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* **Corresponding author**.

lavanyakabilan01@gmail.com

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Comparative study on Synergistic effect of plant growth promoting microalgae and Panchagavya in reclaiming the wasteland and growth induction of *Vigna radiata*

K Jagathy[1], K Lavanya[2]*

1 Head of Department, PG and Research, Department of Microbiology, Sri Akilandeswari women's college, Vandavasi, Tamilnadu, India
2 PG & Research Department of Microbiology, Sri Akilandeswari Women's College, Vandavasi, Tamilnadu, India

Abstract

Objectives: The present comparison investigation determines the synergistic effects of employing Plant Growth Promoters [Spirulina platensis and organic fertilizer (Panchagavya)] in a waste land soil bag containing *Vigna radiata* seeds. **Method**: Soil from the waste landfill were collected and stored for the experiment. The plants were cultivated in experimental pots (Control, A, B, and C groups) using waste land soil supplemented with PGP [Plant growth promoters] in volume separately. Group A was treated with Spirulina platensis. Group B was treated with Panchagavya. Group C were treated with the consortium (Spirulina platensis + Panchagavya). Control Pots were treated only with water. The crop's growth characteristics were measured after 14 days. Biomass production [Phosphorous], and phytohormone quantification were done. The relationship between various growth and yield were determined using Pearson's correlation coefficients. **Results:** Overall growth and physiology of *Vigna radiata* were significantly influenced by the addition of PGP in Consortium. The available phosphorus status of the soil is 40 kg / ha, after consortium treatment. Significant positive correlations of grain yield were observed with consortium ($r = 0.716$; $p < 0.05$) while non consortium showed comparatively weaker correlation, microalgae – Spirulina platensis ($r = 0.448$; $p > 0.05$) and panchagavya ($r = 0.407$; $p > 0.05$). As a result, using biofertilizer in a consortium mode will be an effective strategy for maintaining biomass production in an alkaline environment or in wasteland management.

Keywords: Spirulina platensis; Phyto hormones; Panchagavya; consortium; Vigna radiate; Auxins

1 Introduction

Soil fertility is an ability of soil to provide all essential plant nutrients in available forms and in a suitable balance. For enhancing soil fertility, microalgae and plant growth-promoting bacteria (PGPB) have been established as viable alternatives to chemical fertilizers.[1] This is due to their fertilizing effects (e.g., phytohormones, amino acids, and carotenoids) as well as their ability to suppress plant pathogens.[2]

The current interest to produce organically grown vegetables ensures environmental quality maintenance and consumers' health protection.[3] As an alternative to chemical fertilizer, organic products like panchagavya are developed and used.[4] The use of organic nutrients for plant growth enhances the quality of the product and sustain the shelf-life period. Authors report that the preparation and usage of panchagavya had been reported in Vedic literature, and such Vedic organic agriculture re-enliven natural law in agriculture, bringing the farmer, the process of farming, and the environment in complete harmony with each other.[2] Panchagavya preparation varies with the people although the ingredients are common. Panchagavya has been reported to contain micronutrients, macronutrients, many vitamins, essential amino acids, growth-promoting factors, and beneficial microbes.[5]

Spirulina platensis contains several nutrients i.e. macronutrients like nitrogen, phosphorus, potassium, and micronutrients which are required for the growth and development of plants and also contain various amino acids, vitamins, growth regulators like Auxins, Gibberellins, and cytokinins.[4] Despite their unique chemical composition, microalgae are not as often used for agricultural purposes as macroalgae (seaweeds).[6] This can result from the availability of seaweed biomass—it is usually abundant in many marine and freshwater reservoirs, whereas microalgae are usually cultivated in artificial conditions.[7] Therefore, they constitute a more expensive source of biomass for the production of biostimulants of plant growth.

The effectiveness of a synergistic spirulina and panchagavya approach to stimulate plant growth was examined in the current study. Spirulina platensis and Panchagavya were also utilised as bio-fertilizers in this study to improve plant growth and soil fertility in *Vigna radiata*. The impact of plant growth and soil fertility was calculated, and the study's findings revealed that there was a link between the two.

2 Methodology

Study design

Comparative study.

Study area

The soil samples were collected from the waste landfill of Cheyyar Municipality, Tamil Nadu, India.

Collection of Soil samples

A total of 5 soil samples were collected from the waste landfill of Cheyyar Municipality. Samples (soil mixed with waste) were collected in sterile zip-lock plastic maintaining aseptic conditions, stored at 4 °C, and marked accordingly to their source and location. The measurement of soil physico-chemical properties followed the methods by Zheng.[8] Soil pH and electrical conductivity (EC) were determined by pH meter.

Soil preparation for experiment

Drying of soil sample

Samples received in the laboratory may be moist. They should be dried in wooden trays. Care should be taken to maintain the identity of each sample at all stages of preparation.

Soil testing

Soil testing kits [Rapitest] were purchased from the Sisco research laboratory, Chennai.

Materials

HPLC grade methanol, acetonitrile (CAN), water, ortho-phosphoric acid (OPA), and dimethyl sulfoxide (DMSO), acetic acid were purchased from Merck (Darmstadt, Germany). Solvents were filtered through 0.45 μm membranes (Cat No. HVLP04700, Millipore) and degassed prior to use in HPLC.

Experimental set up

The experimental design for the present study contains four setups comprising of plant i.e. *Vigna radiata*. As a control, simply plant seeds were grown in waste land soil without addition of PGP.

Group A - Preparation of microalgal extract

Dried biomass of Spirulina platensis obtained from SANAT industry, Kodai road.

Homogenate was prepared by a suspension of dry Spirulina platensis in deionized water and mixing at 37 °C for 40 min. The obtained solution was centrifuged for 20 min. (4600 rpm). The supernatant was separated and treated as an algal filtrate 25 gram mixed with water at 100ml of water concentration, w/v.

Group B - Panchagavya

Requirements of ingredients added for the preparation of Panchagavya

Fresh cow's dung – 3kg
Fresh cow's urine – 5L
Cow's milk – 1L
Cow's curd – 1L
Cow's ghee – 500mg
Tender coconut water – 2L
Sugarcane juice – 1L
Well Ripe banana fruit – 5
Water – 5L

Mode of Preparation

Panchagavya liquid fertilizer was prepared according to the method of Summet and Neelam.[9] After 30 days, the panchagavya was filtered properly sieving through a fine cloth. The filtrates were kept as 100% stock of panchagavya and 3% solution was prepared with mixing of appropriate distilled water and used for further treatment.

Group C - Preparation of Consortium

Equal proportion of the biofertilizers in group A and B were mixed to form consortium [1:1]

Preparations for plant growth

The entire plant growth studies were carried out under the green house environment. Best Concentrations of PGP used in the present study were verified from previous reports.[10–13]

The effect of homogenate (Each experimental pot mixed with required doses of PGP [100ml] in soil) in 10g of *Vigna radiata* seeds used for cultivation. The doses of different PGP and their concentrations were shown in below table. Kept the control and test pots evenly moist and free from weeds until germination.

Table 1. Details about treatment

Treatment [Triplicates]	Doses
Control	Uninoculated
Group A	Spirulina platensis [25g/100ml]
Group B	Panchagavya – 100ml [3% - 3 litres in 100 litres of water]
Group C	1:1 [v/v]

The shoot and root length of each groups were measured in centimeters. After a specified period of time (7-14 days) seedlings were collected from each groups, their length was measured and mean seedling length was calculated. Seed lots producing the taller seedlings were considered more vigorous than the seed lots producing shorter seedlings. Biomass concentration of Phosphorus in plant sample was carried out by Vanadomolybdo-phosphoric yellow colour method.[14]

Phyto hormones evaluation by HPLC – PDA

Analysis of mung bean extract was carried out using Waters HPLC integrated with photo diode array detector. The wavelength of detector scan range was set between 210–400 nm and the chromatograms were extracted at 270 nm. An analytical Hypersil octadecyl-silica (ODS) column (150 × 4.6 mm, Thermo Scientific) with thermostated compartment was used for the separation of compounds. The temperature of column oven was set as 30°C. Waters Empower 2 (Waters Pvt Ltd., USA) software was used for the operation of the system.[15]

An elution gradient of 0.1% OPA (v/v; solvent A) and CAN (solvent B) were used at a flow rate of 1.0 mL/min as follows: Solvent A was decreased from 95 to 64% in 18 min and the initial conditions were further set at 95% (solvent A) in last 2 min. Twenty microliters of diluted and filtered mung bean extract was injected into the HPLC sample injection port. The chromatograms were extracted at 270 nm using a PDA detector. Data were analyzed using Empower software and the compounds were identified in accordance with the retention time and absorbance spectra of pure standards.

Method Validation

Stock solution containing analytes were prepared and diluted to appropriate concentration in the range of 5–100 ppm for establishing calibration curves. For quantitative analysis, seven different concentrations of analytes were injected five times. The calibration curves were obtained by plotting the peak areas versus concentration of each analytes. The relationship between concentrations of different phytohormones and peak area has been reported as *a, b*, and *r* values. Here *a* and *b* are the coefficient of the regression equation $y = ax + b$
where *y* corresponds to the peak area and *x* corresponds to the concentration of the particular phenolic compounds and in this case *r* represents the correlation coefficient of the equation.

Statistical analysis

Experimental results were subjected to an analysis of variance (ANOVA) and pearson correlation coefficient was applied to determine the significant differences among the treatment groups. The tests were performed using online Social science statistics tool. Level of significance was tested at $p < 0.05$, r = < 0.7

3 Results and Discussion

Physical and chemical characteristics of soil samples:

The pH, Electrical conductivity and other essential trace minerals of the collected 5 soil samples from the waste landfill were shown in Table 2

Table 2. Soil testing results

Soil tests	Results				
	S1	S2	S3	S4	S5
pH	7.98	7.65	7.54	7.76	8.84
ELECTRICAL CONDUCTIVITY	>4	>4	<4	>4	>8
NITROGEN	6	5	4	3	1
NITRATE	0.64	0.54	0.88	0.23	0.14
AMMONIUM	1.13	1.11	2.6	2.89	1.02
PHOSPHOROUS	0.02	0.21	0.71	0.05	0.27
POTASSIUM	32	36	33	43	28
SULFUR	5	7	9	4	2
CALCIUM	91	99	105	129	87
MAGNESIUM	24	22	45	43	21
SODIUM	0.2	4	2	1	0.4
IRON	3	4	3	5	6
MANGANESE	5	4	4.2	3.6	2
ZINC	0.24	1	0.23	0.18	0.33

Among the 5 samples, S5 samples with high electrical conductivity, low micro and macronutrients, and high Ph were subjected to the present study.

According to Indumathi et al.,[(16)] soil samples with a high alkaline nature have very low significant effects on soil health, nutrient availability, pollution, and possible contaminants' dangers as well as their destiny in the food chain. As a result, knowledge of this can serve as a foundation for making decisions about waste soil management, remediation, rehabilitation, and soil quality maintenance. The discovered soil pH-biogeochemistry connections offer insight into potential applications for higher yields for certain crops via nutrient recycling and availability, which improves crop development.

Growth yield and Biomass production

1. Control – Poor Growth yield seen. The maximum biomass production 0.23 g/plant was observed at 7 days and about 0.49 g at 14 days.
2. Group A - Growth seen in inoculation of PGP (Spirulina platensis) significantly increased the dry matter accumulation at different days after soaking. The maximum biomass production 1.03 g/plant was observed at 7 days and about 1.19 g at 14 days.
3. Group B - Growth viewed in use of Panchagavya. The use of PGP - Panchagavya alone significantly increased Phosphorous in soil. The biomass produced were 0.87 at after 7 days and 0.77 at after 14 days,
4. Group C - PGP in consortium also seen with plant growth. [Figure 1] Biomass production was 1.21 at after 7 days and 1.33 at after 14 days respectively.

Significant interactions effect between the Spirulina platensis and Panchagavya was observed for the total production of P, both at after 7 days and 14 days. This is supported by Zaidi et al., [21] in their experiment which was conducted to evaluate the effects of nitrogen fixing bacteria [Bradyrhizobium sp. (*Vigna radiata*)], phosphate solubilizing bacterium (*Bacillus subtilis*), phosphate solubilizing fungus (*Aspergillus awamori*) and AM fungus (*Glomus fasciculatum*). Triple inoculation of AM fungus, Bradyrhizobium sp. (*Vigna radiata*) and B. subtilis significantly increased dry matter yield, chlorophyll content in foliage and N and P uptake of green gram plants. Also triple inoculation of Bradyrhizobium + G. fasciculatum + B. subtilis increased seed yield by 24%, than control.

The important agronomic parameters (shoot length, root length, plant height) were significantly influenced by the addition of PGP packages.

Synergistic effect of Biofertilizers

The available phosphorous in soil was increased significantly at consortium treatment after 7 days and after 14 days of the mungbean over control, irrespective of the inoculation and the build-up was increased with increasing doses of consortium [Group C] in soils. The available phosphorus status of the soil is 40 kg / ha, after consortium treatment. This was high when compare with other group soil. [Table 3] Mean values of the triplicates set up were calculated. Significant positive correlations of grain yield was observed with consortium ($r = 0.716$; $p < 0.05$) while non consortium showed comparatively weaker correlation, microalgae – Spirulina platensis ($r = 0.448$; $p > 0.05$) and panchagavya ($r = 0.407$; $p > 0.05$). [Table 3] The results indicated a positive role of Spirulina platensis in association with panchagavya, in productivity improvement of mungbean crops in waste land soil, cheyyar municipality, Tamil nadu.

Akin to our study, Bhabai et al.,[11] conducted a study in improvement of mungbean under Terai agro-ecological region of West Bengal. The total P absorption by mungbean was considerably enhanced when they utilized biofertilizer alone with graduated dosages of phosphorus. The overall absorption of P showed significant interactions between the biofertilizer and P. Regardless of inoculation, accessible phosphorus in soils rose considerably. As a result, using biofertilizers in a consortium mode will be a useful tool for sustaining biomass production in an acidic environment with judicious application of phosphatic fertilizers, where the biomass thus produced could be a source of organic matter on decomposition, as well as supplying nutrients like N and P for subsequent cereal crops. This present study correlates with Abdul-Baki and Anderson.[17] In their study, Germinated seeds were counted on 14th day and germinated seedlings were selected from each replication of the treatment and the data were statistically analyzed.

Fig 1. Plant growth in Consortium

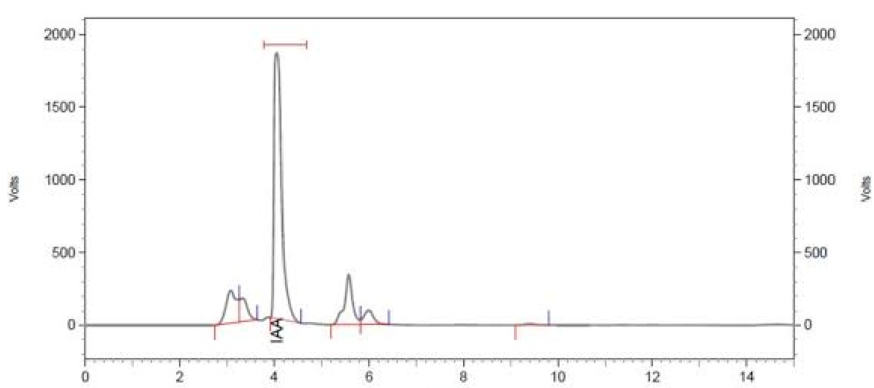
Table 3. Mean value of Agronomic parameters of green gram at 14 days

Groups	Treatment	Root length [cm]	Shoot length [cm]	Seedling length [mm]	Available phosphorous	r and p value
Control	Water and natural light	0.10 cm	1.21 cm	68	12 kg / ha	$r = 0.221; p > 0.05$
A1	Spirulina platensis	0.43 cm	7.25 cm	74	33 kg / ha	$r = 0.448; p > 0.05$
B1	Panchagavya	0.32 cm	5.13 cm	71	28 kg / ha	$r = 0.407; p > 0.05$
C1	Consortium	0.87 cm	10.44 cm	85	40 kg / ha	$r = 0.716; p < 0.05$

Plants with Good agronomic parameters and biomass were chosen to perform HPLC. In this study, group C shown the most vigorous and high significant growth.

Quantitative evaluation of phytohormones

Chromatography techniques, such as HPLC - PDA, have enabled the quantification of hormones and their metabolites with much greater precision, sensitivity, and speed. Throughout 14 days, Consortium [Group - C] of 2 filtrates results in showing isovitexin, phenolics, and indole acetic acid.



Indole acetic acid is a key substance in the tryptophan pathway that is Lenovo auxin biosynthesis. Biosynthesis of phenolics and flavonoids in plants initiates through the deamination of L-phenylalanine to trans-cinnamic acid and ammonia by a strategic enzyme phenylalanine PAL.[18,19] In the present study, IAA increased 171% from 7 to 14 days.

Limitation

However, there are a few issues that need to be looked into more in order to better understand how and when microalgal extracts could be used in crop production to promote agricultural sustainability. If these manures are to be used as synergistic biofertilizers, the pH range of panchagavya and micro algae should be concentrated. As a result, the symbiotic relationship is a crucial aspect of manure applications in soil.

Future outcome

Microalgae extracts are more popular in crop cultivation than Vigna radiata. Various formulations are now commercially accessible, and a rising number of agrochemical companies are including microalgal extracts into their formulations. Microalgal extracts, in particular, showed a number of positive benefits, including improved nutritional intake, enhanced tolerance to abiotic stressors, and increased nutritional intake. For better results surplus amount of consortium preparation should be done.

4 Conclusion

The study emphasized on a possible combination of micro algae and panchagavya, which showed enhanced plant growth and was concluded as synergism. In this context, synergistic application of Spirulina platensis and Panchagavya could potentially double the productivity of mungbean. Thus, application of consortium - biofertilizer is a recommendable option to boost the productivity of mungbean in alkaline soils of waste land soil, Cheyyar.

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