Identification, Isolation and Cultivation of Microalga, *Scenedesmus*

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Abstract: This research deals with identification, isolation and cultivation of beneficial microalga *Scenedesmus* from the ponds in Bawdigone Pagoda, Mandalay. In this research, twelve species of *Scenedesmus* were identified. Among them, *Scenedesmus dimorphus* (Turp.) Kuetzing was isolated by micropipette method to obtain pure culture strain. Cultivation of *Scenedesmus dimorphus* (Turp.) Kuetzing was done by using four different media such as Armo, BG-11, Scenedesmus and Bold's Basal (BBM), at 24˚ C. The doubling time were achieved on 2nd day of the experimental period in these media. The maximum growth was observed in Armo at 14th day. The result showed that Armo is the best medium for cultivation of *Scenedesmus dimorphus* (Turp.) Kuetzing in laboratory.

Key words: Microalga, *Scenedesmus*, media, cultivation

INTRODUCTION

Algae are primarily oxygen releasing photosynthetic organisms with simple body plants no roots, stems, or leaves (Richmond and Hu 2013).There are two main forms of algae, micro and macro algae. Microalgae are divided into two general groups: phytoplankton and periphyton. Phytoplankton lives suspended in the water column. Periphyton live attached to rocks, sediments, plant stems, and aquatic organisms. Algae are usually single celled (unicellular) with these cells either solitary or grouped in clusters (colonies) or strings (filaments) (Addy and Green 1996).

Algae have been shown to have a higher photosynthetic efficiency than terrestrial plants (2-8% compared to 1-2%), resulting in a higher solar to chemical energy conversion (Taylor 2013).

Algae cultures have been principally developed as an important source of many products, such as aquaculture feeds, human food supplements, and pharmaceuticals and they have been suggested as a very good candidate for fuel production. Algae are a large and diverse group of simple typically autotrophic organisms, ranging from unicellular to multicellular forms (Makareviciene *et al*. 2011).

Neenan *et al*. (1986) stated that photosynthetic microalgae are potential candidates for utilizing excessive amount of CO_2, since these organisms are capable of fixing CO_2 to produce energy and chemical compound upon exposure to sunlight. Microalgae have high growth rates and tolerance for varying environmental conditions.

Microalgae have an important role in aquaculture as a means of enriching zooplankton for on-feeding to fish and other larvae. In addition to providing protein (essential amino acids) and energy, they provide other key nutrients such as vitamins, pigments and sterols, which are transferred through the food chain (Brown 2002).Microalgae are not only sources of fuel, food for humans and animals, but are also the sources of a wide range of chemical compounds used in industry, food technology, and pharmaceuticals as well (Bruton *et al.* 2009).

Scenedesmus is a genus of algae, specifically of the chlorophyceae. Colony a flat (rarely curved) plate of usually 2-4-8 (rarely 16-32) cells which are always in multiples of two. Cells acicular, ellipsoid, ovoid or cylindrical, arranged in one or two rows and in lateral contact. Cell well smooth or granulate, with or without lateral ridges, lateral teeth or spines.

Chong *et al*. (2000) stated that a variety of aquatic microalgae, including the green alga*Scenedesmus*, have been studied for their possible efficacy as bioresources for applications as fish feed, human food, supplemental human nutrients and pharmaceutical products, and also for the bioremediation of polluted water. *Scenedesmus* is an ubiquitous organism, and frequently is a dominant microalgae in freshwater lakes and rivers. The green algae are among the most common and taxonomically diverse of the chloroccalean genera, within which over 200 species, and almost 1200 infraspecific taxa, have been identified.

Scenedesmus can easily be cultivated in cement tanks of suitable shape and size using bricks and cement or any other comparable material. Cultures of *Scenedesmus* should be agitated suitably to prevent the settling of the algae. This also helps to make light and nutrients available uniformly for all growing cells (Becker and Venkataraman 1978).

Nitrogen fixation in algae which have the capacity to fix gaseous nitrogen requires the following elements, boron, calcium, iron, molybdenum, and perhaps cobalt. The critical concentration of an element for its two or more functions is usually always much higher for nitrogen fixation. Algae which are forced to fix

nitrogen have very high boron and calcium requirements. There is also some evidence that small amounts of manganese, calcium, boron, cobalt, and copper are required for other growth functions (Jackson 1964).

In Myanmar, there is no research on isolation and cultivation of *Scenedesmus* sp. Therefore, in the present researchfour different media, Armo, BG-11, Scenedesmus and Bold's Basal medium were used for cultivation. The study was designed to investigate the most suitable media for the significant growth of *Scenedesmus dimorphus* (Turp.) Kuetzing in laboratory.

The aims and objectives of this study were to identify some *Scenedesmus* speciesfound in Bawdigone Pagoda, Mandalay and to isolate pure culture of*Scenedesmus dimorphus* (Turp.) Kuetzing.

MATERIALS AND METHODS

3.1 Collection and identification

Algae specimens were collected from the fresh water ponds of Bawdigone pagoda, Mandalay. It is situated at east longitude 90º 10' and north latitude 22º 00' (Figure 3.1). Water samples were taken from the surface of the water and carried by bottles, during the October 2014 to January 2015.

Algae specimens were studied by using light microscope at the laboratory, Department of Botany, University of Mandalay. The specimens were recorded by the digital camera. Morphological identification was performed using microscope and the literatures ofPrescott (1962), Philipose (1967) and Dillard (1989).

3.2 Isolation

Among twelve species of *Scenedesmus, S. dimorphus* was selected and isolated to get pure culture. Pure culture of *Scenedesmus dimorphus* (Turp.) Kuetzing was obtained by agar plate, subculture and micropipette method. The apparatus such as petridishes, bottles, slides, wire-loop and culture media were sterilized in an autoclave for 20 min at 121º C in order to prevent contamination. The micropipettemethod was used to isolate *Scenedesmus dimorphus* (Turp.) Kuetzing. The specimens were picked up by using a sterilized micropipette and inoculated in the microwell plate containing BG 11. The microwell plate was sealed with the parafiln to prevent from other undesirable contamination.

In agar plate method, 2g of agar was added into a beaker containing 100ml BG-11 media solution and stirred with glass rod, then autoclaved. The agar medium was poured into sterilized petridishes. Then, the water samples were cultured on the agar plates.

After one week, green color appears in culture plates and the cells were checked under the microscope. Then single colony were picked up by using wire loop and transferred to another sterile liquid medium.

Subcultures were made using BG-11 medium. The sample was poured into sterile 100ml bottles with BG-11 medium. After one week, a dominant species will appear. Repeat the process by transferring from each bottle to new bottle. The desired species was produced in the 3^{rd} to 4^{th} subcultures.

After subculture, micropipette method was used to pick up individual cells. The single cells were picked up individually from sample and transferred into the microwells plate (48 wells). After 7-10 days, the desired species was multiplied. Strain was maintained separately in 7 ml screw-capped test tubes with BG-11 and Scenedesmus medium under low light (2000 Lux).

3.3 Experimental procedure for culture of *S. dimorphus* (Turp.) Kuetzing

In the experiment, *Scenedesmus dimorphus* (Turp.) Kuetzing was cultured in four different media, Armo, BG-11, Scenedesmus and Bold's Basal (BBM) medium.

Pure species of*Scenedesmus dimorphus* (Turp.) Kuetzing on the agar plates were picked up with sterilized wire loop and poured into the bottles containing BG-11 medium. 100ml bottles were used for experiments. Algal suspensions were poured into experiment bottles containing 50 ml of four different media and adjusted initial optical density (OD) reached to 0.20 gl^{-1}.Theseculturebottles were placed on the 3-tiers shelves and illuminated by 4 feet fluorescent light tube (2000 lux) about1 feet distance from the bottles throughout the study period. The experimental temperature was approximately 24±1ºC under continuous aeration. These experiments were performed for 14 days. These experiments were done in three duplications and three replications.

3.4 Measurement of cell concentration

The optical densities of the algal suspension were measured as absorbance at 560 nm with the help of UV-VIS Spectrophotometer (spectro UV-2550) daily, at the Department of Botany, University of Mandalay.

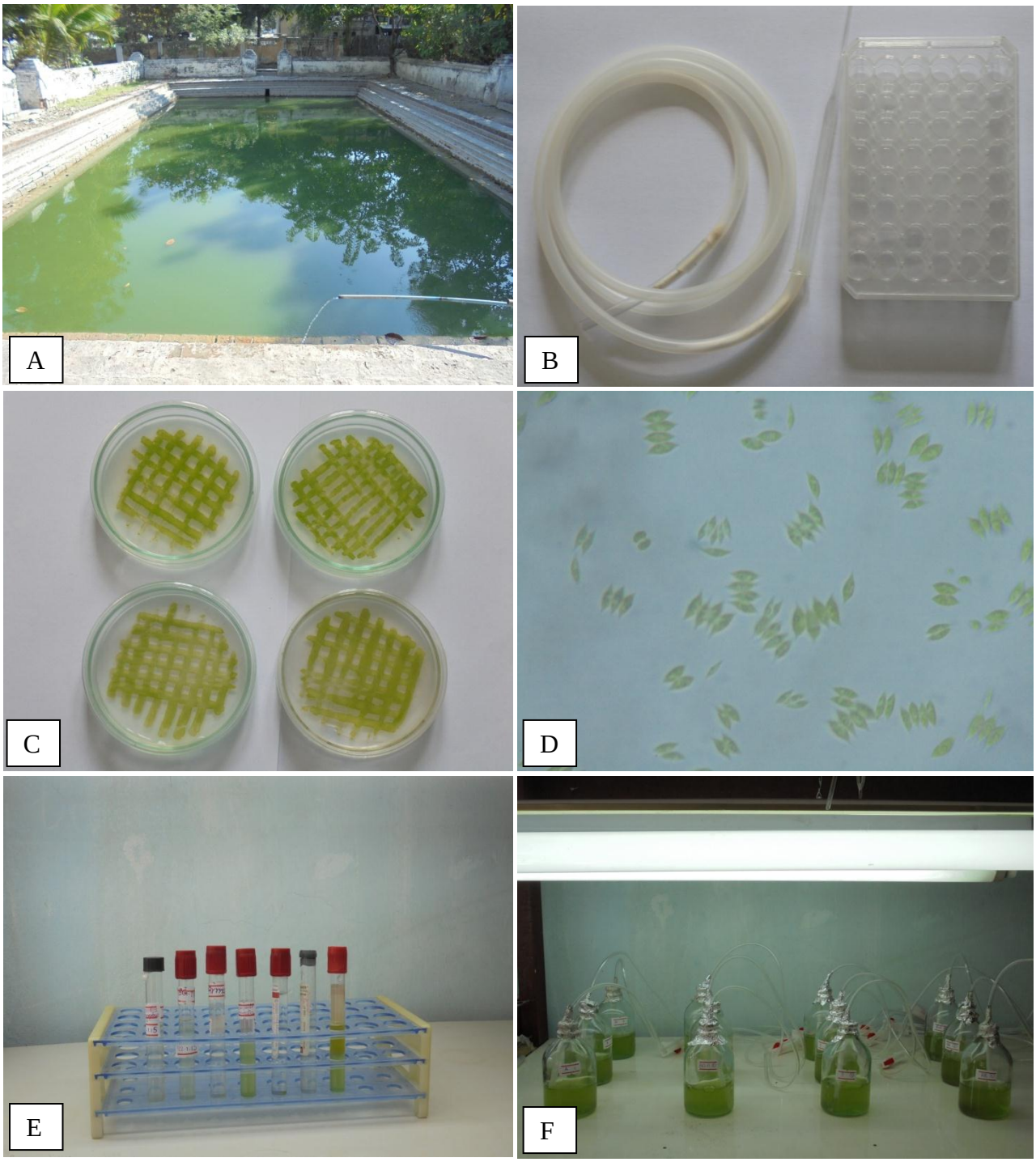

Figure 3.2 A. Sampling Site

B. Micropipette and Microwell plate (48 wells)

C. Pure cultures of *Scenedesmus dimorphus* in agar plates

D. Pure strain of *Scenedesmus dimorphus*

E. Pure strain in test tube

F. Culture bottles under 2000 Lux

RESULTS

***Scenedesmus* Meyen 1824**

Colony of 2-4-8-32 ovoid, fusiform, crescent-shaped, or oblong cells lying side by side in a single series, or in a double row with the cells alternating, cell walls smooth or with spines, teeth, and ridges; chloroplast a parietal plate covering most of the cell wall and often showing a medium lateral notch, 1 pyrenoid. This genus contains some species which are perhaps more widely distributed than any other fresh water algae.

Totally 12 species of *Scenedesmus*were identified and recorded in this study.

Table 4.1 List of *Scenedesmus* Species

Genus	Species
Scenedesmus	*S.abundans*(kirchner)Chodat
	*S. abundans*var. *brevicauda* Smith
	*S.arcuatus*Lemmermann
	*S. arcuatus*var.*capitatus* Smith
	*S. armatus*var.*bicaudatus* (Guglielmetti) Chodat
	S. bijugatus(Turp.) Kuetzing
	*S. bijugatus*var.*graevenitzii*Benard
	S.dimorphus (Turp.) Kuetzing
	S. obliquus(Turp.)Kuetzing
	S. quadricauda (Turp.) Brebisson
	*S. quadicauda*var. *quadrispina*(Chodat) Smith
	S. qutwinskii Chodat

***Scenedesmus abundans* (Kirchner) Chodat (Figure 4.1 A)**

Colonies four-celled, arranged in a linear series. Cells ovoid to oblong. External cells with one or more median lateral spines from the outer face in addition to spines from the four corners of the colony, 2.5-5 μ broad, 7.5-12.5μ long. Internal cells with one to two spines from their poles, 3.5-7.5 μ long.

***Scenedesmus abundans* var. *brevicauda* Smith (Figure 4.1 B)**

Colonies four-celled with much shorter spines which are not more than three between the polar spines of terminal cells. Cells 5-7.5 μ broad, 10-12.5 μ long.

***Scenedesmus arcuatus* Lemmermann (Figure 4.1 C)**

Colonies eight-celled in two series, oblong-ovoid, sometimes slightly angular at the base due to mutual pressure, 3.5-7.5 μ borad, 12.5-17.5 μ long,cell wall smooth and without teeth or spines.

***Scenedesmus arcuatus* var.*capitatus*. Smith (Figure 4.1D)**

Colonies four-celled in a linear or sublinear series. Cells slightly curved with one side convex and the other straight or slightly concave, 7.5-10 μ broad, 10-20 μ long. End of cells stumpy and with nodular thickenings.

***Scenedesmus armatus* var. *bicaudatus* (Guglielmetti) Chodat (Figure 4.1 E)**

Colonies four-celled, 5-10 μ broad, 10-17.5 μ long. Differ from the type in having a long spine from one of the poles of the terminal cell only, the spines of the two terminal cells alternating with each other, 3.5-7.5 μ long. Longitudinal ribs usually seen only in the internal cells.

***Scenedesmus bijugatus* (Turp.) Kuetzing (Figure 4.1 F)**

Colonies four-celled, flat or slightly curved, arranged in a single linear series. Cells oblong-ellipsoid to ovoid with the ends broadly rounded; 3.5-5μ broad, 10-17.5μ long.

***Scenedesmus bijugatus* var. *graevenitzii* Benard(Figure 4.2A)**

Colonies eight-celled. Cells fusiform, ellipsoid, oblong-ellipsoid to ovoid with obtuse poles and arranged in an alternating series with adjacent cells in contact only along a short portion of their length, 5-7.5 μbroad and 12.5-15 μ long.

***Scenedesmus dimorphus* (Turp.) Kuetzing (Figure 4.2 B)**

Colonies four-celled in a linear or subalternating series. The outer cells of the colony being more or less lunate and the apices of the cells being attenuated, 2.5-7.5 μ broad, 15-32.5 μ long.

***Scenedesmus obliquus* (Turp.) Kuetzing (Figure 4.2 C)**

Colonies four-celled, erect cells arranged in a linear or sublinear series. Cells fusiform with acute or slightly rounded ends and usually with straight sides, 2.5-7.5 µ broad, 10-17.5 µ long.Outerside of terminal cell concave or slightly convex. Cell wall smooth and without terminal teeth or spines.

***Scenedesmus quadricauda* (Turp.) Brebisson (Figure 4.2 D)**

Colonies four-celled, oblong cylindrical with rounded ends and arranged in a linear series, cells 3-7.5 broad, 10-17.5 µ long. Poles of terminal cells with a long, more or less straight or curved spine, 7.5-12.5 µ long. Cell wall smooth and without ridges.

***Scenedesmus quadricauda* var. *quadrispina* (Chodat) Smith (Figure 4.2 E)**

Colonies four-celled. Cells broadly ovoid and about twice as long as broad, 5-7.5 µ broad, 10-15 µ long. Poles of terminal cells with a single short recurved spine, 2.5-5 µ long.

***Scenedesmus qutwinskii*Chodat (Figure 4.2 F)**

Colonies four-celled, a single linear series, elongate-ellipsoid to cylindrical, 2.5-5 µ broad, 5-12.5 µ long. Outer face of outer cells with several spines, longer than width of cell.

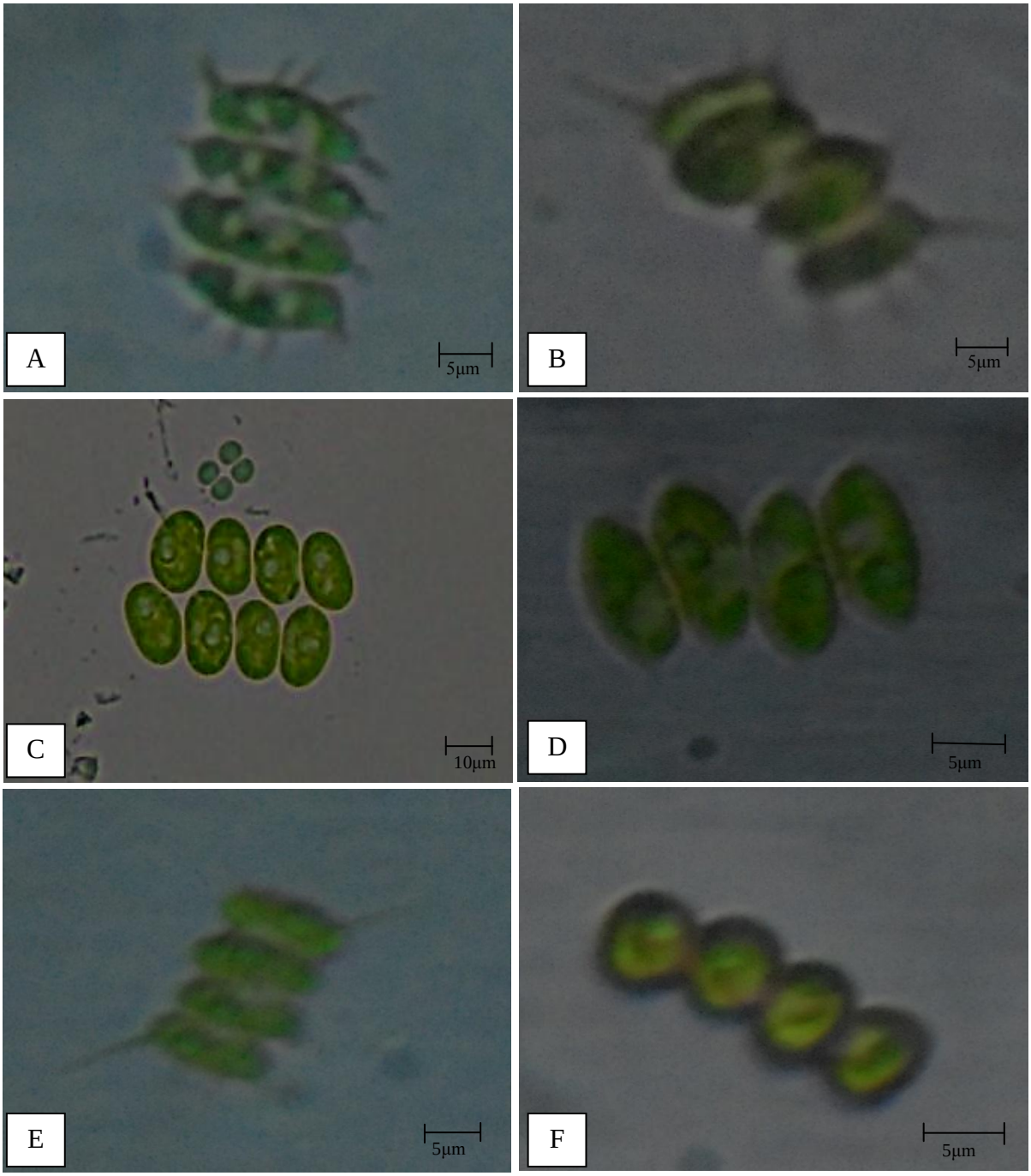

Figure 4.1 **A. *Scenedesmus abundans* (Kirchner) Chodat**

B. *Scenedesmus abundans* var. *brevicauda* Smith

C. *Scenedesmus arcuatus* Lemmermann

D. *Scenedesmus arcuatus* var.*capitatus* Smith

E. *Scenedesmus armatus* var. *bicaudatus* (Guglielmetti) Chodat

F. *Scenedesmus bijugatus* (Turp.) Kuetzing

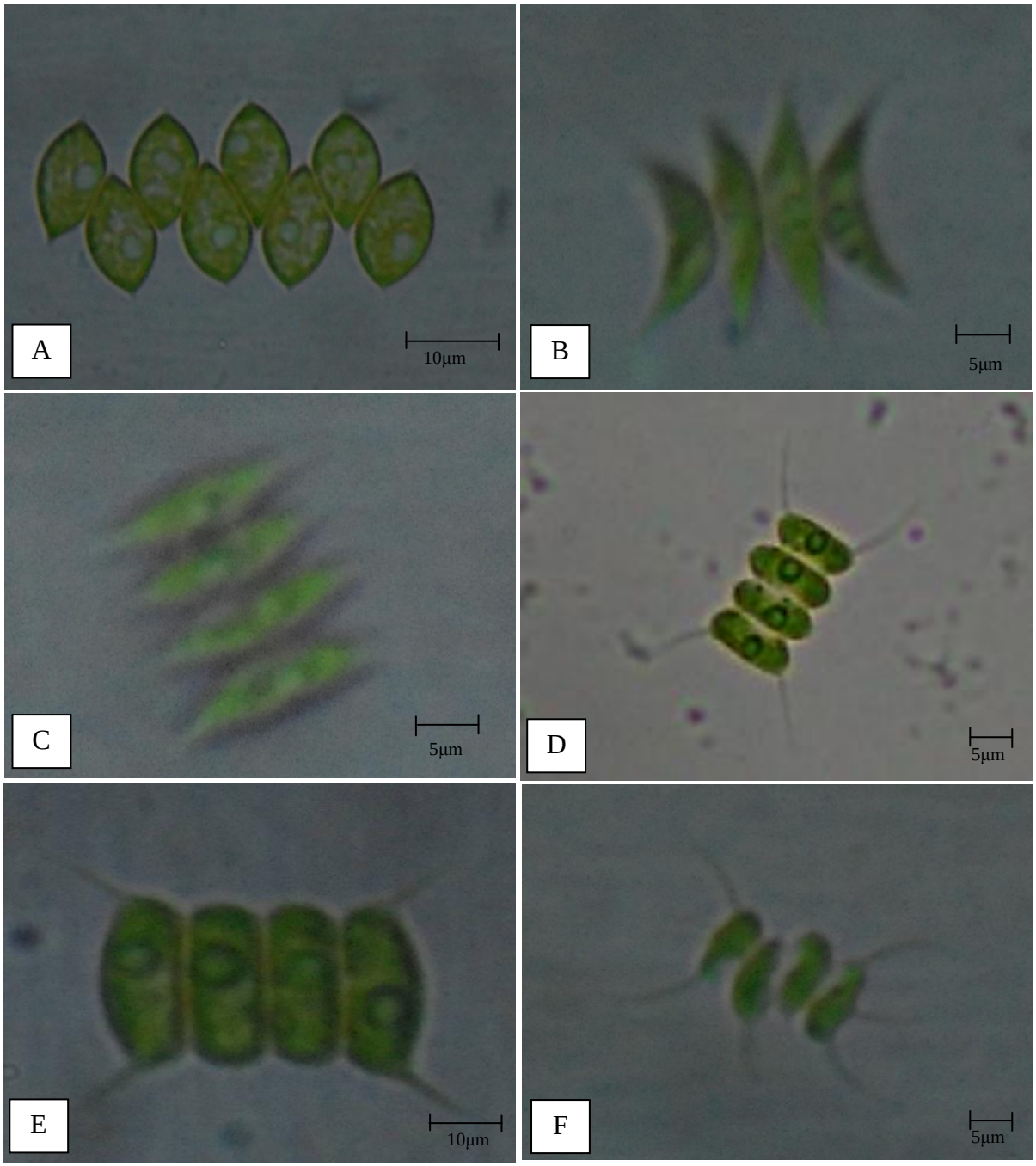

Figure 4.2 **A. *Scenedesmus bijugatus* var. *graevenitzii* Benard**

B. *Scenedesmus dimorphus* (Turp.) Kuetzing

C. *Scenedesmus obliquus* (Turp.) Kuetzing

D. *Scenedesmus quadricauda*(Turp.) Brebisson

E. *Scenedesmus quadricauda*var. *quadrispina* (Chodat) Smith

F. *Scenedesmus qutwinskii* Chodat

4.2 Effect of Different Nutrients media on Growth of *S. dimorphus*(Turp.) Kuetzing

It was observed that the growth of cell densities was increased in all different media. In this experiment, the initial optical density (OD_{560}) was $0.20gl^{-1}$ and cultured under continuous light of 2000 lux for 14 days of experimental period in January. The doubling cell density achieved on 2^{nd} day of the experimental period in each medium.

Among four different media, Armo is the best for the growth of *Scenedesmus dimorphus* and BG-11 medium is better than the Scenedesmus and Bold's Basal medium. These results were shown in Table 4.2 and Figure 4.3.The experimental period should be extended to show the stationary phase and death phase for clear result.

Table 4.2 Comparison on the Daily Increase Optical Density of *Scenedesmus dimorphus* (Turp.) Kuetzing at initial OD_{560} 0.20 gl^{-1}in Different Media

Time(Day)	MEDIA			
	ARMO	BG11	BOLD'S BASAL	SCENEDESMUS
0	0.20	0.20	0.20	0.20
1	0.36	0.35	0.31	0.35
2	**0.57**	**0.54**	**0.50**	**0.41**
3	0.82	0.77	0.50	0.72
4	1.02	0.84	0.59	0.86
5	1.15	0.90	0.63	0.89
6	1.31	1.03	0.70	0.94
7	1.43	1.13	0.78	1.02
8	1.51	1.23	0.84	1.09
9	1.62	1.37	0.95	1.19
10	1.68	1.48	1.04	1.29
11	1.72	1.57	1.15	1.35
12	1.73	1.57	1.20	1.40
13	1.76	1.60	1.24	1.44
14	1.76	1.62	1.29	1.49

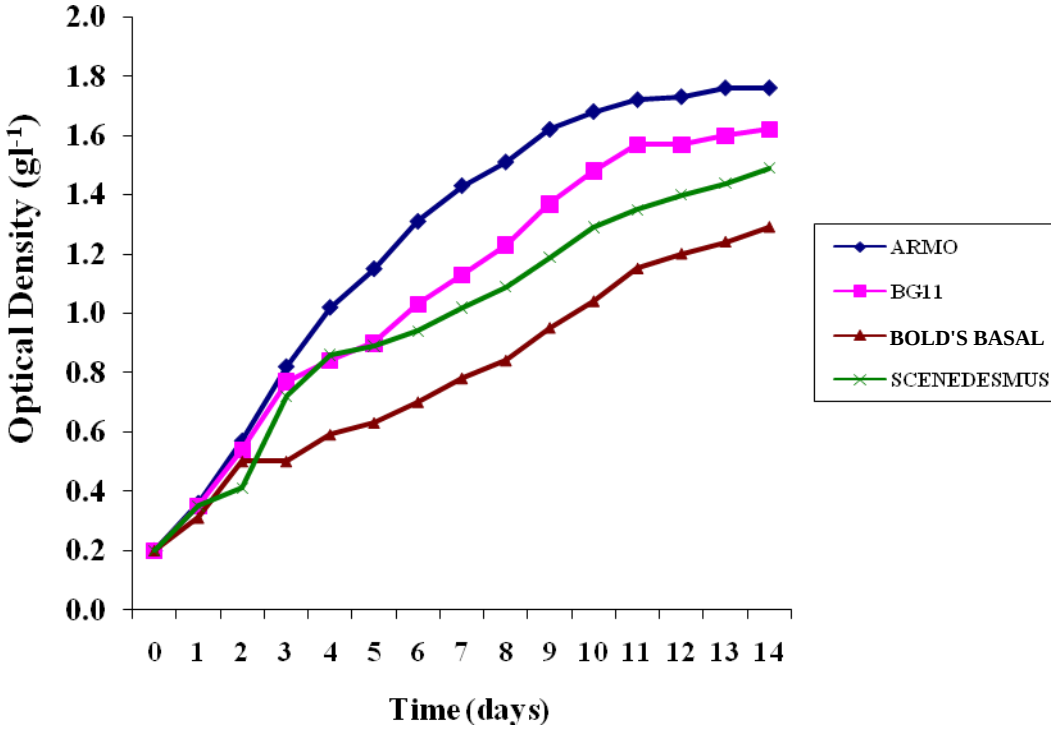

Figure 4.3 Comparison on the Daily Increase Optical Density of *Scenedesmus dimorphus* (Turp.) Kuetzing at initial OD $_{560}$ 0.20 gl^{-1} in Different Media

DISCUSSION AND CONCLUSION

In this study, twelve species of genus *Scenedesmus* were observed in the ponds of Bawdigone Pagoda, Mandalay and only species *Scenedesmus dimorphus*(Turp.) Kuetzing was isolated and culturedby using four different media Armo, BG-11, Scenedesmus and Bold's Basal medium.

In the present study, Armo medium was better for the growth of *Scenedesmus dimorphus* than that of BG-11, Bold's Basal, and Scenedesmusmedium. Richmond (1988) indicated that photoautotrophic microalgal growth is mainly dependent on nutrient such as carbon, nitrogen, phosphorus, and micronutrients. Richmond and Hu (2013) reported that the nitrogen is the most important nutrient contributing to the biomass produced. Armo contains 21% of nitrogen source that it was agreed with Richmond and Hu (2013).

EDTA and citric acid were used as chelating agent in these media. These agents play an important role in stabilizing the sufficient supply of trace metal elements and in the prevention in inhibitory effects of some metal (Becker and Venkataraman 1978). The BG-11 medium contains EDTA and citric acid. BBM-medium contains only EDTA.

Rousch *et al*. (2003) reportedthat the main environmental factors influencing microalgal growth and chemical composition are light, nutrients, temperature and pH.Light and temperature management are important for algal growth. Microalgae cultures can be grown in various kinds of transparent containers, including test tubes, bottles and flasks (Richmond and Hu 2013).

Xin *et al*. (2010) reported the growth and lipid accumulation properties of a freshwater microalga*Scenedesmus* sp. under different cultivation temperature, 10ºC, 20ºC, 25ºC and 30ºC for 16 days. They evaluated that the maximum microalgal densities at the temperature of 20, 25 and 30ºC were significantly higher than the one at 10ºC. In this research, the cultivation temperature was 24 $\pm$1ºC. It was agreed with Xu *et al* (2015), Arumugam *et al*. (2011), Goswami and kalita (2011), Shatri *et al*. (2014), kim *et al*. (2007), Makareviciene *et al*. (2011), Cicci and Bravi (2014) and Velichkova *et al.* (2013).

Xin *et al*. (2010) evaluated that their results indicated that at 25ºC which is the normal temperature for microalga cultivation, the lipid content per microalgal biomass was about 25% (w/w). At lower temperature of 10 and 20ºC, the lipid content per microalgal biomass was about 31% (w/w) and 35% (w/w) significantly. At higher temperature of 30ºC, the lipid content per microalgal biomass was 22% (w/w). Of this results, in the present research should be analysis the lipid content of cells.

Becker and Venkataraman (1978) evaluated that the pH of the *Scenedesmus* culture is maintained between 7 and 8. In this research, the pH value of Armo was pH 7.2, BG-11 was pH 7.4, Scenedesmusmedium was pH 7.2 and Bold's Basal medium was pH 7.2, respectively. Therefore it was agreed with that of Becker and Venkataraman (1978).

Ren *et al*. (2013) studied the effects of carbon and nitrogen sources and initial pH on the biomass and lipid production of *Scenedesmus* sp. under pH 3.0, 4.0, 6.0, 7.0, 9.0, 11.0 and 12.0.

In the present research, a result indicated that the Armo was the best medium and BG-11 was better than Scenedesmus and Bold's Basal medium. It may be

concluded that the difference of the algal growth rates in each medium was due to the difference of the contents of the nutrients used in media.

Xin *et al*. (2010) showed that the *Scenedesmus* sp. was cultivated in the BG-11 medium, the exponential growth phase was observed on the 2nd day followed by the stationary phase. In this study, the doubling of cells density was occurred on 2nd day of the experimental period in all media.

The result indicated that Armo is the best medium for *Scenedesmus* growth. The Armo contains nitrogen 21%,phosphate (P_2O5) 11%, potassium (K_2O) 21% and micronutrients, such as Fe, Mn, B, Zn, Cu and Mo.Goswami and Kalita (2011) evaluated that the nitrogen is known to have the strong influence on metabolism of lipids and fatty acids in various microalgae.

Geldenhuys *et al.* (1988) reported that agricultural, agro-industrial and municipal wastes contain all the macro and micronutrients important for algal growth. Both inorganic and organic nutrients give very good growth rate of alga species in the laboratory where the most successful genera are *Chlorella*, *Scenedesmus* and *Ankistrodesmus. Scenedesmus* contains all the essential amino acids and a good amount of protein, lipid and mineral content.

Becker and Venkataraman (1978) stated that the slaughter house wastes are available near an alga cultivation set up, these can be profitably used to get better yields of algae. Cow's urine, as a source of nitrogen, is used instead of chemical urea. This application has good promise in an integrated system of algal cultivation coupled with a dairy. And then this observation was agreed with Goswami and Kalita (2011).

Dragone *et al*. (2010) reported that the biofuel production from renewable source is widely considered to be one of the most sustainable alternatives to petroleum source fuels and available means for environmental and economic sustainability. Microalgae are currently being promoted as an ideal third generation biofuel feedstock because of their rapid growth rate, CO_2fixation ability and high production capacity of lipids, they also do not compete with food or feed crops, and can be produced on non-arable land.

Wang *et al.* (2008) evaluated that the utilization of microalgae for biofuel production has the potential for additional benefits, including waste water remediation, CO_2sequestration, and production of valuable co-products such as ethanol, methane, fertilizer and livestock feed.

The biomass obtained from microalgae could be transformed into various kinds of products for human beings.A good selection of microalgal species is available to support the aquaculture industry, and industry sectors and with improved nutritional quality. According to literature records, in recent years, *Scenedesmus* spp.was one of the most known species of biodiesel production. However this alga has not been used in yet locally as biodiesel in Myanmar.Therefore*Scenedesmus dimorphus* (Turp.)Kuetzing should be cultured by using Armo medium because it was as not expensive as compared with other media for mass production.

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