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Studies on the *Centella Asiatica* Growth and Yield After the Application of Filtrates from *Spirulina Species* Isolates in Mangrove Ecosystem

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ABSTRACT

An experiment was conducted at the Experimental Farm, Department of Microbiology, Faculty of Agriculture, Annamalai University to study *Centella asiatica* growth and yield after the application of filtrates from *Spirulina species* isolates Mangrove ecosystem. The present study aims to investigate the growth parameters of *Spirulina species*. The results of present include *Spirulina* isolates from MS1-MS10, among which MS7 strain recorded highest growth parameters. The experiment was laid out in randomized block design (RBD) with seven treatments and three replications using *Centella asiatica* variety IIHR CA-1. The results showed that the application of Spirulina extract significantly influenced the leaf area index (LAI) of the plant. The highest plant height was recorded under (T_7) (Seedling root dipping + Foliar spray) at 30 and 60 days after transplanting, with the lowest stolon length recorded at harvest. The maximum leafy yield of 1.755 kg/pot was achieved under (T_7). The phosphorous content in the leaves was significantly increased by applying Spirulina extract at different concentrations. The N, P, K, and asiaticoside content of the leaves were also significantly increased after applying a combination of treatments. The results also indicated a significant increase in Chlorophyll content and asiaticoside content. The application of Seedling root dipping + Foliar spraying of Spirulina extract (T_7) was found to be more promising as an efficient method for maintaining soil fertility and ecosystem.

Keywords: Mangrove, *Spirulina platensis, Centella asiatica*, Asiaticoside, Productivity

INTRODUCTION

Mangroves are among the world's most productive ecosystems and a part of the “blue carbon” sink. In India mangroves cover over 4,975 sq km, including the coasts, Andaman and Nicobar, and Lakshadweep islands. India has 2.66 per cent of the world's mangroves and among them about 57 per cent are found on the East Coast. The east coast is endowed with the world's largest mangrove forest, the Gangetic Sundarbans in West Bengal that has 30 of 50 true mangrove species. In Tamil Nadu coast, mangrove mangroves forest exist in Muthupet and Pichavaram areas. Extensive scientific research covering all aspects has been carried out about these three mangrove ecosystems. As per the report of ‘EnviStats India 2022’ released by the Ministry of Statistics and Programme Implementation, Tamil Nadu had mangrove coverage of 23 sq km in 1987, but it was increased to 45 sq km in 2021 over the years. However, data available on the existence and importance of Vedaranyam mangroves has been very much limited. Prioritizing assessment studies through this preliminary study on the existing biodiversity helps to understand the wealth and well-being of Vedaranyam mangroves.

In **Agriculture** algal bio-fertilizers will be the best alternative source of nitrogenous chemical fertilizers as they are fully independent, cost effective and easily available. Microalgae are the cheap and best source of Nitrogen, which does not cause soil and water pollution. Cyanobacteria are capable of fixing the atmospheric nitrogen which is very useful for agriculture and has various positive effects for soil and plants like build-up of soil

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fertility and consequently increase and yield as a natural biofertilizer (Dineshkumar *et al*., 2018). ***Centella asiatica***, commonly known as "Gotukola" in Sinhala language ,**Indian pennywort** and **Asiatic pennywort**, is a herbaceous , perennial plant in the flowering plant and the family Apiaceae. It is native to tropical regions of Africa, Asia, Australia, and islands in the western Pacific Ocean. It is consumed as a culinary vegetable and is used in traditional medicine.

The plant contains bioactive compounds such as asiaticoside and madecassoside, which have been found to possess a wide range of pharmacological properties including neuroprotective, anti-inflammatory, antioxidant, and wound healing activities. *Centella asiatica* has been traditionally used for the treatment of various conditions including memory enhancement, nervous system disorders, skin diseases, and skin lesions. (Shinjini *et al*., 2023). The plant has been extensively studied for its therapeutic advantages and its potential use in dermatology, including the treatment of skin abnormalities, burn injuries, and skin whitening. *Centella asiatica* has shown promising medicinal properties and has the potential for further research and development in various fields. In China, it is indigenously called as Gotu kola, and over 2000 years ago, it was one of the documented **"miracle elixirs of life"** (Diwan *et al*., 1991)**.** The aim of the present study is to develop new strain of *Spirulina platensis* from Mangrove ecosystem for mass production and development of by products and application of filtrates on the growth and yield of Medicinal crop *(Centella asiatica).*

MATERIALS AND METHODS

The present investigation was carried out in Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar. The algal samples were collected from Mangrove ecosystem aquatic habitats like Marine and Brackish water in different locations of Cuddalore district mainly in Pichavaram. The predominant algal genera in the 10 samples were identified based on their morphological characters. The ten *Spirulina* strains were isolated and designated as MS1, MS2, MS3, MS4, MS5, MS6, MS7, MS8, MS9 and MS10.The isolated *Spirulina* strains were characterized based on habitat, average no. of spirals, direction of the helix, distance between the spirals, the diameter of the spirals, the width of the spirals, filaments, shape of the spirals, pH tolerance and temperature tolerance. The pure culture of *S. platensis* strain MSp7 was grown in 1000 ml of Enriched liquid medium for 30 days under laboratory condition for maintaining stock cultures for indoor cultivation. The *S. platensis* growth was evidenced by thickening of the culture marked by the development of anintense green colour and they are shaken a few times every day. After 30 days, the inoculum was ready for outdoor cultivation. The *S. platensis* cultivation was carried out in 1000 litre shallow cement tank during the summer (Jan-Mar, 2023). The stock culture (1.0 g /l) of *S. platensis* (MSp7) was inoculated in Enriched medium. The growth medium was mixed by manual stirring with a plastic stick to prevent algal accumulation at the surface and to ensure uniform distribution of nutrients.After the incubation period (60 days) the algal mat was filtered through the fine nylon filter by gravity filtration. Filtered algal mats were washed in sterile distilled water to remove the impurities.

The used algae in the present study *Spirulina platensis* is photosynthetic and multicellular blue – green microalgae that grown in wide range fresh, marine and brackish water. The freah algae material (one kg) was cut into small pieces and weighed. The sample was extracted using a blender. The blended material was filtered through a double –layered of muslin cloth to remove debris and designated as 100% and different used concentrations in the study were prepared by adding tap water and refrigerated between 0- 4˚C until use. The plants were sprayed with algae extract after 30,60 and 90 days and also a seedling root dipping with some plants. *Spirulina* extract was prepared by mixing *spirulina* filtrate powder and water at a ratio of 1:0.5, homogenizing. The percentage level of preparing foliar spray of Spirulina extract is 0.5% and 1%. Add the measured *spirulina* extract to a container containing the desired amount of water. Stir or agitate the mixture to ensure that the *spirulina* is well-dissolved in the water. Use clean, non-chlorinated water. If possible, use water with a pH level close to neutral.

RESULTS AND DISCUSSION

The results of present investigation include collection, isolation and identification of *Spirulina species* from Mangroveecosystem. The mass cultivation (Table 1) are similarly, Soletto *et al.,* (2005), Cultivation medium has a great impact on the productivity of biomass and other compounds of *Spirulina platensis*. Pelizer *et al.,*

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(2003) reported that there are several parameters, which can influence growth could be optimized ie physiochemical factors like medium composition, temperature, pH, light, agitation. The two most important techniques widely used to cultivate microalgaeis open raceway pond system and closed system photobioreacter system. The open pond system is less favorable due to limitation in controlling contamination from predator while, like photobioreacter provided an easy system of controlling nutrients for growth, cultivation parameter such as temperature, dissolved CO_2, pH and prevent contamination (Groddelaar, 2007). Similarly, in the present study, the mass cultivation of *S. platensis* MSp7 inenriched medium was conducted in cement tank with surface area $4m^2$(capacity, 2000L), during summer season, (Jan-March 2023) average day time temperature normally above 40^0C.The growth was significantly increased with increasing the period up to 60 days. During the period, the highest biomass and proximate composition of *S. platensis* MSp7 was recorded in (Jan-Feb).

Application of Seedling root dipping + Foliar spraying of Spirulina extract at 1% (T_7) recorded the highest leafy yield of *Centella asiatica*. (Table 2). This might be due to adequate supply of nutrients at different growth stages of crop which helped in better absorption and translocation into the plant system more efficiently to developing leafy structures contributing to the proper photosynthetic pigments processes filling up the chlorophyll content there by resulting in higher leafy yield. The least leafy yield recorded the T_1(Control- no treatment). The application of algal fertilizers enhanced the vegetative growth of plants and this was supported by Sharma and Ubadhyay (1992) who stated that increase in leaf area contributes to the improved photosynthesis rate thereby increasing the bulb length, bulb diameter and bulb weight that taken in the production of the vegetative part and functioning bulbs. Spirulina extract could increased asiaticoside content value of *Centella asiatica*. (Table 3). Wang *et al.* (2010) on Chinese cabbage conducted experiments to asses the effect of increasing doses of spirulina extract applications and found a significant increases in the nutritional value. The increment of NPK in cucumber leaves increased by increase the concentration of Sprulina that contain a higher capacity of macronutrients or NPK as reported by Abd El-Moniem EA *et al.* (2008). The beneficial effect of *Spirulina platensis* extract symbiosis in improving nutrient supply, especially P, might increased the levels of asiaticoside. It may also participate in the biosynthesis regulation of secondary metabolites in plants. Moreover, *Spirulina* extract induced the accumulation of asiaticoside (Trisilawati *et al.*, 2019).

Table 1: Growth of Spirulina platensis MSp7 Stain in outdoor condition during Summer Season (Jan-Mar)2023

Growth Period	**Biomass (g/l)**	**Protein (g/l)**	**Lipid (mg/ml)**	**Chlorophyll (mg/ml)**	**Total Phycobiliprotein (mg/ml)**	**Total Carotenoid (mg/ml)**
0	0.112	0.061`	18.70	17.08	31.40	6.80
10	0.187	0.117	22.74	19.87	33.71	7.33
15	0.231	0.139	24.50	23.45	34.70	7.67
20	0.262	0.168	26.91	25.59	35.64	8.10
25	0.305	0.184	29.69	27.55	36.82	8.56
30	0.339	0.210	31.80	29.24	38.00	8.90
35	0.383	0.232	34.57	31.16	39.37	9.41
40	0.405	0.246	35.26	33.06	40.25	9.77
45	0.424	0.268	36.97	34.81	41.29	10.16
50	0.437	0.273	38.45	36.79	41.83	10.36
55	0.454	0.290	39.83	38.50	42.56	10.70

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60	0.470	0.304	41.35	40.33	43.17	10.90
Mean	0.334	0.207	31.73	29.78	38.22	9.055
SEd	**0.092**	**0.065**	**1.720**	**1.293**	**1.352**	**0.231**
CD (p=0.05)	**0.189**	**0.137**	**3.445**	**2.592**	**2.744**	**0.465**

Table 2: Effect of Spirulina extract at different concentrations on the leafy yield of Centella asiatica variety IIHR CA -1

T. No.	Treatment Details	Leafy yield (kg pot^{-1})
T_1	Control (no treatment)	0.260
T_2	Foliar spray of Spirulina extract at 0.5 %	0.800
T_3	Foliar spray of Spirulina extract at 1%	1.053
T_4	Seedling root dipping with Spirulina extract at 0.5%	1.268
T_5	Seedling root dipping with Spirulina extract at 1%	1.382
T_6	Seedling root dipping + Foliar spray of Spirulina extract at 0.5%	1.567
T_7	Seedling root dipping + Foliar spray of Spirulina extract at 1%	1.755
S.Ed		0.028
CD(P=0.05)		0.062

Table 3: Effect of Spirulina extract at different concentrations on the asiaticoside content of Centella asiatica variety IIHR CA -1

T. No.	Treatment Details	Asiaticoside content (%)
T_1	Control (no treatment)	1.24
T_2	Foliar spray of Spirulina extract at 0.5 %	1.38
T_3	Foliar spray of Spirulina extract at 1%	1.46
T_4	Seedling root dipping with Spirulina extract at 0.5%	1.71
T_5	Seedling root dipping with Spirulina extract at 1%	1.83
T_6	Seedling root dipping + Foliar spray of Spirulina extract at 0.5%	2.03
T_7	Seedling root dipping + Foliar spray of Spirulina extract at 1%	2.07
S.Ed		0.032
CD(P=0.05)		0.071

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CONCLUSION

From the above Study, it can be concluded that the isolation of *Spirulina platensis* from mangroven ecosystem- the *S. platensis* MSp7 recorded highest level. It can be concluded that the Spirulina extract application of Seedling root dipping + Foliar spraying of *Spirulina* extract at 1% (**T_7**) was performed well and economic method for enhancing the nutrient development and yield of centella asiatica. The combined application of seedling root dipping and foliar foliar spraying of Spirulina extract improved the soil health and steadily supplies the nutrients. This may be an eco-friendly, environmentally sound and economically viable method. Microalgal biofertilizers represent a promising and sustainable alternative to traditional chemical fertilizers. By harnessing the natural capabilities of algae, these biofertilizers offer a range of environmental and agricultural benefits, including improved soil fertility, enhanced plant growth, and reduced environmental pollution. The application of algal biofertilizers also supports the principles of organic farming, contributing to more sustainable agricultural practices. However, further research is needed to optimize production processes, increase efficiency, and explore the full potential of different algal species. With continued advancements in biotechnology and agricultural practices, algal biofertilizers could play a significant role in the future of sustainable agriculture, helping to address global challenges such as food security and environmental degradation.

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