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Research Journal of Agricultural Sciences
Volume 14; Issue 05 (Sep-Oct 2023); pp 1357–1361

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Bioformulations of Spirulina Effluent for Plant Growth Promotion and Combating Phytopathogens: A Sustainable Agriculture

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Abstract

The soil is a living being and a valuable natural resource not only in agriculture and food security but it is also towards maintenance of all life process in current scenario. Comparative study was undertaken to evaluate efficiency of the soil organisms like Spirulina in two combinations along with spirulina waste to formulate it into a bio fertilizer and in enhancing the growth of green gram plants. Sustainable agriculture is proving as one of the toughest jobs in these days. There is no uniformity in agricultural practices all over the world, but one thing which is more or less common is the use of chemical pesticides and fertilizers. The present study consists of three experimental groups such as Control and a Test control with different combinations. Group 1 contains control, Group 2 contains the Spirulina waste, Neem seed powder and cow dung, Group 3 contains the Spirulina waste. Spirulina is a rich protein as well as nitrogen provider for plant growth. The study results reveal that group 2 has shown a greater impact on the growth of the plant in terms of germination percent, length of shoot and root of the plant and the level of chlorophyll and protein in the leaf of the group 2 plant was higher when compared with other experimental test and control groups. So, the spirulina waste, cow dung and Neem seed powder see along with efficient soil organisms is potential in promoting growth of crops and its play an important role to increase in soil fertility for development of eco-friendly sustainable agriculture.

***Key words*:** Spirulina waste, Bio fertilizer, Green gram plants, Soil fertility

Microalgal research has been an area of great interest as microalgae have higher productivities than land plants and can be used for the production of valuable commodities such as biofuel, animal feeds and agricultural fertilizers, among others. To enhance the economic feasibility of algal-based commodities, the growth of microalgae can be coupled to wastewater remediation. The remediation potential of *S. platensis* was found to be good for ammonia and nitrate removal, but inadequate for nitrite removal. Currently, considering the growing global population, among agricultural issues, a crucial challenge is to meet food demands, improving agricultural sustainability. Hence, whilst farmers are called to increase agricultural production along with issues related to climate change, researchers must be called to develop innovative products and technologies able to increase crop yields and quality, while decreasing their agricultural carbon footprint also improved significantly in terms of seedlings' dry weight. Microalgae are classified mainly considering their pigmentation, life cycle and cell structure. It was estimated that ~800,000 microalgae species exist, of which ~50,000 species are described. This high number of species might provide a wide range of possible uses. In fact, is possible to select different strains having different biochemical compositions and which are able to grow in different environments.

Spirulina are ubiquitous, spirally coiled or filamentous prokaryotic cyanobacteria and possess significant morphological similarity, the blue-green color of Spirulina is due to two pigments: phycocyanin (blue) and chlorophyll (green) [1]. Spirulina effluents can enhance soil nutrient availability for crop growth, improve soil water-holding capacity, increase the content of plant antioxidants, enhance cellular metabolism and increase leaf chlorophyll. Until nowadays, microalgae were largely investigated as a practical approach for the production of lipids and for environmental purposes, such as the mitigation of carbon dioxide (CO_2) emitted by industrial processes, and for wastewater treatments. On the other hand, investigations of microalgal products suitable for crop productions remain largely unexploited. In the light of this point, this study highlights some of the current researches and future development priorities, summarizing the biological activity of microalgal components, and examining the factors supporting the use of Spirulina effluents for managing crop productions and abiotic stresses [2].

Spirulina effluent is used as fertilizer to plants, booster in

Received: 13 May 2023; Revised accepted: 14 Sep 2023; Published online: 25 Sep 2023

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Citation: Sridevi B, Kalaimathi J, Vidya K, Shazneen Farhana S, Suriya M. 2023. Bioformulations of spirulina effluent for plant growth promotion and combating phytopathogens: A sustainable agriculture. *Res. Jr. Agril. Sci.* 14(5): 1357-1361.

compost and as feed to animals or fish in aquaponics system. Spirulina is grown in optimum pH of 9-10, it will neutralize the acid rain effects in soil and in high pH, and other microbes just simple cannot live. Cow dung, also known as cow pats, cow pies or cow manure, is the waste product (faeces) of bovine animal species. These species include domestic cattle ("cows"), bison ("buffalo"), yak, and water buffalo. Cow dung is the undigested residue of plant matter which has passed through the animal's gut. The resultant faecal matter is rich in minerals. It helps in promoting the plant growth [3-5].

The fertilizer benefits the soil as it promotes faster growth, reduces insects and keeps pests away. Neem with its anti-bacterial properties acts as a natural pest repellent. It improves the quality of the soil and enriches it to ensure the healthy growth of the plants. Neem seed powder organic manure protects plant roots from nematodes, soil grubs and termites, probably due to its residual limonoid content. It also acts as a natural fertilizer with pesticidal properties. Neem seed powder is widely used in India to fertilize paddy, cotton and sugarcane. Usage of neem seed powder have shown an increase in the dry matter in *Tectona grandis* (teak), *Acacia nilotica* (gum Arabic), and other forest trees. Neem seed powder used as the manure and can also reduce alkalinity in soil, as it produces organic acids upon decomposition [6]. Being totally natural, it is compatible with soil microbes and *rhizosphere microflora* and hence ensures fertility of the soil. Neem seed powder improves the organic matter content of the soil; helps improve soil texture, water holding capacity, and soil aeration for better root development.

MATERIALS AND METHODS

The two soil samples were collected from the normal soil and waste landfill of Tindivanam, Tamil Nadu, and India. Soil pH and electrical conductivity (EC) were determined by pH meter.

Collection of Spirulina platensis culture

Spirulina platensis culture will be collected from Krishi Vigyan Kendra, Puducherry.

Neem seed powder: the ground and dried powder of the neem seed (includes oil, active ingredients, and seed cake).

Microalgae culture

During production of Spirulina, and also of other photosynthetic microorganisms, macronutrients, such as nitrogen and phosphorus, are commonly supplied in the form of commercial inorganic fertilizer. Replacing these inorganic chemical compounds with residue streams would increase both sustainability and economics during production. During the experiment process, the contents in the aquarium tank are stirred by glass rod daily 6-7 time.

The physico-chemical parameters are measured at three different periods (7^{th}, 14^{th}, 21^{st}day) and growth parameters of *Spirulina platensis* are measured after harvesting in 21^{st} day (Fig 1). After concerning 3 to 6 weeks of growth, Spirulina will be all set to harvest and need to scoop some of the culture water out and make it run through the net or mesh cloth. The Spirulina will be collected on the net. Gently squeeze out any excess liquid to avoid intense alkaline water. Ultimately, it will have the green paste.

Identification of Spirulina effluents

Effluent from the spirulina and is filtered in polyamide filters in several steps with a final mesh size of 5 µm to remove particles. The concentrations of nitrate and ammonium in the filtered effluent are determined calorimetrically on an automatic analyzer.

The concentrations of phosphorus and other nutrients are analyzed.

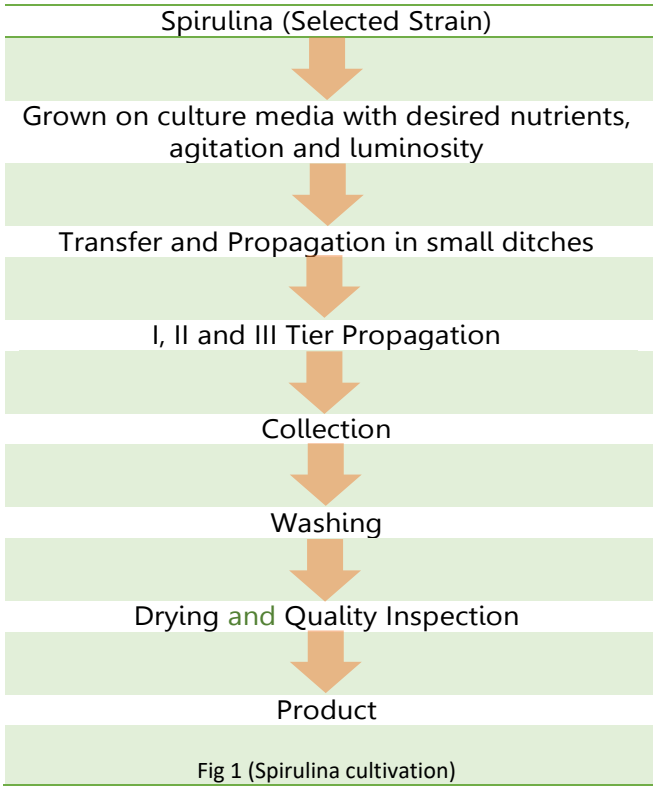

Fig 1 (Spirulina cultivation)

Experimental setup

The experimental setup included the effluent-based medium described above and the control treatment based on Spirulina medium. All experiments are performed in a greenhouse with a16 h/8 h day/night regime with an added light intensity of 100 µmol m^{-2} s^{-1} and the temperature set to 25 °C.

Plant material and culture conditions

Three plant species were used in the current study, green bean, lettuce and baby beetroots seeds are purchased from Villupuram agriculture department. Seeds were kept in different planters containing mixture of sandy soil, organic manure and spirulina effluents plants are sprayed with total effluents (3 g. dissolved in water, pH 6.0) twice with 3 days interval and non-treated plants served as control.

After 30 days, plant growth is evaluated by measuring: plant size (shoot size), plant weight (shoot and roots dry weight: 70°C at 72h), foliar area as well as number of nodes per plant.

Every experience is repeated twice with 10 independent replicates / treatment.

In vitro studies

Effect of N fertilization and Spirulina algae levels on yield and growth of green bean, lettuce and baby beet roots plants

Measurement of nitrogen using micro-kjeldahle method

Weight 100 mg of the sample (containing 1-3 mg nitrogen) and transfer to a 30 mL digestion flask. Add 1.9±0.1 g potassium sulphate and 80±10 mg mercuric oxide and 2 mL conc.H_2SO_4 to the digestion flask.

If sample size is larger than 20 mg dry weight, 0.1 mL H_2SO_4 should be added for each 10 mg dry materials. Add boiling chips and digest the sample till the solution becomes colorless (The time of digestion will vary with regard to the size of the sample, temperature, and the mode of digestion).

After cooling the digest, dilute it with a small quantity of distilled ammonia-free water and transfer to the distillation apparatus (When the nitrogen content of the sample is high, the digest can be made up to a known volume and an aliquot may be transferred to the distillation flask). The kjeldahl flask should be rinsed with successive small quantitative of water. Place a 100 mL conical flask containing 5 mL of boric acid solution with a few drops of mixed indicator with the tip of the condenser dipping below the surface of the solution to the test solution in the apparatus.

Add 10 mL of sodium hydroxide-sodium thiosulphate solution to the test solution in the apparatus. Rinse the tip of the condenser, and titrate the solution against the standard acid until the first appearance of violet colour, the end point. Run a reagent blank with an equal volume of distilled water and substrate the titration volume from that of sample litre volume.

Estimation of phosphorous molybdenum blue method and determined by spectrophotometer

0.5 to 2.0 ml of working standard was pipette out into the series of test tubes and makes it as respectively. From that 1 ml of biowaste material (orange, pomegranate and peach peel) sample is taken in a test tube and marked T. The volume was made upto9ml with distilled water and marked as blank. Then add 1ml of ammonium molybdate and 0.4ml of amino naptholsulphonic acid to all the test tubes.

The tubes were kept at room temperature for 10minutes. The intensity of the blue colour was calorimetrically at 640 nm. From the standard graph the amount of inorganic phosphorus present in the given biowaste material (orange, pomegranate and peach peel) sample was calculated.

Potassium is determined by flame photometer

Grind about 25gm of are derived soil to a find powder with an automatic mortar and pestle. Place a 0.5gm. Aliquot of this powder in a smell agate thoroughly mix with 0.5mg of NaCl. Divide 4.0gm. Of Caco3 (Low in alkali so that about 3gm added to and mixed with the soil-NaH_4Cl in the mortar, 4gm is, retained to rinee the mortar, and 4gm. Is placed in the bottom of the J. L. Smith platinum crucible. At the soil NH_4Cl -$CaCo_3$ mixer to the crucible followed by the $CaCo_3$ used to rinse the mortar. Place the cap the crucible and best the crucible allow with a wing- tipped burner for about 10 minutes or until fumes do longer appear. Then bring the lower 3 quarters of the crucible to the full heated of the wing- tipped burner for 50 minutes. After cooling the crucible transfer the contents to an evaporating dish.

Use hot water to rinse the remaining particles from the crucible. Slake the sinter cake with hot water and thoroughly grind with a pestle. Wash the mass 5 times.

Identification of nutrients in green bean, lettuce and baby beetroots plants (control and experimental plants)

Estimation of chlorophyll

Procedure

Chlorophyll was extracted from 1g of the sample using 20ml of 80% acetone. The supernatant was transferred to a volumetric flask after centrifugation at 5000 rpm for 5 minutes. The extraction was repeated until the residue became colorless.

The volume in the flask was made up to 100ml with 80% acetone. The absorbance of the extract was read in a spectrophotometer at 645 and 663nm against 80% acetone blank. The amount of total chlorophyll in the sample was calculated using the formula:

Total chlorophyll = 20.2 (A645) + 8.02 (A663) × V / 1000 ×W

Estimation of protein

Procedure

The sample preparation from each assay was used as the protein source for estimation. Standards corresponding to 0.2 to 1.0ml (20-100µg) were pipetted out into a series of test tubes and 10µl of the sample extract was used for the estimation. The volume was made up to 1.0ml in all the tubes with distilled water. To this, 5.0ml of solution 3 was added, mixed well and incubated at 37°C for 3 minutes. Then 0.5ml of Folin-Ciocalteau reagent was added, mixed well and incubated at 37°C for 3 minutes. The blue colour developed was read at 660nm in a spectrophotometer.

RESULTS AND DISCUSSION

The percent of germination rate was tabulated in (Table 2). The average lengths of root and shoot calculated and mentioned in (Table 3). The amount of chlorophyll estimated has been shown in (Table 4). Protein estimation results tabulated in (Table 5). The spirulina waste is highly organic, rich in calcium, niacin, potassium, magnesium and proteins. Neem seed powder converted the waste into a reusable fertilizer by improving the quality of compost with adequate amounts of organic carbon and nitrogen and acts as a pest repellant. By application of this fertilizer the seeds of green beans, lettuce and baby beetroot has shown better rate of germination and growth [7-9]. The soil characters which have been tested are shown clear cut evidence of variation for soil nature, pH, salt concentration, organic carbon, phosphorous, potassium and nitrogen for different cultures inoculated into the spirulina waste [10-11].

Table 1 Soil + spirulina waste compost analysis report

S. No.	Characters	Group-I Control	Group –II Spirulina effluent + cow dung + Neem seed oil	Group -III Spirulina effluent
1.	Soil nature	S -I	S -I	S -I
2.	pH	6.90	7.40	7.40
3.	Salt Conc (mM/cm)	0.72	0.21	0.21
4.	Organic carbon	H	L	M
5.	Phosphorous (Kg/ha)	33	28	45
6.	Potassium (Kg/ha)	259	116	259
7.	Nitrogen (Kg/ha)	H	L	M

The values of shoot length and root lengths of different setups show the clear differentiation and the highest values observed in case of group II. The very nearer values have been shown by group II and group III have shown comparatively

lower values than the other two but, higher or similar with the test control / control. The values of chlorophyll estimation and protein are also higher in case of group II. Because of presence of spirulina which is a potential source of chlorophyll and nitrogen and other bacteria which can fix atmospheric nitrogen and the antibacterial activity of neem seed powder pest control on the plants then improves the productivity [12-13]. The increase of chlorophyll and protein in the product was observed. The increase in chlorophyll shows good growth of plants. By overall study of these results, it is clearly proved that the protein and chlorophyll content of green beans, lettuce and baby beetroots plants has been enhanced by the application of spirulina waste compost with different cultures. The test control with chemical fertilizer has shown slightly higher or nearer values with control and are less values with microbial culture [14-15].

Table 2 Germination rate of plants

Groups	Percent (%) of germination rate
Group - I	99%
Control Group - II	80%
Group - III Test control	85%

Table 3 Mean shoot and root lengths of the seedlings with different groups

Groups	Mean shoot length in cms	Mean root length	Total length of plantlets in cms
Group-I Control	23.3	9.0	32.3
Group -II	16.0	5.0	21.0
Test control Group -III	20.0	5.0	25.0

Table 4 Estimation of chlorophyll from leaves of the plants

Types of culture	Estimation of total chlorophyll mg/g of sample
Group - I	0.123
Control Group - II	0.050
Group - III Test control	0.059

Table 5 Protein estimation of the product

Types of culture	Volume of sample	Volume of NAOH (ml)	Conc. of sample (mg/m)	O.D at 650nm	Volume of BSA (ml)	O.D of BSA at 650nm
Group - I	1ml	5ml	12mg	0.14	Blank	0.0
Control Group - II	1ml	5ml	5mg	0.8	0.8	1.02
Group - III Test control	1ml	5ml	8mg	0.10	1.0	1.34

CONCLUSION

The present invention meets the need by providing a water soluble NPK fertilizer having a neutral pH that combines Seaweed Extract containing trace elements and microelements and Spirulina that provides essential amino acids to the plants. The said fertilizer consists of sources of Nitrogen and sources for Phosphorous and Potassium are provided. Source for microelements and trace elements is cow dung, neem seed powder acts as the pest repellent and Source for amino acids is Spirulina. Finally, analysis of the chemical composition of the *S. platensis* biomass obtained under optimal conditions revealed an abundance of proteins and lipids, indicating its great potential for plant growth. The ability of spirulina fertilizers to accelerate plant growth has been demonstrated in leafy vegetables such as green beans, lettuce and baby turnips, and the dry weight of seedlings has been significantly improved. The utility of platensis in aquaculture wastewater treatment was studied and the subsequent use of algal biomass in fertilizer research was demonstrated. The culture of S. platensis was carried out indoors with an illuminance of not more than 1000 lux. At these conditions, the algae were able to remove the ammonia and nitrate concentrations in soil, indicating its ability to treat the water despite its inadequacy in removing nitrite. Potentially, the efficacy of water treatment can be much higher under sunlight where illuminance is typically about 100,000 lx. Platensis for leafy greens showed improved plant growth in all tested vegetables compared to the controls. Compared to the chemical fertilization effect, the Spirulina fertilizer performed comparable on most plant growth parameters and positively for one of the tested types of green beans, lettuce and young turnips. Seed germination (measured by the dry weight of the seedlings) also improved for all tested vegetables. This work has shown the utility of *S. platensis* in water treatment and its applicability as agricultural fertilizers. The present study demonstrated that cow dung manure and Neem seed powder along with the Spirulina waste. Cow dung manure can be a cheap alternative source of microelements like nitrogen, phosphorous and potassium. Spirulina waste is rich in amino acids and the neem seed powder acts as a pest resistant to the enhance the growth of the plants. Spirulina waste alone cannot produce that much amount of nutrients to the plant growth because it lacks the microelements like nitrogen, phosphorous and potassium. The study emphasized on a possible combination of spirulina waste, cow dung manure and neem seed powder, which showed enhanced plant growth and was concluded as synergism. In this context, synergistic application of *Spirulina platensis* could potentially double the productivity of green beans, lettuce and baby beetroots. Thus, application of biofertilizer is a recommendable option to boost the productivity of green beans, lettuce and baby beetroots in waste land soil. There is a need of cost effective, biodegradable, potential, ecofriendly and safe sustainable agricultural products alternating to the chemical fertilizers and pesticides.

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