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ORIGINAL RESEARCH ARTICLE

Growth and Bio-Pigment Production of Three Microalgal Species in Organic and Inorganic Media and Determination of Generation Time – A Comparative Study

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ABSTRACT

Microalgae has been used as food for centuries by different populations and only rediscovered in recent years. Phycocyanin is a water soluble blue pigment that gives *Spirulina* its bluish tint. It is widely found in Blue green algae like *Spirulina*. Phycocyanin is a powerful water soluble antioxidant, scientists in Spain showed that an extract of *Spirulina* containing phycocyanin is a potent free radical scavenger and inhibits microsomal lipid peroxidation. This present study was aimed to evaluate the growth of three micro algal species (blue green algae- *Spirulina platensis, Spirulina platensis* var *lonar* and the green alga- *Chlorella* sp.) on organic media. The organic media were formulated from Spent Wash (SW) and Swine Dung (SD). The inorganic media (Zarrouk's medium for *Spirulina* and Sarokin & Krauss medium for *Chlorella*) were used as control (C). The biomass yields of all the three algae were recorded. In addition, the C-phycocyanin yield of the two species of *Spirulina* also compared to the control medium. The cultivation was carried out for a period of 15 days, at 27°C and at constant light intensity of 1.7 klux. The physico-chemical characteristics of both organic media were analyzed. Sizeable reduction in hardness, calcium and chloride content were observed in the media, after cultivation of the microalgae. The generation time of three micro algae in organic and inorganic media were also calculated. Overall, Spent wash (SW) showed better prospects than the control in terms of both biomass and C-phycocyanin production.

Keywords: Microalgae, Physico-chemical analysis, Organic medium, Generation time, Biomass production and C-Phycocyanin.

1. INTRODUCTION

Spirulina platensis has been used as food for centuries by different populations and only rediscovered in recent years. The annual production of the algae is about 10,000 tons which makes it the largest microalgal cultivation industry in the world. Due to its richness in protein, phycocyanin, essential amino acids, polysaccharides, carotenoids, minerals, vitamins and essential fatty acids has been regarded as an ideal bioresource and has drawn increasing attention in recent decades. The growing demands for natural products for the health, cosmetics have attracted the interest of micro algal biotechnology during the last two decades. The major concern in micro algal biotechnology is the low efficiency in the yield of mass cultures outdoors so there is a need for improvement in the growth performance of microalgae in these conditions (Richmond-2000)[1]. The micro alga *Spirulina platensis* are cultivated commercially by indigenous companies around the world and the product is mainly sold as a food supplement and animal feed (Belay *et al.* 1993)[2]. This unicellular alga possesses high protein content (60-70%) and considerable lipid content constituted of polyunsaturated fatty acids like Linoleic acid and Linolenic acid in the proportion of 1.24% and 1.04% respectively. Further more this alga also contains a high percentage of several pigments like

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phycobilliprotiens, carotenoids, chlorophyll-a and xanthophylls.

Phycocyanin is a water soluble blue pigment that gives *Spirulina* its bluish tint. It is widely found in Blue green algae like *Spirulina*. Phycocyanin is a powerful water soluble antioxidant, scientists in Spain showed that an extract of *Spirulina* containing phycocyanin is a potent free radical scavenger and inhibits microsomal lipid peroxidation. Phycocyanin in *Spirulina* that is though to help protect against renal failure caused by certain drug therapies. Phycocyanin has also shown promise in treating cancer in animals and stimulating the immune system. A human clinical study showed that a hot water extract of *Spirulina* rich in phycocyanin increased interferon production and Nk cytotoxicity (cancer killing cells) when taken orally. Zarrouk's medium was the first accepted nutrient base for cultivating *Spirulina*. Although alterations were made in the basic composition, the media so developed commercially were inorganic in nature and not economical. An attempt has been made to develop low cost organic media to cultivate *Spirulina* and *Chlorella* using spent wash (SW) and hygienically raised Yorkshire swine dung (SD).

2. MATERIALS AND METHODS

2.1. Culture collection and maintenance:

Spirulina platensis culture was obtained from CAS Botany University of Madras, Guindy campus Chennai. The *Spirulina platensis* var *lonar* culture was obtained from Arovil, Pondicherry and the *Chlorella* sp. was obtained from Krishnamurthy Institute of Algology, Nungambakkam, Chennai. These cultures were maintained in the modified inorganic media. Sub-cultures of *Spirulina* sp. and *Chlorella* sp were done by taking 10% as inoculums, performed every 30 days in modified Zarrouk's medium and Sarokin & Krauss medium at ambient temperature with initial pH 9.2. Cultivation was done in 1 litre Erlenmeyer flask and subjected to moderate mixing provided by a small aquarium aerator. Cultures were exposed to artificial light source and operated in cycles of 12 hours light and 12 hours dark period.

2.2. Media preparation:

The Spent wash was obtained from a distillery unit, Cuddalore, Tamilnadu, India. The swine waste was obtained from Post Graduate Research Centre (Formerly known as Live Stock Research Station), Kattupakkam, a unit of Tamilnadu University of Veterinary and Animal Sciences (TANUVAS), Chennai. The SW culture medium was prepared by mixing the SW with sterile distilled water in the ratio of 1:20. The colour of the medium was light brown. The pH of the medium was adjusted to 9.2 by adding sodium bicarbonate. The swine dung was solar dried and made into a powder form. An extract was obtained from the powdered organic waste by dissolving it in sterile distilled water in the ratio of 1:10 and filtered through cotton followed by ordinary filter paper. The colour of solution is light brown. The pH of the medium was adjusted to 9.2 by adding sodium bicarbonate.

2.3. Cultivation:

In each flask 5% of *Spirulina platensis*, *Spirulina platensis* var *lonar* and *Chlorella* sp. were inoculated respectively. The cultivation was carried out in a series of Erlenmeyer flasks (1000 ml) containing the formulated media in quadruplicates. Artificial lighting was provided with an ordinary white fluorescent lamp for 12 hrs (day). Uniform aeration was maintained in culture vessel by an aquarium aerator. The incubation temperature was maintained at 27ºC ±2 throughout the study. The growth of the culture was monitored as per the protocol of Venkataraman (1983)[3] for a period of 15 days. The generation time was calculated as per Prescott *et al*. (2008)[4].

2.4. Physic-chemical characterization.

In both organic medium, the physico-chemical parameters such as color, odour, pH, hardness, alkalinity, calcium content, and chloride content were determined according to standard methods (APHA-1989)[5].

2.5. Phycocyanin extraction.

The C- phycocyanin was extracted from fresh biomass (Sarada *et al*. 1999)[6]. Fresh biomass was homogenized with 50mM sodium phosphate buffer, the homogenate was subjected to alternate freezing and thawing(3 to 4 cycles) and centrifuged at 5000rpm for 10 minutes. The phycocyanin content was estimated by the method of Sigelman and Kycia(1978)[7].

3. RESULTS AND DISCUSSION

Spirulina platensis was found in waters containing from 85 to 270 g of salt per liter, but growth seemed to be optimal at salt concentrations ranging from 20 to 70 g/liter, and it is possible that the population of *Spirulina platensis* found at the highest salt concentrations. *Spirulina* can be grown in alkaline conditions and the organism appears to be capable of adaptation to very different habitats and colonizes certain environments in which life for other

microorganisms is difficult. Phycocyanin is a water soluble blue pigment that gives *Spirulina* its bluish tint. It is found in blue green algae like *Spirulina*. Phycocyanin is a powerful water soluble antioxidant, scientists in Spain showed that an extract of *Spirulina* containing phycocyanin is a potent free radical scavenger and inhibits microsomal lipid peroxidation.

The physical parameters of spent wash (SW) and swine dung (SD), i.e., color, odor, and pH were analyzed. The SW has dark brown color, disagreeable odor with pH of 5.5. From the figure (Fig 1) it was learnt that, the organism cannot efficiently reduce the alkalinity but can efficiently reduce the hardness of the medium i.e., from 330mg/l to 20mg/l. there was a three fold increase in the calcium content of the medium where as the chloride concentration showed three fold reduction. The SD has dark brown color, ammonia tinged odor and the pH was 6.0. The total alkalinity of SD before inoculation was 800mg/l, but the filtrate showed increase in alkalinity (1550mg/l). The hardness of the medium was 200mg/l and it was completely reduced to 60mg/l at the end of incubation. The calcium and chloride concentration of the medium was initially 14.028mg/l and 15.016mg/l respectively. After inoculation, their concentration was 12.024mg/l and 10.0mg/l.

The generation time of *Spirulina platensis* in control was 0.41h/g, in SW 1.2 h/g and in SD 1.09 h/g. The generation time of *Spirulina platensis* var *lonar* in control was 1.4 h/g, in SW 1.03 and in SD 1.4 h/g. and for *Chlorella* in control was 0.41 h/g, in SW 0.47 and in SD 0.38. The biomass production of *Spirulina platensis* on control was 0.3 g/l, in SW 0.3 g/l and in SD 0.2 g /l., the biomass production of *Spirulina platensis* var *lonar* in control was 0.36g/l in SW 0.34 and in SD 0.2g/l. for *Chlorella* 0.2g/l in C, 0.12 in SW and 0.1 in SD. The bio-pigment C-phycocyanin production of *Spirulina platensis* was 0.076 mg/ml in control, 0.085 mg/ml in SW and 0.077 mg/ml in SD. For *Spirulina platensis* var *lonar* it was 0.08 in control, 0.093 in SW and 0.076 in SD. Based on the above findings, the three microalgae could efficiently absorb the nutrients found in the organic waste and convert them into biomass as well bio-pigments.

Kaushik *et al*. (2006)[8] cultivated *Spirulina platensis* (ARM 730) in different dilutions (5, 10, 20, 30 and 50%) of Anaerobically digested distillery effluent diluted with distilled water. They found that 50% dilution was optimum for the growth of the microalgae and decline beyond that concentration. Murugan *et al*. (2007)[9] conducted similar experiment and found that 7.5% was optimum. In accordance with our previous trial the concentration of SW medium was fixed at 7.5% for *Spirulina platensis*, *Spirulina platensis* var *lonar* and *Chlorella* sp.

The efficacy of pig wastewater treatment by the Green algae *Scenedesmus quadricauda* and Blue green alga *Spirulina platensis* was investigated by *Gantar et al*. (1991)[10]. They used 10, 20, 30, 40 and 50% diluted swine waste as medium. They found that 20% dilution provided favorable growth condition not only for the growth of *Spirulina platensis* but also for the autochthonous alga *Chlorella*. Manikandavelu *et al*. (2009)[11] formulated a medium using swine dung waste and found that 10% favour the growth of the blue green alga *Spirulina platensis*. In accordance with our prior study the organic media was formulated by using 10% swine dung waste. The growth of three different microalgal species was determined in the above said culture media and also their pigment production efficiency. It was found that *Spirulina platensis* var *lonar* adapted to the newly formulated medium much better than other two microalgae (*Spirulina platensis* and *Chlorella*). The growth performance of the three experimental microalgae showed good response in the spent wash medium and the two cyanobacterial species (*Spirulina platensis* and *Spirulina platensis* var *lonar)* adopt much better than *Chlorella*.

Bohra (2009)[12] investigated the growth pattern of *Spirulina platensis* in standard and modified media based on seawater-chemicals and seawater fertilizers. During the cultivation, the cell concentrations were analyzed at 540nm along with protein and chlorophyll-*a* estimation. Growth patterns of different species and strains were monitored for 25 days and specific growth rate, mean daily division rate and doubling time were calculated. *Spirulina platensis* was observed to have different specific growth characteristics in different media at same environmental parameters.

Yi-Ming Zhang (2004)[13] separated C-phycocyanin and allophycocyanin from *Spirulina platensis* and purified by precipitation with ammonium sulphate, ion exchange chromatography and gel filtration chromatography. Pure C-phycocyanin and allophycocyanin were finally obtained with an A620/A280value of 5.06 and an A655/A280 value of 5.34, respectively. Silveira *et al.* (2007)[14]

extracted C-phycocyanin from cyanobacteria S*pirulina platensis* was optimized using factorial design and response surface techniques. The effects of temperature and biomass-solvent ratio on phycocyanin concentration and extract purity were evaluated to determine the optimum conditions for phycocyanin extractions. The optimum conditions for the extraction of phycocyanin from S*pirulina platensis* were the highest biomass-solvent ratio, 0.08g/ml/l, and 25^0C. Under these conditions it's possible to obtain an extract of phycocyanin with a concentration of 3.68mg.mL/1 and purity ratio (A615, A280) of 0.46.

Fig 1: Chemical characteristics of organic media (SW and SD)

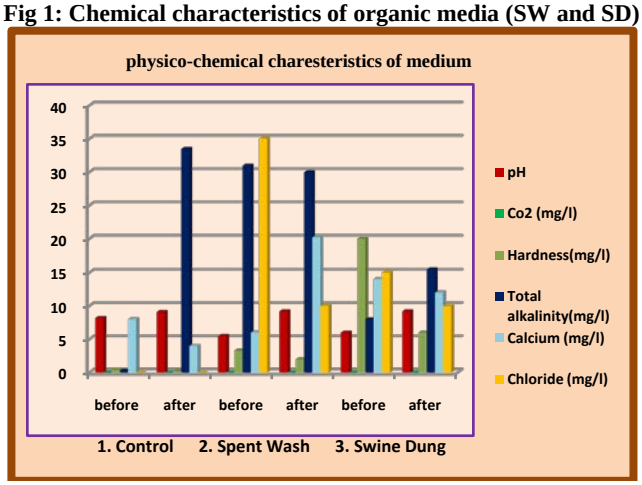


Fig 2: Growth performance of *Spirulina platensis* on control media by DMC

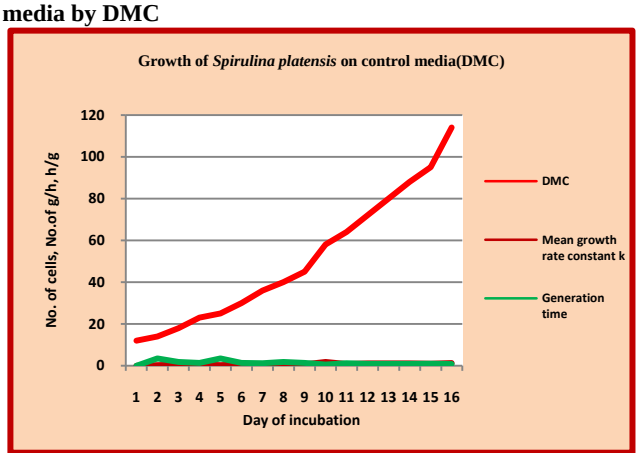


Fig 3: Growth performance of *Spirulina platensis* on SW media by DMC

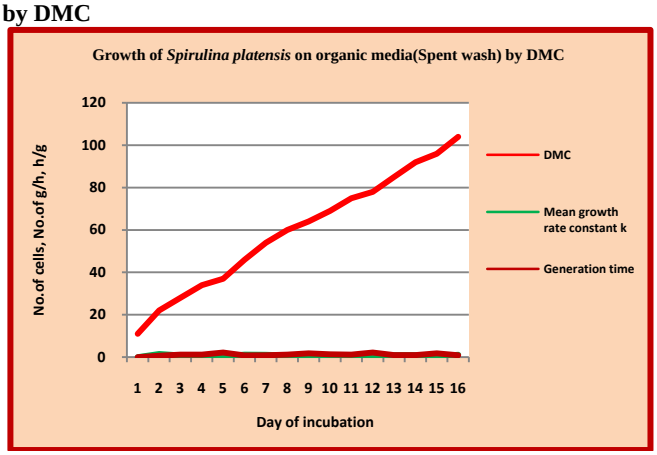


Fig 4: Growth performance of *Spirulina platensis* on SD media by DMC

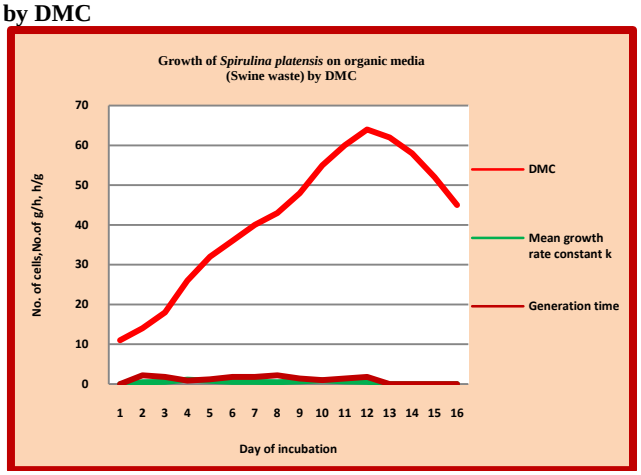


Fig 5: Growth performance of *Spirulina platensis* var *lonar* on control media by DMC

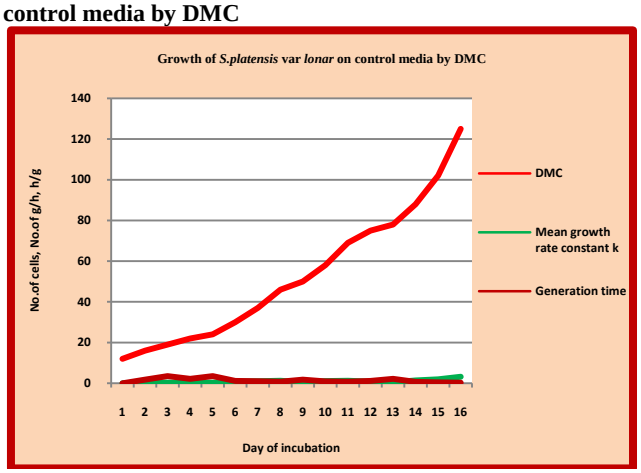


Fig 6: Growth performance of *Spirulina platensis* var *lonar* on SW media by DMC

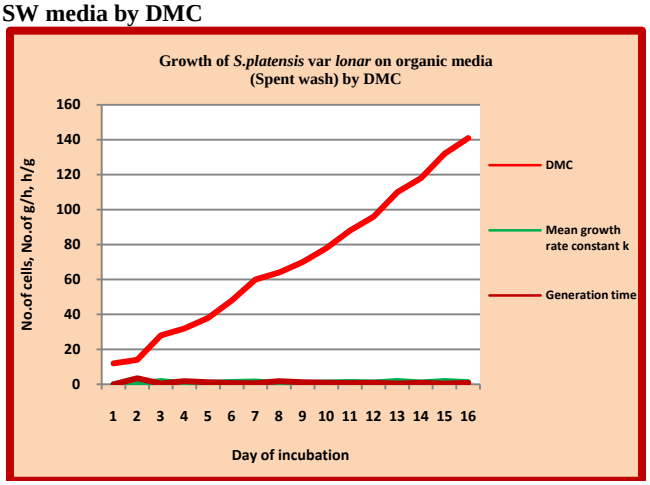


Fig 7: Growth performance of *Spirulina platensis* var *lonar* on SD media by DMC

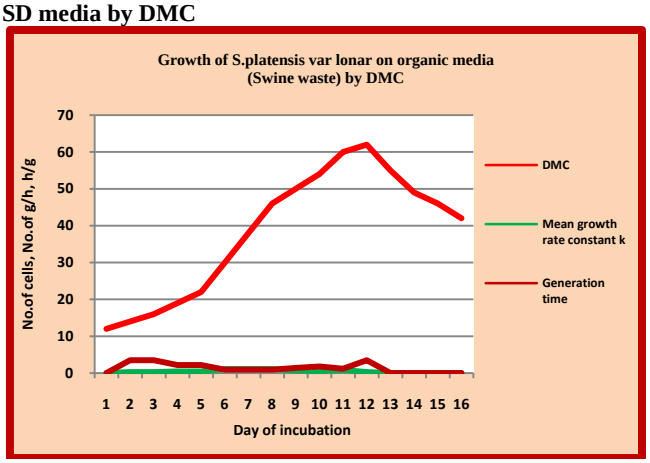


Fig 8: Growth performance of *Chlorella* on control media by DMC

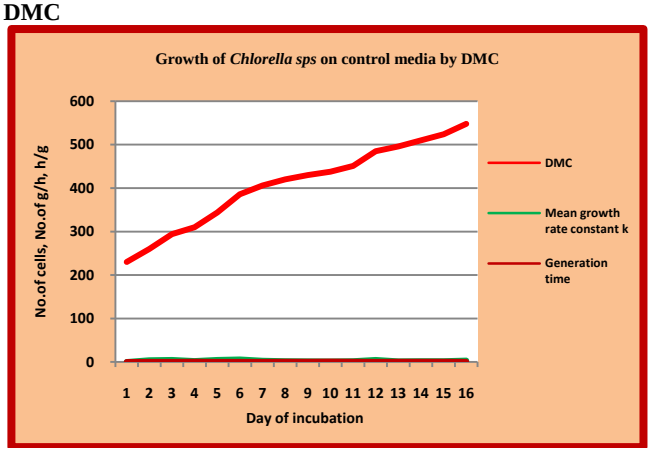


Fig 9: Growth performance of *Chlorella* on SW media by DMC

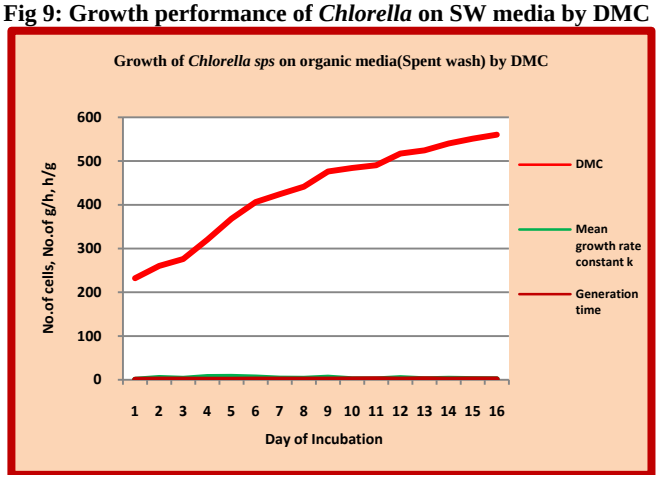


Fig 10: Growth performance of *Chlorella* on SD media by DMC

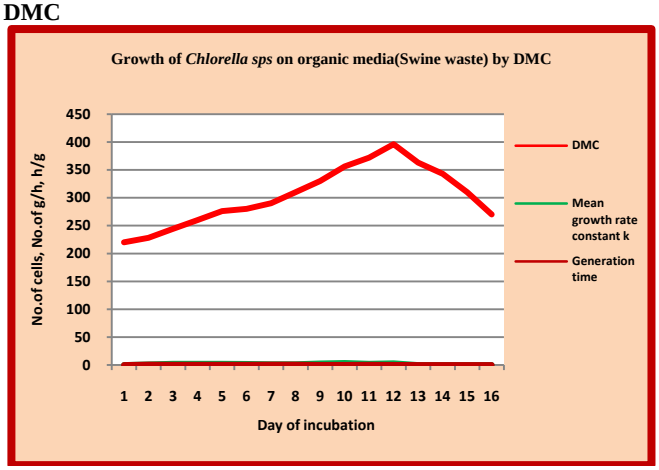


Fig 11: Comparison of Generation time on organic and control media

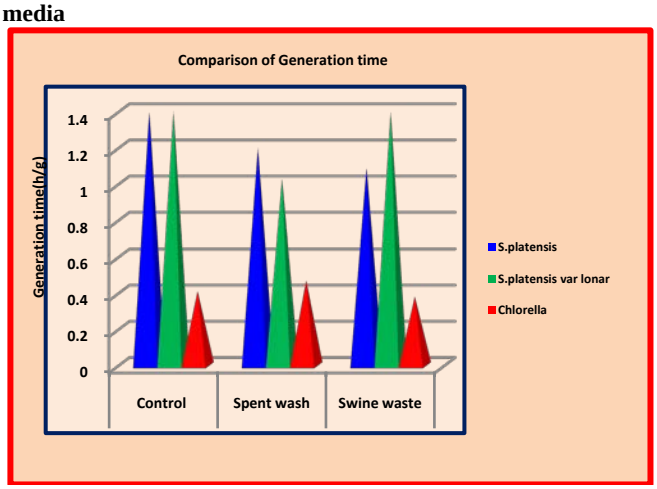


Fig 12: Growth performance of *Spirulina platensis* on media by optical density

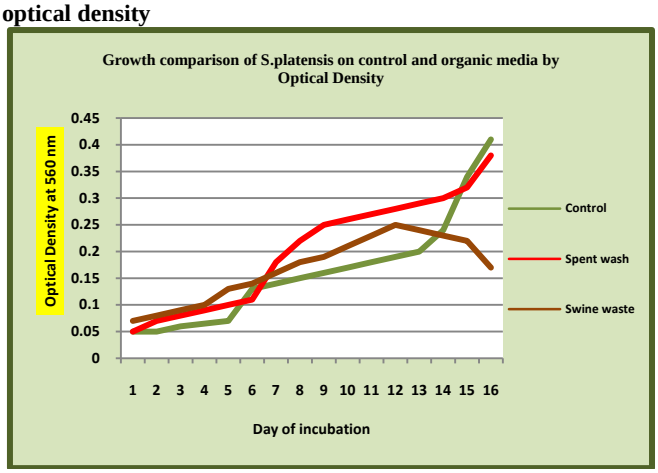


Fig 13: Growth performance of *Spirulina platensis* var *lonar* on media by optical density

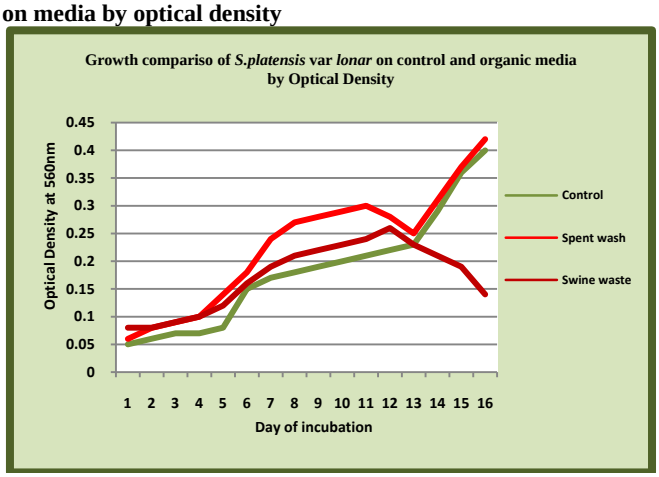


Fig 14: Growth performance of *Chlorella* on media by optical density

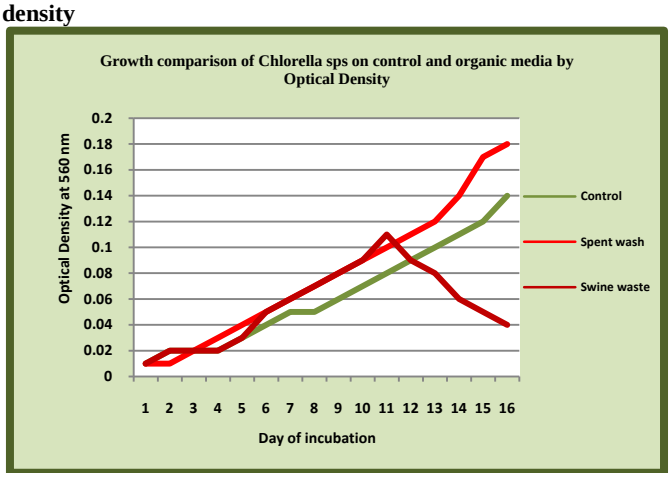


Fig 15: Biomass weight on organic media

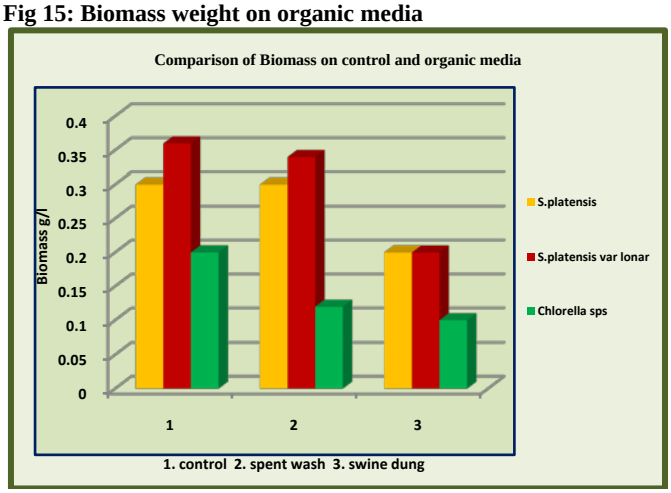
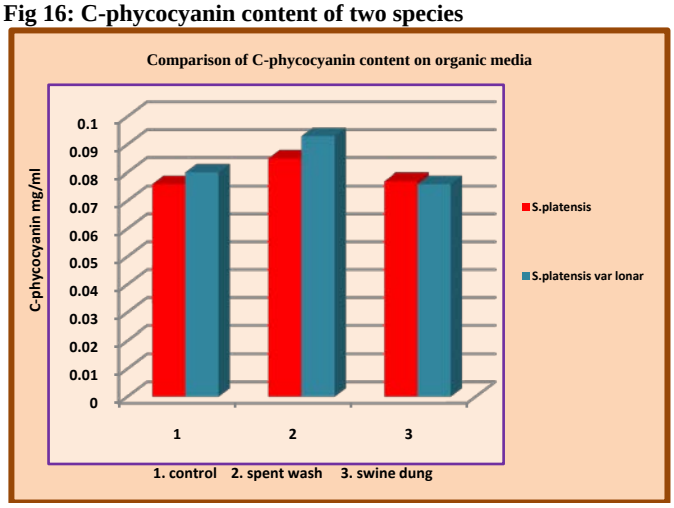


Fig 16: C-phycocyanin content of two species



4. CONCLUSION.

From the results of the present study, it could be concluded that SW medium was more conducive than Sd medium for these three algae. Out of three micro algae tested *Spirulina platensis* var *lonar* responded well to the formulated organic media. In conclusion, the SW medium proved to be profitable for adopting this practice at small level to have it on a sustainable scale.

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