4: 8 ml extract + 200 ml *Chlorella* culture = 400 mg extract/Litre = 40%; Level 5: 10 ml extract + 200 ml *Chlorella* culture = 500 mg extract /Litre = 50%; Control: 200 ml *Chlorella* culture without extract = 0%.

Estimation of the population growth

Growth was monitored over a period of ten days. The cell population was determined at two-day intervals by counting method using 0.1 mm deep hemacytometer with improved Neubauer ruling (Guillard, 1973). The culture population density per millilitre of culture sample was calculated as follows:

Table 1. Analysis of variance (ANOVA) of the effect of aqueous and ethanolic leaf extracts of *Moringa oleifera* on *Chlorella sorokiniana* biomass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Extract	1	62720.0	62720.0	497.34*	<.001
Concentration	5	468244.4	93648.9	742.59*	<.001
Day	4	1747930.0	436982.5	3465.06*	<.001
Extract × Concentration	5	13566.7	2713.3	21.52*	<.001
Extract × Day	4	9474.4	2368.6	18.78*	<.001
Concentration × Day	20	59850.0	2992.5	23.73*	<.001
Extract × Concentration × Day	20	4372.2	218.6	1.73*	0.037
Residual	120	15133.3	126.1		
Total	179	2381291.1			

Grand mean 195.22. *Significant difference at P < 0.05. d. f.= degrees of freedom, s. s.= sum of squares m. s. mean square, v. r.= variance ratio, F pr.= Probability > Frequency.

Table 2. Effect of aqueous and ethanolic extracts of *Moringa oleifera* on the biomass of *Chlorella sorokiniana.*

Extract	Biomass (× 10^4 individuals per millilitre) Population (Mean ± SE)	% increase over control
Aqueous	176.56 ± 10.96[b]	61.49
Ethanolic	213.8 ± 13.0[c]	95.55
Control	109.33 ± 18.33[a]	
$LSD_{0.05}$ = 3.315		
% difference, aqueous against leaf extract		21.09

Different letters indicate significant difference at P < 0.05.

$d = (10^4 \times Q) f$

Where **'**Q' is the average number of cell in each 1 mm square; 'f' is the dilution factor; and'd' is the culture density.

Statistical analysis

The results of the culture population growth were analysed using GENSTAT statistical package. Analysis of Variance (ANOVA) was used to test for significance at P < 0.05 while LSD was used to compare the means of the treatment groups.

RESULTS

Analysis of variance (ANOVA) on the effects of aqueous and ethanolic extracts on *Chlorella* biomass revealed that there were significant differences (P < 0.05) in all the main effects and all the interactive effects of the extracts (Table 1). There was a significant difference (P < 0.05) between the effect of the aqueous and ethanolic leaf extracts of *M. oleifera* on the population of *Chlorella* (Table 2). The aqueous and ethanolic extracts had mean populations of 176.56 ± 10.96 and 213.8 ± 13.0 (× 10^4 individuals/mL) respectively. These represent 61.49 and 95.55% increases over the control respectively. Moreover, the ethanolic extract had 27.09% more biomass than the aqueous extract and it exhibited higher population growth than the aqueous extract. Table 3 shows a significant difference in the interaction between the aqueous and ethanolic leaf extracts and levels of concentrations at P < 0.05. Significant differences were observed in the interaction between the aqueous and ethanolic extracts and day in the population growth at P < 0.05 (Table 4). There were significant differences in the interactions between the leaf extracts, levels of concentration and day of the population growth of *Chlorella* (P < 0.05) except on days 8 and 10 for both extracts (Table 5). Within the first four days, the population growth increased significantly in both leaf extracts irrespective of level of concentration but dropped significantly on day 10 at 20, 30 and 40% levels of concentrations. It remained the same at 10% and increased at 50% level of concentration for the aqueous extract. It significantly dropped at 50%, remained the same at 10 and 30%, and increased at 20 and 40% for the ethanolic extract.

DISCUSSION

The *Moringa* leaf extracts used in this study promoted the growth of *Chlorella*. The aqueous and ethanolic leaf extracts of *M. oleifera* led to a significant increase in the population

Table 3. Interactive effect of aqueous and ethanolic extracts of *Moringa oleifera* and levels of concentration on *Chlorella* biomass.

Levels of concentration (%)	Population (× 10^4 individuals per millilitre)	
	Aqueous extract	Ethanolic extract
0.00	109.33 ± 18.83	109.33 ± 18.83
10.00	141.33 ± 20.07	178.67 ± 23.50*
20.00	163.33 ± 20.89	209.33 ± 26.70*
30.00	186.00 ± 22.41	238.00 ± 30.40*
40.00	217.33 ± 24.20	265.33 ± 33.80*
50.00	242.00 ± 27.50	282.67 ± 36.60*
$LSD_{0.05}$ 8.119		

*Significant difference at P < 0.05

Table 4. Interactive effect of aqueous and ethanolic extracts of *Moringa oleifera* and day on the *Chlorella* biomass.

Day	Population (× 10^4 individuals per millilitre)	
	Aqueous extract	Ethanolic extract
2	66.67 ± 7.37	84.44 ± 8.64*
4	92.78 ± 7.09	115.00 ± 8.13*
6	157.78 ± 11.19	201.67 ± 15.09*
8	283.89 ± 14.80	333.89 ± 20.00*
10	281.67 ± 14.80	334.44 ± 19.90*
	$LSD_{0.05}$ = 7.411	

*Significant difference at P < 0.05.

Table 5. Interactive effect of leaf extracts (aqueous and ethanolic) of *Moringa oleifera*, levels of concentration and day on the population of *Chlorella.*

Extract	Population (× 10^4 individuals per millilitre)					
	Concentration (%)	Day 2	Day 4	Day 6	Day 8	Day 10
Aqueous	0.00	23.33^{a}	46.67^{b}	96.67^{c}	190.00^{d}	190.00^{d}
	10.00	43.33^{e}	66.67^{f}	123.33^{g}	236.67^{h}	236.67^{h}
	20.00	56.67^{i}	90.00^{j}	133.33^{k}	270.00^{l}	266.67^{l}
	30.00	70.00^{m}	103.33^{n}	163.33^{o}	300.00^{p}	293.33^{p}
	40.00	96.67^{q}	123.33^{r}	200.00^{s}	336.67^{t}	330.00^{t}
	50.00	110.00^{u}	126.67^{v}	230.00^{w}	370.00^{x}	373.33^{x}
Ethanolic	0.00	23.33^{ab}	46.67^{bb}	96.67^{cb}	190.00^{db}	190.00^{db}
	10.00	53.33^{eb}	103.33^{fb}	170.00^{gb}	283.33^{hb}	283.33^{hb}
	20.00	90.00^{ib}	126.67^{jb}	180.00^{kb}	323.33^{lb}	326.67^{lb}
	30.00	103.33^{mb}	130.00^{nb}	223.33^{ob}	366.67^{pb}	366.67^{pb}
	40.00	116.67^{qb}	136.67^{rb}	263.33^{sb}	400.00^{tb}	410.00^{tb}
	50.00	120.00^{ub}	146.67^{vb}	276.67^{wb}	440.00^{xb}	430.00^{xb}
$LSD_{0.05}$ = 18.152						

Different letters indicate significant difference at P < 0.05. The same letters indicate not significant at P < 0.05.

growth of Chlorella when compared with the untreated culture (control). The ethanolic extract exhibited more pro-motion effect on the population growth than the aqueous extract. The promoter effects also increased as

the level of concentration of the extracts increased with time in days. These findings are in line with the observations of Makkar and Becker (1996) on the use of *M. oleifera* leaf extract as growth enhancing principle in higher plants. However, they contrast previous reports that the seeds of this same plant species have been used locally and industrially in water purification as a natural coagulant (Muyibi and Evison, 1995; Ndabigengesere et al., 1995; Schwarz, 2000; Amagloh and Benang, 2009).

Furthermore, these results support the use of some plant species as enrichment in algae culture media. Nichols (1973) reported that peat moss was used in conjunction with soil in soil water cultures for successful cultivation of Charophyceae or desmids. Most algal culture media can be modified with a variety of components (organic or inorganic) to improve growth population. Such components are called “enrichments’’.

Although Gibson et al. (1990) and Ferrier et al. (2005) observed in *ex situ* and *in situ* that small quantity of barley straw (*Hordeum vulgare*) promote the growth of certain algal population in water bodies, it has been reported that the presence of large quantity of decomposing barley straw in water can reduce the growth of a range of algal species in the field (Newman and Barrett, 1993; Waybright et al., 2009). In contrast, results from this study did not show a decrease in population of *Chlorella* with increase in concentration of *Moringa* leaf extracts.

The constituent minerals and nutrients reported in the leaf extract of *M*. *oleifera* include sodium, potassium, calcium, magnesium, zinc, iron, manganese and nutrients such as carbohydrate, protein, fat, crude fibre, moisture and ash *(*Krishnaiah et al., 2009; Oluduro, 2012; Nweze and Nwafor, 2014). Appreciable amount of nutrients and minerals were undoubtedly the reason for the increase in population growth of *C*. *sorokiniana* when compared with the untreated culture (control). These minerals (especially, Na, K, Mg, and Ca) constitute the major elements for green algal growth medium as noted by Ogbonna et al. (1997). Nichols (1973) suggested that nutrients such as carbohydrate and ash can also serve as the carbon source for the growth of green algae.

The ethanolic extract had higher growth response on the population of *C. sorokiniana* proving that it is a better solvent for extraction of nutrients from *M. oleifera* leaves than water for enrichment purposes. This is contrary to the observations of Kasolo et al. (2010) and Oluduro (2012) that the aqueous extract had more antimicrobial activities on both Gram -ve and Gram +ve bacteria than the ethanolic.

In all the levels of treatment, the population doubled within six days of treatment. This response could be harnessed to multiply the yield of *Chlorella* during commercial production thereby saving costs and time. *Moringa* is used extensively in food and herbal preparations (Kasolo et al., 2010), hence the results of this study, suggest its use in facilitation of *C. sorokiniana* biomass production in commercial quantities at minimal cost.

Conclusion

The extracts of *M. oleifera* at the levels of concentration used showed appreciable promoter activity on the population growth of *C. sorokiniana* when compared with the untreated samples (control). The phytochemical, mineral and proximate composition of *M. oleifera* may be responsible for the promotion effects and variations in the effecttiveness of this plant leaf extracts. Further studies need to be conducted to determine the suitability of the extracts as natural enrichments in commercial production of *Chlorella* and other micro-algae used for food supplements and source of biofuel.

Conflict of Interests

The author(s) have not declared any conflict(s) of interests.

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