International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 3(H); March 2018; Page No. 10983-10986
DOI: http://dx.doi.org/10.24327/ijcar.2018.10986.1888

Research Article

EVALUATING THE ROLE OF *SPIRULINA PLATENSISAS* BIOFERTILIZER AND BIOSTIMULANT ON *TRIGONELLA FOENUM-GRAECUM*

Shakila H* and Krishnapriya S

Department of Botany, Stella Maris College (Autonomous), Chennai-86

A R T I C L E I N F O

Article History:

Received 10th December, 2017
Received in revised form 20th January, 2018 Accepted 4th February, 2018
Published online 28th March, 2018

Key words:

Spirulina platensis, biofertilizer, biostimulant, *Trigonella foenum-graecum*Zarrouk's medium

A B S T R A C T

The basic aim was to provide a series of mediato produce*Spirulina platensis* and evaluate the results in terms of chlorophyll content. Among all the media Zarrouk's medium is more suitable for the growth of *S.platensis*. It is very well known that chemical fertilizer adversely affects soil, plant and environment. To reduce this effect in recent years, interest to organic farming has been increasing. In the present study, *S.platensis* is used as a biostimulant to enhance protein levels in *Trigonella foenum-graecum*. The protein content of the plant was estimated and the study results indicated that there was significant increase in protein. Also, the carbohydrate and lipid content of the plant was estimated. At the end, it is concluded that *S.platensis* which is a blue green alga can be helpful in agriculture as a biofertilizer and as a biostimulant when compared with chemical fertilizers as an enhancer of plant growth in terms of protein content.

INTRODUCTION

Modern world is moving towards a life with balanced ecosystem which is obtained by modern generation bio fertilizer made of algal species which shows better results. Some of the extremely good algal species were checked based on nutrient content and utilized as bio fertilizer in laboratory as well as in field and shown outstanding results. They are used as soil conditioner somehow differs from fertilizer. Fertilizers only supply nutrient to the soil but soil conditioner enhances the physical, chemical and biological health of soil.

Blue green algae are simplest autotrophic plant having a high value as a soil conditioner because of its ability of building up food materials from in organic matters and its ability to photosynthesize food and fix atmospheric nitrogen shows its symbiotic relation in paddy fields (Saurabh Prakash and Kumar Nikhil, 2014). The result includes good water holding capacity of soil, well aeration to root part, good soil binding capacity and supplement of continuous and calculated nutrient to the soil.

Spirulina is a multicellular spiral shaped chain of cells. It is a blue-green alga belonging to the family oscillatoriaceae. It consists of 6-8 µm diameter cylindrical cells in unbranched helicoid trichomes. The filaments show movement, gliding along their axis. The trichomes elongate by intercalary cell divisions.

Corresponding author:* **Shakila H
Department of Botany, Stella Maris College (Autonomous), Chennai-86

They do not have heterocysts. *Spirulina* can grow in widely differing environments such as soils, marshes, brackish and sea waters and thermal springs. It can even grow in waters whose alkalinity is so high (upto pH-11). In this alkalinity, other microorganisms cannot exist (John Jothi, 2006). There are two species, *Arthrospira platensis* and *Arthrospira maxima*. *A. platensis* is the most widely distributed and is mainly found in Africa but also in Asia. *Arthrospira maxima* is believed to be found in California and Mexico.

Many species of *Spirulina* are cultivated for the rich protein and the β-carotene they have. *Spirulina* can be used as a soil conditioner to reclaim alkaline and saline soils (Venkataraman, 1991). It is very well known that chemical fertilizer adversely affects soil, plant and environment. To reduce this effect in recent years, interest to organic farming has been increasing. In the present study, *Spirulina platensis* is used as a biostimulant to enhance protein levels in *Trigonella foenum-graecum*. The protein content of the plant was estimated and the study results indicated that there was significant increase in protein.

Fenugreek (*Trigonella foenum-graecum*) is an annual plant in the family Fabaceae, with leaves consisting of three small obovate to oblong leaflets. It is cultivated worldwide as a semiarid crop, and its seeds are a common ingredient in dishes from South Asia. Fenugreek leaves are basically a kind of green leafy vegetables unless you dry them for storage (kasuri methi). The fresh leaves of the Methi plant have a slightly bitter under taste and can be cooked just like we cook spinach. In the Eastern cultures, especially India, fenugreek leaves are more widely used as compared to seeds primarily due to its immense nutritional benefits. While the seeds of this plant are

used for only for the flavour, the leaves have herb nutrition value in them.

MATERIALS AND METHODS

Media optimization of Spirulina platensis

Media I – Bold's, Media II – Zarrouk's, Media III – OFERR and Media IV – NPK
All the above media were prepared with distilled water and maintained
with alkaline pH. Themedia is then inoculated with the starter culture.

Starter culture*:* The alga *Spirulina platensis* was bought from MCRC (Murugappa Chettiar Research Centre, Taramani, Chennai) and the starter culture was prepared with 1g of algae and 200 ml of distilled water. The starter culture was grown in a conical flask in in-vitro condition for about 1 day and then examined microscopically in 10X and 40X resolutionusing Olympus (HB) microscope and photomicrograph using Nikon digital camera (Coolpix L-16)

30 ml of the alga is inoculated with 500 ml of each of the above-mentioned media. The pH is maintained in alkaline condition to enhance the growth of the alga and the temperature is maintained in normal room temperature. It is then kept in dark and light at regular interval of time. The media were agitated by shaking with hands daily and left for 25 days in the culture room. The algal biomass is directly related to the growth of algae (cell count). Microalgae cells were counted every 5 days with the aid of Haemocytometer. The cell count will reach maximum at 20^{th}, 25^{th} days and gradually decrease thereafter due to nutrient depletion. After 21st day, microalgae biomass reaches peak level (Dayananda *et al*., 2007).

Estimation of Chlorophyll

Microalgae cells were pelleted by centrifugation at 2000rpm for 15 minutes. To the pellet, 10 ml of 90% acetone was added and homogenized for 15 minutes using tissue homogenizer. After complete extraction, it was again centrifuged at 2000 rpm for 15 minutes and the clear supernatant was used for spectrophotometric estimation of chlorophyll pigments using double beam UV-visible spectrophotometer (Varian).

Chlorophyll 'a', Chlorophyll 'b' and total Chlorophyll was estimated from the extinction coefficients given byJeffrey and Humphrey (1975).
Chlorophyll 'a' (mg/L) = 12.7(663 O.D)-2.69(645 O.D)
Chlorophyll 'b' (mg/L) = 22.9(645 O.D)-4.88(663 O.D)
Total Chlorophyll= 20.2(645 O.D)-8.02(663 O.D)

Screening the growth and germination rate of Trigonella foenum-graecum

The seeds of *Trigonella* was soaked in the extracted algal mass solution overnight and then sown in the pot for studying the rate of germination and the biostimulant ability of *Spirulinaplatensis*. Then 3 equal sized pots were taken with soil, first pot was filled with a mixture of soil and compost second pot was filled with a mixture of soil and dried flakes of *Spirulina* and the third pot was filled only with soil and treated as control for studying the biofertilizer ability of *Spirulina platensis*. All the pots were sown with equal quantity of soaked *Trigonella* seeds. The pots were sprinkled with water daily. After 5 days, the growth of the plant was measured and which was repeated in the 10^{th} day.

Germination rate = (seeds sprouted/ total seeds sown) X 100
The plants of *Trigonella* from all the three pots were taken separately for estimating the carbohydrate, protein and lipid contents.

Screening of Biomolecules

Extraction and estimation of total protein (Lowry et al., 1951)

1g of *Trigonella*plants (leaf and stem) was homogenised with mordant and made into 10 ml with distilled water.It was centrifuged and the pellet was re-suspended with 5 ml of tris HCl buffer and the suspension was centrifuged at 2000rpm for 15 minutes. The supernatant was treated with 10% Trichloro Acetic Acid (TCA) and the precipitate is obtained by centrifugation at 5000rpm for 15 minutes. Then the precipitate is neutralized in known quality of 2N NaOH for protein analysis.

Reagents

1. 1N NaOH - 4g of NaOH was dissolved in 100ml of double distilled water.
2. 0.1N NaOH -10ml of 1N NaOH solution was made up to100ml with double distilled water.
3. 1% potassium sodium tartarate- 1g potassium sodium tartarate in 100 ml. of double distilled water.

Reagent A: 2% Sodium carbonate in 0.1N NaOH
Reagent B: 0.5% Copper sulphate ($CuSO_4.5H_2O$) in 1% potassium sodium tartarate. (Alkaline Copper Solution)
Reagent C: 1ml of Reagent B was mixed with 50 ml of reagent A.
Reagent D: Folin-Ciocalteau reagent.

Procedure

Pipette out standard BSA solution (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml) into a series of test tubes. Pipette out 0.5 and 1.0ml of the sample extracts in other tubes. Make up the volume to 1ml. in all tubes. Distilled water is considered as blank. Add 5ml. of reagent C and allow to stand for 10 minutes. Add 0.5ml. of Folin's reagent, mix well and keep in dark at room temperature for 30 minutes. Color developed was read at 650nm. Draw a standard graph and calculate the amount of protein in the test samples using the standard.

Extraction and estimation of carbohydrates by anthrone method (Pons et al., 1981)

1g of *Trigonella* plants (leaf and stem) was homogenised, made into 10 ml with distilled water and centrifuged at 2000 rpm for 15 minutes and the pellet was hydrolysed with 5 ml of 2.5 N –Hydrochloric acid. Kept in boiling water bath for three hours and then cooled to room temperature. After cooling, the samples were neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 10 ml using distilled water and centrifuged as above. 1 ml supernatant was used for analysis.

Reagents

1. 2.5 N-Hydrochloric Acid
2. Anthrone Reagent: Dissolve 200mg anthrone in 100ml of concentrated sulphuric Acid. Prepare fresh before use.
3. Standard Glucose.

Stock standard: Dissolve 100mg in 100ml double distilled water.

Working standard: 10ml of stock was diluted to 100ml with double distilled water. Store it in refrigerator after adding a few drops of toluene.

Procedure

- Prepare the standards by taking 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml of the working standard. '0' serves as blank.
- Make up the volume to 1ml in all the tubes including the test samples by adding distilled water.
- Then add 4ml of anthrone reagent.
- Heat for 10 minutes in a boiling water bath.
- Cool rapidly and read the green to dark green color at 620nm. in a spectrophotometer.
- Draw a standard graph by plotting concentration of the standard on X-axis versus absorbance on the Y-axis.
- From the graph calculate the amount of carbohydrate present in the samples.

Note: Cool the contents of all tubes on ice before adding anthrone reagent.

Extraction and estimation of total lipids (Bligh and Dyer, 1959)

1g of *Trigonella* plant (leaf and stem) was homogenised with a mordant and made up into 10 ml using distilled water and centrifuged at 2000 rpm for 15 minutes. Then 4 ml of 2:1 chloroform-methanol was added and kept overnight at room temperature preferably in the dark condition. After the 12 hours standing time, 10 ml of chloroform and 10 ml of double distilled water were added and mixed well and centrifuged at 2000rpm for 15 minutes. A clear lower layer of chloroform containing the entire lipid was obtained, which was aspirated carefully using a pasture pipette and poured in a pre-weight petri plate. The weight of total lipids was calculated.

RESULTS AND DISCUSSION

Spirulina platensis was subjected to various analyses like cell count and chlorophyll content (Fig 1 and 2). Microalgae culture was grown in separate conical flasks and above parameters have been determined on 20th day and 25th day of growth. Temperature is a major factor in the growth of algae cultures. Temperatures from 15-30°C are needed for optimal growth of blue green alga *Spirulina* and 25°C to promote the highest growth rate (Cho *et al*., 2007). In the present study, it was observed that Zarrouk's medium (M – II) showed good yield when compared with other media (Fig 2 and 3).

Green algae have two membranes around the chloroplast and chlorophyll *a* and chlorophyll *b* both serve asphotosynthetic pigments. Their energy reserve product is starch stored in the chloroplast. Chlorophyll content is the key criteria for photosynthesis. Chlorophyll content of the microalgae is given in table (1). Total Chlorophyll content of *Spirulina platensis* was 3.3004 mg/ml at the 20th day in Zarrouk's medium.

The rate of germination and the biostimulant ability of *Spirulinaplatensis* on *Trigonella* was done by agermination test which determines the percentage of seeds that were alive in the seed lot. The level of germination in association with seed vigour provides a very good estimate of the potential field performance. In this study *spirulina* treated soil showed the highest germination percentage (84%) when compared with compost soil and control soil (Fig 5 and Table 3) The rate of germination is an indicator of vigour. It was also clear from the present study that there was increased growth rate of *Trigonella* seeds in *spirulina* treated soil than the other soil (Fig 4 and Table 2).

Carbohydrate is the sole source for the glycolysis reaction and it can be produced from photosynthesis. From table (4) the carbohydrate content of *Trigonella* plant showed an increased level in *spirulina* treated soil. Similarly, the protein content of the plant was also increased maximum in *spirulina* treated soil when compared with that of control, but there was not much variation seen in the lipid content of *Trigonella* plant grown in different soil. From the present study, it may be concluded that on evaluating the biomolecules, *Spirulina platensis* can be used as a biostimulant to enhance protein levels in *Trigonella foenum-graecum*. The protein content of the plant was estimated and the study results indicated that there was significant increase in protein than carbohydrate and lipid.

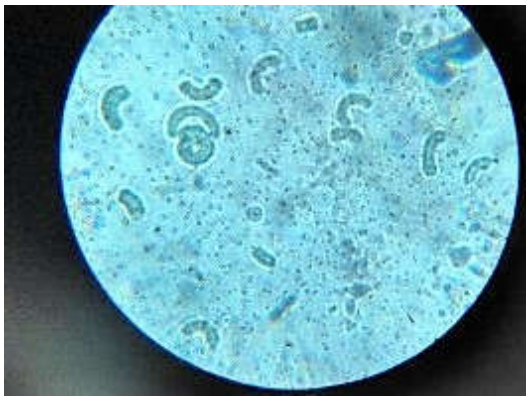

Fig 1 Microscopic view of *Spirulina platensis* in 40X magnification

Fig 2 Culture flasks showing the growth of *Spirulina platensis* in different media

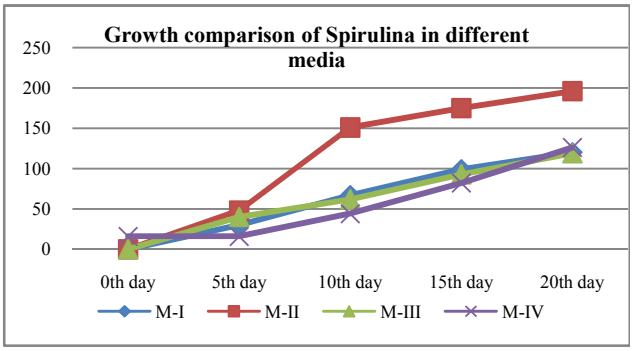

Fig 3 Microalgae growth analysis in different media

Table 1 Chlorophyll content of *Spirulina platensis* on 25th day in different media

S.NO	Media	Total chlorophyll (mg/ml)
1.	NPK	2.8042
2.	Zarrouk's	3.3004
3.	OFERR	1.1042
4.	Bold's	2.1004

Fig 4 Growth rate of *Trigonella* seeds that germinate over the 5- and 10- day periods in different soil.

Table 2 Measurement of Growth of *Trigonella* seeds that germinate over the 5- and 10- day periods in different soil.

S.NO	SOIL TYPE	5TH DAY(cm)	10TH DAY(cm)
1.	Control	2	5.5
2.	Compost	3.5	7.8
3.	*Spirulina* treated	5.5	12.3

Fig 5 Rate of Germination of *Trigonella* seeds in different soil

Table 3 Comparison of Germination rate of *Trigonella foenum-graecum* in different soil

S.no	Soil type	Germination rate
1.	Control	24%
2.	Compost	45%
3.	*Spirulina* treated	84%

Table 4 Evaluating the effect of*Spirulina platensis* on Biomolecules in *Trigonella* in mg/g

S.No	Biomolecules	Control	Compost	Spirulina treated
1.	Carbohydrate	0.022	0.030	0.033
2.	Protein	0.037	0.055	0.072
3.	Lipid	0.008	0.012	0.018

CONCLUSION

The present study was carried out to evaluate the role of *Spirulina platensis* in the field of agriculture as biofertilizer or bioconditioner and biostimulant. It may be concluded that the blue green algae significantly increase the protein level in *Trigonella foenum-graecum* so, as a result the work can be extended in the field of agriculture to improve the nutritional requirements in high yielding cereal crops.

References

Bligh,E.G. and Dyer, W.J. 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37. 911-917.

Padhya *et al*. 2000. Allelopathic potential of Eucalyptus leaf litter leachates on germination and seedling growth of Finger millet. *Allelopathy J*., 7(1):69-78

Lowry *et al*. 1951. Protein measurement with Folin phenol reagent. *Biochemistry*, 15:529-536

Cho.S.H., S.C. Ji. S.B. Hur, J. Bae. I.S. Park, and Y.C. Song. 2007. Optimum temperature and salinity conditions for growth of green algae *Chlorellaellipsoidea, Spirulina platensis* and *Nannochloris oculata*. *Fisheries Science* 73.1050-1056.

Dayananda, Chandrappa, Ravi Sarada, Vinod Kumar, Gokare Aswathanarayana, Ravishankar.2007. Isolation and characterization of hydrocarbon producing green alga *Botryococcus braunii* from Indian freshwater bodies. *Electronic Journal of Biotechnology*.10

Jeffrey, S.W., Humphrey, G.F. 1975. New spectrophotometric equation for determining chlorophylls a, b, c & c_2 in higher plants, algal and natural phytoplankton. *Biochem. Physiol. Pflanz,*167. 191-194.

Oswald, W.J. 2003. My sixty years in applied algology. *J. Appl. Phycol*.15.99-106.

Pons, A., Rola, P., Agvilo, C., Garcia, F.J., Alemarry, M., Paloo, A., 1981. *J. Biochemical and Bio Physical methods*. 4. 227-231

Venkataraman L.V. 2005. Algal industrial application- Dose it has a future? *Indian Hydrobiology*.7.43-47.

How to cite this article:

Shakila H and Krishnapriya S (2018) 'Evaluating The Role of Spirulina Platensisas Biofertilizer And Biostimulant on Trigonella Foenum-Graecum', *International Journal of Current Advanced Research,* 07(3), pp. 10983-10986.
DOI: http://dx.doi.org/10.24327/ijcar.2018.10986.1888

****