Bangladesh J. Bot. **47**(3): 523-531, 2018 (September) **- Short communication**

USE OF ALGAE FROM AN OASIS IN SAUDI ARABIA IN PRODUCTION OF BIOFUEL AND BIO-FERTILIZER

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Keywords: Biodiesel, Biofertiliser, *Chlorococcum*, *Hormophysa cunieformis*

Abstract

AlAhsa oasis in Saudi Arabia is one of the largest oases in the world. Algae, from this region have been under-explored in the past decades. A study was conducted with *Chlorococcum* strain to produce biofuel alongside the seaweed *Hormophysa cuneiformis*. Gas Chromatography/Mass Spectrometry of fatty acid composition showed that the biodiesel obtained had limited number of unsaturated fatty acids as compared to the number of saturated fatty acids present, which indicates the stability of the produced biodiesel. Thereby the use of algal biomass for the production of biofuel is feasible. Moreover, the biomass may serve other different biotechnological applications. To further test this hypothesis, the aqueous extract of two different algae; one derived from the blue green alga (cyanobacterium) *Phormidium* sp. and the other from brown alga *Hormophysa cuneiformis* was used as liquid biofertiliser at concentrations of 50 and 10% of both algae. Sterilized *Vigna* seeds were soaked in the extracts for two days. Seeds were sown in sterilized soil and the germination percentage as well as shoot and root lengths were recorded for developing seedlings. The results showed that there was a significant increase in seed germination rate compared to control. Similarly, there was a significant increment in the length of root and root system compared to control with the 50% aqueous extract concentration being highest in growth parameters for brown alga followed by blue-green alga possibly due to the presence of growth stimulants in these extracts.

Algal biodiversity has not been fully unravelled as very few reports are available that are mostly site-based. A list of different types of algae in the Gulf region was generated as a result of screening study funded by Aramco (Basson 1979a,b, Basson 1992) as well as a list of animals and seaweeds in the book Atlas of the West Gulf maritime (http://www.saudiaramco.com/content/dam/Publications/Marine_Atlas).

Another study explored the composition of seaweeds in Jubail protectant (De Clreck and Coppejans 1996) as well as individual efforts, which added new records of marine algae (Kareem 2009). Algae present in the soil of palm plantations were only recently explored (El Semary and Khalifa 2016) despite their importance to soil systems of those commercially important plants. Algal species inhabiting those habitats have unique metabolism that enables them to endure harsh conditions including temperatures that can reach up to 60°C, dry weather and exposure for a long time to high light intensities. Moreover, the Eastern province has a long coastline along the Gulf which is usually covered by seaweeds, mostly brown algae. This in turn represents a freely available source of organic matter and bioactive compounds for biotechnological purposes such as generation of biodiesel and biofertilisers. Different biofuels can be derived from algae which include bio-diesel, bio-ethanol, and hydrogen (Parmar *et al*. 2011). The environmental advantages of using algae for that purpose include their carbon neutrality as they take up carbon dioxide and use it for organic matter production during photosynthesis.

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In the same process, oxygen is generated which in turn would reduce global warming. As bio-fertilisers, algae not only represent a source of useful nutrients and minerals but they also contain growth stimulants such as vitamins, polyamines and growth regulators. Biodisel is generated through the transesterification of fats in algal biomass. Biodiesel is actually the methyl esters of fatty acids that are usually found in phospholipid bilayer and in fat globules within cytoplasm (Benemann 2009). The present research highlights the dominant algal taxa present in different niches in the Eastern province and explore the biotechnological usages of some of them. The use of algae as biofuel or bio-fertilisers would reduce the negative effects of the use of fossil fuel or chemical fertilizers that are both polluting and hazardous as well as giving the economic alternative.

Water samples (El Semary 2016) were collected from different locations. One litre sample was obtained from agricultural wastewater and sewage Canal in AlAhsa. Microscopic examination was conducted to determine the types of algae present. For marine water and macro algal samples, collection commenced in winter time 2015 and 2017. Water samples (3 litre) were collected to examine microscopic algae and for macroscopic algae from littoral zone from Uquair and Half Moon beach.

Bold's medium was used for growing microscopic algae that was based on distilled water in case of freshwater and marine water for marine microalgae. Solid medium was prepared by adding 20 g agar to 1 litre growth medium. Occasionally, marine medium F/2 was later used for mass production (Gulliard and Ryther 1962).

Fatty acids were extracted using a method modified from Gunasekaran and Hughes (1980).The algal biomass of green alga C*hlorococcum* and brown algal *Hormophysa cuneiformis* was treated with hydrochloric acid in the presence of methanol in water bath at 80ºC for 2 hrs. The hydrlysate was then treated with hexane, centrifuged and the supernatant was analysed using mass-GC to identify the composition of fatty acid methyl esters (Central Lab of Faculty of Agriculture, King Faisal University). Programming included injector temperature (200ºC), temperature transmission line (250°C), first rate (80°C/min) and the slope (3ºC/min) (temperature 230ºC/5 min). Carrier gas was He2 (1.5 ml/min) ionization 70 volts. The composition of methyl esters of the fatty acids (bio-diesel) were identified through comparison to reference material.

To test the use of aqueous extract as bio-fertiliser, biomass from one marine microalga *Phormidium* sp. and macroalga *Hormophysa cuneiformis* was dried, ground and extracted with hot water and left for two days. The slurry was centrifuged, and the aqueous supernatant was used to prepare 10 and 50% concentration of the extract after dilution with distilled water.

For germination experiment, seeds were soaked in algal extract as ten seeds of *Vigna* sp. (cowpea) were used in 3 replicates. Those seeds were sterilized using diluted sodium hypochlorite solution for five min, rinsed thoroughly and then soaked in the two concentrations (10 and 50%) of the aqueous algal extract of both *Phormidium* sp. and *Hormophysa cuneiformis* for two days. The seeds were sown in plates containing sterilized sand and observed for a week. Three replicates were used for each algal extract concentration (10 and 50%) in addition to control.

A-Types of algal taxa dominating different niches: Agricultural drainage water was mainly dominated by green algae including *Desmodesmus*, *Chlorococcum* and *Selenstrum* sp. The latter is also known as *Kirchneriella* sp. This particular alga is so sensitive to water pollution and is being used as a bioindicator for pollution especially with heavy metals hence indicating the nearly the absence of heavy metals in drainage water. *Euglena* sp. has dominated the wastewater sample as well as the blue green alga *Oscillatoria* sp. Regarding marine microalgae, the *Lyngbya majuscula, Phormidium* sp.*, Oscillatoria* sp.*, Chroococcus* sp. and *Synechocystis* sp. dominated marine samples with a few diatoms observed. *Lyngbya majuscule* is a filamentous non-heterocystous cyanobacterium that is a well-known producer of toxins. Attempt was made to isolate and establish pure cultures to verify the strains that are capable of fast growth. However, most of the pure cultures were slow in growth and did not reach a considerable biomass. Only the green alga *Chlorococcum* sp. was the fastest in growth and was thereby selected for biodiesel generation. *Phormidium* sp. was selected for extraction of its aqueous extract to be used as a biofertiliser as cyanobacteria are known for high phosphate content that is stored in the cytoplasm as well as nitrogen based phycobilins are water-soluble pigments (Van den Hoek *et al*. 1995). The marine macroalga *Hormophysa cuneiformis* was used for biodiesel generation and bio-fertiliser application. The thallus is about 50 cm with tough texture and regular main branching and contains air vesicles (Figs 1 and 2).

Figs 1-2: 1. The green alga *Chlorococcum* sp. 2. The brown alga *Hormophysa cuneiformis.*

B-qualitative analysis of fatty acid composition of Chlorococcum sp. and Hormophys acuneiformis: Qualitative analysis of fatty acids composition of both *Chlorococcum* sp. and *Hormophys acuneiformis* is presented in Table 1.

The qualitative analysis showed that the fatty acid composition was mostly dominated by saturated fatty acids (Figs 3 and 4). C18 fatty acid in particular is involved in most of algal structures (El-Naggar 1999). Despite the presence of some unsaturated fatty acids, saturated fatty acids were the dominant indicating the good quality of biodiesel obtained, as rich diesel-saturated fatty acids are chemically stable. Algae also represent a promising source of biofuel due to renewed growth and their simple growth requirements such as mineral nutrients and solar energy to make organic matter that is lignin-free which impedes the extraction of biofuel in plants (Chisti 2007).

C-The use of algal aqueous extract as a bio-fertiliser: Algal extracts were tested for their enhancement of *Vigna* seeds germination. The germination percentage was highest at 50 of algal extract of *Hormophysa cuneiformis* (denoted on the graph as Phaeophyta) followed by the 50% extract of *Phormidium* sp. (denoted on the graph as Cyanophyta) followed by *Hormophysa cuneiformis* 10% extract (Fig. 3). Those concentrations enhanced germination but not 10% concentration of extract of *Phormidium* sp. The growth parameters followed the same pattern.

Table 1. Qualitative analysis of the composition of bio-diesel in both *Chlorococcum* sp. and *Hormophysa cuneiformis.*

Algae	Methyl fatty acid type	Number of carbon atoms	Saturated	Unsaturated
Chlorococcum	Methyl myrisate	C14	Yes	-
	Methyl palmitate	C16	Yes	-
	Methyl palmitoleate	C16:1	-	Yes
	Methyl stearate	C18	Yes	-
	Methyloleate	C18:1	-	Yes
	Methyllineolate	C18:2	-	Yes
Hormophysa	Methyl myrisate	C14	Yes	-
	Methyl palmitate	C16	Yes	-
	Methyl margarate	C17	Yes	-
	Methyl stearate	C18	Yes	-
	Methyloleate	C18:1	-	Yes
	Methyllineolate	C18:2	-	Yes
	Methyl nonadecanoic	C19	Yes	-
	Methyl nonadecenoic	C19:1	-	Yes
	Methyl nonadiecanoate	C19:2	-	Yes
	Methyl aracedoneotate	C20:4	-	Yes
	Methyl eicospantonate	C20:5	-	Yes

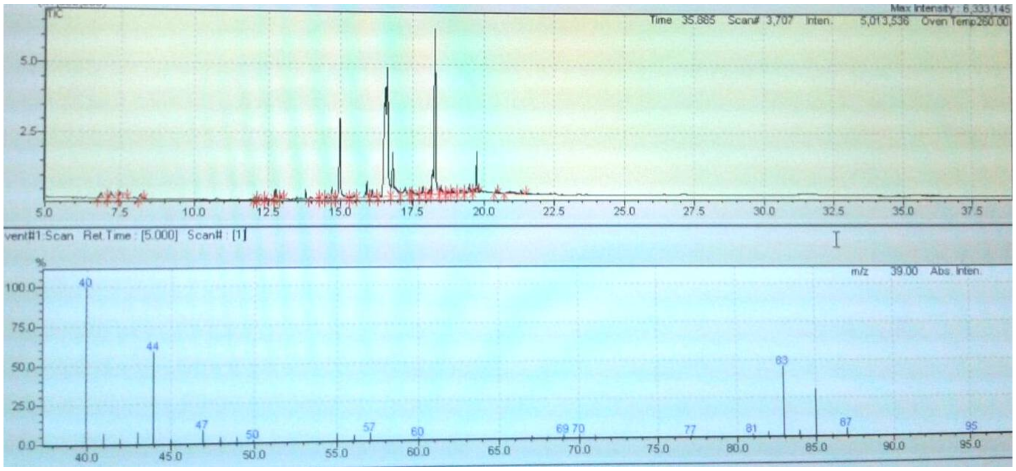

Fig. 3. Chromatogram of methyl ester (bio-diesel) of *Chlorococcum.*

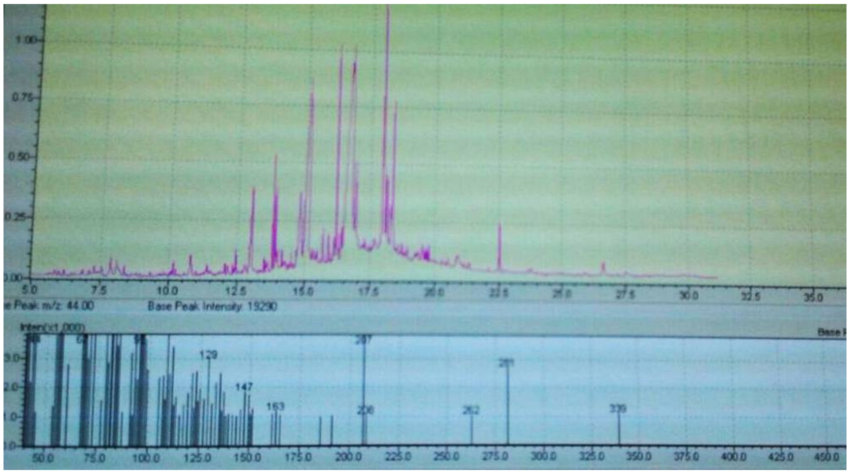

Fig. 4. Chromatogram of methyl ester (bio-diesel) of *Hormophysa cuneiformis*.

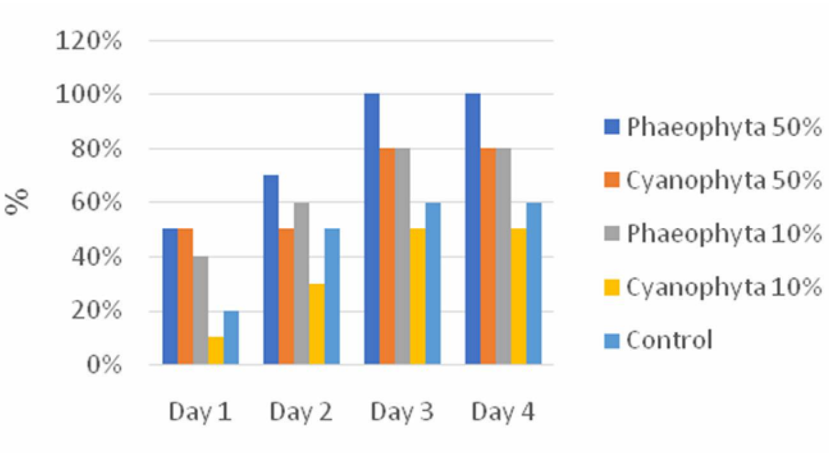

Fig. 5. The effect of extract concentrations on germination % of *Vigna* seeds.

Germination percentage of seeds soaked in brown algae (*Hormophysa*) using 50% extract concentration was higher than the rest of the ratios in the brown alga *Hormophysa cuneiformis* followed by 10 % of the same extract followed by blue-green alga *Phormidium* sp. 50% extract concentration than control. The least percentage was obtained for extract concentration of 10% of the blue-green alga *Phormidium* sp. (Fig. 5).

Length of plumule of cowpea (*Vigna*) seeds in Phaeophyta (*Hormophysa*) 50% extract concentration was higher than that of Cyanophyta (*Phormidium*) 50% extract concentration (Fig. 7).

Al Ahsa Governorate occupies a large area of Saudi Arabia and is considered as one of the largest oases in the world. As a result, there is a wide variation in terms of the topology and climate. This variation has led to a high degree of biological diversity. Therefore, attention was focused on exploring some types of algae that inhabit different environments within the province.

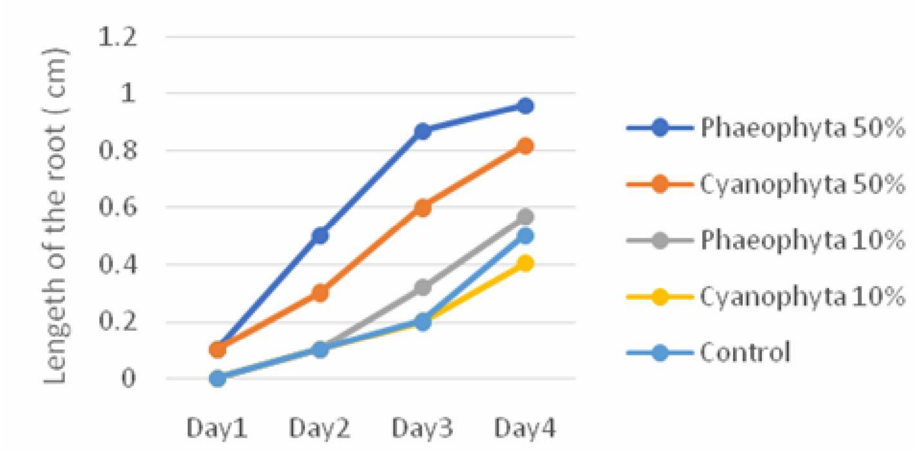

Fig. 6. The effect of certain concentrations of extracts brown alga (*Hormophysa*) and extracts blue green alga (*Phormidium*) on length of the radical of *Vigna* seeds.

Length of the radicle of cowpea (*Vigna*) seeds in Phaeophyta 50% (*Hormophysa*) was found to be higher than that of Cyanophyta 10% (*Phormidium*) (Fig. 6).

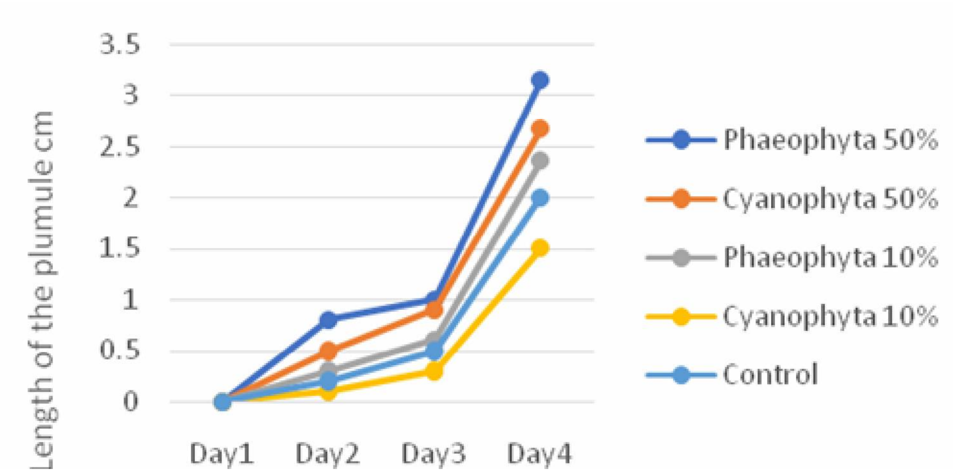

Fig. 7. The effect of certain concentrations of extracts brown alga (*Hormophysa*) and extracts blue-green alga (*Phormidium*) on length of the plumule of *Vigna* seeds.

Euglena sp. and blue green algae dominated wastewater most likely due to the heavy metals and organic load that they can cope with unlike most of algae. On the other hand, agricultural drainage water was dominated by green algae, which are known for their sensitivity to contamination, which may indicate contamination-free water. This is a positive indicator on the status of the groundwater used in agriculture. This is in accordance with Palmer (1969) who used algae as ecological indicators. It is noteworthy that the green alga *Chlorococcum* sp. was the fast growing alga in pure cultures. Regarding marine algae, the filamentous cyanobacterial strains dominated the microalgal marine community e.g. *Lyngbya*, *Phormidium* and *Oscillatoria. Lyngbya majuscule* in particular is a well-known toxin producer (Osborne *et al.* 2001) and hence is not a suitable material for bio-fertilizers production unlike *Phormidium.* With regard to macroalgae, they were identified according to the identification keys (Abdel Kareem 2009 De Clerck and Coppejans 1996).

Green algae such as *Cladophoropsis membrance* , *Cladophora vagabund*a, *Acetabularia* sp. were frequently encountered. The red alga *Gracilaria* sp. and the brown alga *Ectocarpus* sp. were also present. However, the brown macroalga *Hormophysa cuneiformis* accumulated in massive amounts. In that regard, a study regarding marine biology of the Arabian Gulf was funded by Aramco (http://www.saudiaramco.com/content/dam/Publications/Marine_Atlas/Chapter_5a.pdf), and conducted by Sheppard and Borowizka (2001). This study attributed the dominance of brown algae in the Arabian Gulf region to their adaptability to extreme conditions represented by the very high temperature and salinity level thereby outcompeting other algae for the rich nutrients and the wide optical areas used for photosynthesis present in the littoral region. It is worth mentioning that some of the major algae present in AlUquair region were enlisted in the present study. The massive accumulation of *Hormophysa cuneiformis* made it a plausible candidate for both biodiesel and bio-fertilizer production. It is proposed that its dominance is related to its ability to adapt to fluctuations and extremes in environmental conditions such as temperature and salinity. To produce biofuel, algae were selected for their ability of biomass production in short time without the cost of high growth nutrients. One microalga (*Chlorococcum* sp.) and one seaweed (*Hormophysa cuneiformis*) were selected to test the suitability of their fatty acid composition for bio-diesel production as their biomass is suitable for this technological purpose. Both the algae showed more saturated fatty acids proportion than unsaturated fatty acids, a plausible character in producing bio-diesel as it indicates its stability. Algae provide part of nutrients for the plant which is a positive effect of bio-fertilizer. Seaweeds are a source of bioactive compounds and growth promoting substances such as growth hormones and many other organic bioactive compounds (Ramarajan *et al.* 2012, Pacholczak *et al.* 2016a). They are also rich source of nutrients such as nitrogen, phosphorus and potassium as well as minerals (Tuhy *et al*. 2015).

Antimicrobial compounds are found in a vast number of brown algae which help in resisting certain endemic diseases in the soil. Extracts may also improve water absorption by the roots (Ismail and El-Shafay 2015). Therefore, the extract would provide nutrients, minerals, growth stimulants, antioxidants, and antimicrobials. Regarding the stimulatory growth of *Phormidium* sp., Singh *et al*. (2016), reviewed the promotory effects of cyanobacteria on plant growth. They attributed those effects to: (a) Enhance of nutrients solubility and bioavailability, (b) mineralize organic compounds such as amino acids to allow direct uptake, (c) possession of plant growth promotors such as auxins, cytokines, vitamins and gibberellins and (d) possession of antimicrobial and biocontrol agents

Several reports were found on the solubilizing unavailable phosphates to bioavailable forms possibly but not definitely through the activity of phosphatase enzyme (Bose *et al*. 1971, Cameron and Julian 1988). It is also proposed that after phosphate is solubilized, it is taken up by growing cyanobacteria. Nevertheless, after their death, the phosphate is released and mineralized which are taken up by growing plants (Saha and Mandal 1979, Mandal *et al*. 1999). Gantar *et al.* (1995) reported that extracellular substances released by cyanobacteria colonizing wheat plant roots showed significant positive effect on plant growth.

Algae represent a promising source of biofuel production and bio-fertilizers. However, it is of paramount importance to select the algal species that can provide the right biomass in terms of quantity and quality without expensive growth costs. The expansion of the algal screening programmes and growth manipulation experiments to allow the selection of the right algal species are recommended.

Acknowledgements

The authors would like to express their deepest gratitude to King Faisal University, Deanship for Scientific Research in supplying funds under the undergraduate project funds in the year 2015-2016 and 2017-2018. . The research was supported by grants number 175006 and 175063.

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(Manuscript received on 28 February, 2018; revised on 29 April, 2018)