ejpmr, 2024, 11(3), 215-220

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF *SPIRULINA PLATENSIS* ON *VIGNA RADIATA* SEED GERMINATION AND FOLIAR SPRAY APPLICATION

Jansirani D.*, Vinitha S.[1], Subha Ranjana P.[2] and Gayathri Manju B.[3]

*Associate Professor, Department of Biotechnology, P.S.R. Engineering College, Sivakasi-626140.
[1,2]Research Students, Department of Biotechnology, P.S.R. Engineering College, Sivakasi-626140.
[3]Assistant Professor, Department of Physics, P.S.R. Engineering College, Sivakasi-626140.

***Corresponding Author: Dr. Jansirani D.**

Associate Professor, Department of Biotechnology, P.S.R. Engineering College, Sivakasi-626140.

Article Received on 13/01/2024 **Article Revised on 02/02/2024** **Article Accepted on 23/02/2024**

ABSTRACT

The study investigates the impact of *Spirulina platensis*, a nutrient-rich cyanobacterium, on *Vigna radiata* (mung bean) seed germination and seedling growth. Our work assesses the influence of *Spirulina platensis* as a foliar spray and explores the effect of varying soaking durations (1hr, 2hr, 3hr, 4hr, and 5hr) on seed germination. In addition, foliar spray treatments with *Spirulina* extract are administered, with control groups receiving only water treatments. The experiment results and analyzes data on germination rates, seedling vigor, growth parameters, and physiological responses. Surprisingly, results show a slightly significant impact on seed germination and seedling growth regardless of *Spirulina* concentration. Notably, a soaking duration of 4hrs significantly enhances seed germination in *Vigna radiata* compared to other durations. These findings confirm *Spirulina platensis* as an effective bio stimulant, with its bioactive compounds accelerating the germination process. The study suggests the potential utility of *Spirulina platensis* in agriculture, presenting opportunities for sustainable practices and improved crop production.

KEYWORDS: *Spirulina, Vigna radiata*, Soaking, Seed germination, Foliar spray application.

INTRODUCTION

India stands as a major global consumer of fertilizers, primarily relying on chemical inputs since the 1960s Green Revolution. Rising global population, expected to hit 9.7 billion by 2050, poses a 70% increase in food demand. To address this, optimizing fertilizer use on limited arable land is crucial. Yet, economic challenges arise as mineral fertilizer prices surpass those of food, impacting agrarian productivity.[1] Ranking third in fertilizer production and second in consumption, India emphasizes a crucial NPK ratio of 4:2:1 to enhance soil health and crop yield. Bio fertilizers play a crucial role in maintaining soil fertility by facilitating the enrichment of various micro and macro nutrients through processes such as nitrogen fixation, phosphate and potassium solubilization or mineralization, release of plant growth-regulating substances, antibiotics production, and organic matter biodegradation in the soil. This leads to enhanced nutrient uptake and increased resilience to drought and moisture stress.[2] Cyanobacteria have the potential to play a pivotal role in promoting sustainable agriculture, contributing to soil fertility, crop growth, yield, and the enhancement of environmental quality.[3] *Spirulina* serves as a link in the evolutionary chain between bacteria and green plants. Polysaccharides, auxins, cytokines, betaines, amino acids, vitamins, and polyamines have been found to benefit seed germination in micro algal extracts. Leguminoseae is the most important family under dicotyledonous *Vigna radiata*, is a member of the fabacaceae family. It ranks as India's third-most-important crop of legumes used for food. *Vigna radiata* is one of the important short-season grain legumes utilized in the traditional farming practices of tropical and temperate regions.[4] The germination process consists of a series of actions that begin when the mature, dried seed absorbs water and end when the radicle pokes through the seed envelopes. The intricate process of germination requires the ingested mature seed to swiftly transition from a maturation to a germination-driven developmental phase in order to set the stage for the growth of seedlings.[5] An efficient method of arousing the dormant embryo inside a huge, tough seed is to soak it. The best approaches to increase grains' nutritional and bioactive potential while lowering their anti-nutritional components are still through traditional procedures such as soaking. The percentage of seeds can be increased and germination accelerated by soaking. Generally speaking, seeds should be soaked in a dish of warm water for six to twenty-four hours. The size of the seed determines how much time is needed. Smaller seeds

with thinner coatings require less time, while larger seeds with thicker coats require more time.

This study addresses India's overpopulation challenge, specifically the increased demand for seeds, aiming to enhance *Vigna radiata* yield and provide solutions to potential food shortages and malnutrition risks. This predicament poses a looming threat of potential food shortages and an elevated risk of malnutrition. Consequently, our study is dedicated to enhancing the yield of *Vigna radiata*, aiming to contribute solutions to mitigate the pressing issues associated with population growth, food security, and nutritional well-being.

MATERIALS AND METHODS

Sample collection

Spirulina and *Vigna radiata* were collected from SS Biotech *Spirulina* production unit located at Madurai and local market respectively.

Experimental design

Vigna radiata seeds were treated with varying *Spirulina* concentrations (1%, 2%, 3%, 4%, and 5%), with pure water serving as the control. Subsequently, these treated seeds were planted in soil at a depth of 1cm, with three seeds per planting spot in the field.

Effect of different concentration of *Spirulina* on different soaking hours

In this method, *Spirulina* was soaked at various time intervals ranging from 1hr to 10hr. Subsequently, seeds were cleaned and stored in airtight plastic jars. Prior to sowing, the seeds underwent soaking in *Spirulina* suspension (3g/l) for different time intervals, while the control group was soaked in pure water for 1hr. Planting was done at a depth of approximately 1cm, with three seeds per planting spot in the field.

Physical characteristics of *Vigna radiata*

Effect of different concentration of *Spirulina* on water absorption index and water solubility index

Water Absorption Index and Water Solubility Index were studied as described by Aderson and Conway.[6] To find the WAI and WSI, 2.5 g of flour samples weremixed with 30 ml of distilled water and boiled for 15 mins at 90 °C. The heated paste wasallowed to cool at room temperature. Then, centrifuged for 10 mins at 3000 rpm. The Sediment particles were weighed after the supernatant was decanted into a dish that was covered in tare. By evaporating the supernatant at 110 °C, the weight of the dry particles in the supernatant wascalculated. According to equation, the water solubility index (g/100g) and the water absorbtion index.

WAI=Weight of the sediment / Weight of the flour sample (g/100g).

WSI=Weight of dissolved solids in the supernatant / Weight of the flour ×100(g/100g).

Effect of different concentration of *Spirulina* on water absorption capacity

Water absorption capacity of the *Vigna radiata* flour was analyzed by Sosulki and Garratt.[7] 3g of the sample was dissolved in 25ml of distilled water. The dispersions were stirred for 30 min while being held, and then centrifuged for 25 mins at 3000 rpm. The surplus moisture was dried at 50 ℃ for 25 mins after the supernatant was decanted, the sample was allowed to cooled and then weighed.

Effect of different concentration of *Spirulina* on oil absorption capacity

Oil absorption capacity of the *Vigna radiata* flour was measured by the centrifugation method as described by Lin *et al.*[8] 1g of sample was mixed with 6ml of oil. The contents were agitated for 1 min and after 30 mins of incubation period, they were spinned in a centrifuge at 3000 rpm for 25 mins. The separated oil was removed with a pipette. Average values of three replicates were recorded. The OAC were expressed as grams of oil bound per 100gram of the sample on a dry basis.

Effect of different concentration of *Spirulina* on plant leaves

To evaluate the effect of *Spirulina plantesis* on the *Vigna radiata* seed germination, the analysis focused on leaf count in plant saplings. Randomly selected saplings were subjected to manual leaf counting, covering all sections of the shoot from top to bottom. The recorded number of leaves was documented in the data sheet.

Effect of different concentration of *Spirulina* on shoot length

Saplings were selected at random, and their shoot lengths were measured using a ruler or measuring tape. Each shoot was placed on a flat, stable surface to prevent bending or curving. The measurement was taken from the starting point to the tip of the shoot, ensuring precision. The recorded shoot lengths were then documented on the data sheet.

Effect of different concentration of *Spirulina* on root length

The roots were meticulously excavated by digging around the base of the plant using a shovel or trowel. Excess soil was gently shaken or brushed off from the roots. Subsequently, the length of the roots was measured with precision using a ruler or measuring tape, starting from the base to the tip. The recorded root lengths were then noted on a data recording sheet.

Effect of different concentration of *Spirulina* on leaf area index

Collect the leaves from plant saplings. Place the leaves on graph paper, trace their outlines, and count the squares covered to provide an estimate of the surface area for each leaf.

Effect of different concentration of *Spirulina* on number of pods

To assess the pod count of the plant saplings, selected saplings were analyzed. The number of pods in all sections of the saplings was manually counted and recorded in the data sheet.

Effect of different concentration of *Spirulina* on number of seeds

To harvest *Vigna radiata* seeds, select a plant from the field and collect pods from a section of the plant. Extract the seeds from the pods. Weigh the seeds using a balance by following these steps: Place an empty container on the balance pan and press the "tare" or "zero" button to set the balance to zero with the container on it, excluding the container's weight from the final measurement. Transfer the harvested *Vigna radiata* seeds into the container on the balance. The balance will display the weight of the seeds in grams. Record the weight of the seeds on the data sheet.

Effect of different concentration of *Spirulina* on foliar spray application

The fresh algae material, amounting to one kilogram, underwent chopping into small pieces, following which it was weighed for evaluation. Subsequently, the sample underwent extraction through a blender. The blended material was then filtered using a double layer of muslin cloth to eliminate any debris, and this resulting filtrate was labeled as 100%. Various concentrations used in this study were achieved by diluting the 100% filtrate with tap water. The prepared solutions were refrigerated at 0 – 4ºC until they were ready for use. The application of algae extract occurred on the plants at the intervals of 30 and 45 days from seedlings, while the untreated plants (control group) received a spray of tap water.[10]

Quality analysis of soil after foliar spray application

Before sowing *Vigna radiata* seeds, the physical characteristics of the soil were analyzed by using the method of Jackson.[10] The soil sample analysis was conducted at the TNAU Soil Testing Centre in Kovilpatti.

RESULT AND DISCUSSION

The *Vigna radiata* seeds treated with *Spirulina* exhibited enhanced germination rates. The treatment further contributed to improvements in various parameters, including root and shoot length, number of leaves, number of branches, and leaf area. Additionally, the physical characteristics of the *Vigna radiata* seeds showed an increase after the *Spirulina* treatment.

Optimizing concentration of *Spirulina*

Optimal performance was achieved when the process was fine-tuned to operate below the 50% threshold, demonstrating superior outcomes compared to the 3% benchmark. This discerning result points toward the efficacy of the soaking method as the preferred approach in this specific context. Fine-tuning the optimization parameters below the 50% threshold not only showcased improved results but also underscored the superiority of employing the soaking method over alternative approaches.

Effect of different concentration of *Spirulina* on different soaking hours

The effects of *Spirulina* suspension at different concentrations revealed optimal germination and seedling growth in the 5% treatment for *Vigna radiata*. The findings from this plot experiment suggest a positive impact of *Spirulina* suspension on the growth and yield of *Vigna radiata*.

Physical characteristics of *Vigna radiata*

The physical characteristics of *Vigna radiata* was tabulated in table 1. From the table 1, We concluded that *Vigna radiata* seeds treated with *Spirulina* exhibit increased Water Absorption Index (WAI), Water Solubility Index (WSI), Water Absorption Capacity (WAC), and Oil Absorption Capacity (OAC) compared to untreated samples. The maximum level of WAI (210.3/100g), WSI (15.33g%), WAC(58.23ml/100g) and OAC(88.3ml/100g). The effect of varying concentrations of *Spirulina* on WAC and OAC in *Vigna radiata* seeds is visually represented in Fig. 1 and Fig. 2, demonstrating significant differences.[11]

Table 1: Physical characteristics of *Vigna radiate.*

PHYSICAL CHARACTERISTICS	CONTROL	TREATMENT
WAI	192.8/100g	210.3/100g
WSI	12.76 g%	15.33 g%
WAC	56.86ml/100g	58.23ml/100g
OAC	82ml/100g	88.3ml/100g

Fig. 1 & Fig. 2 Effect of different concentration of *Spirulina* on WAC and OAC.

Effect of different concentration of *Spirulina* on number of Plant branches

The number of branches at T1 have 5 branches, T2 have 6 branches, T3 have 7 branches, T4 have 8 branches, T5 have 9 branches and control have 4 branches and are represented in Fig. 3. when compared to control, T5 showed more number of branches.[12]

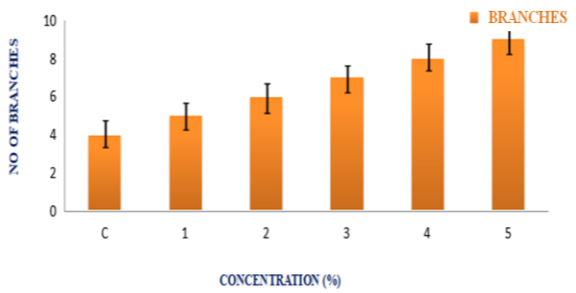

Fig. 3: Effect of different concentration of *Spirulina* on number of plant branches.

Effect of different concentration of *Spirulina* on shoot and root length

The *Spirulina* treated seeds showed increase in shoot and root length because *Spirulina* helps in nitrogen fixation of the soil. The effect of different *Spirulina* suspension treatment on shoot & root length of *Vigna radiata* was measured on 50 days is represented in Fig. 4 and Fig. 5. It demonstrated the highest values for shoot and root lengths at 48±2 cm and 38±5 cm, respectively, observed with the T5 concentration. Similar work was carried out by Fusun Akgul.[13] Auxin, Gibberellins, and Cytokinins are present in substantial amounts, contributing significantly to the augmentation of plant height.[14]

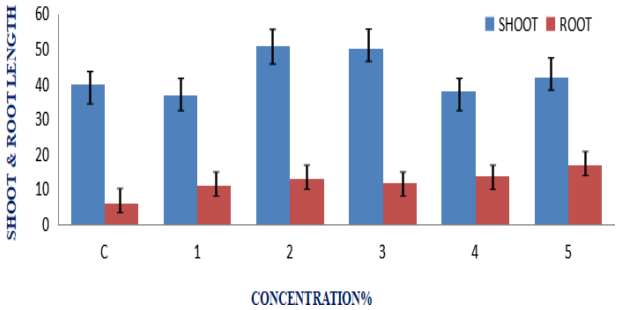

Fig. 4: Effect of different concentration of *Spirulina* on shoot and root length.

Fig. 5: Effect of different concentration of *Spirulina* on shoot and root length.

Effect of different concentration of *Spirulina* on plant pods and seeds

Figure 6 illustrates the maximum number of pods observed at T5, with a count of 30 pods, and the minimum number of pods at T1, totaling 11 pods. In the control group, at 50 days of *Spirulina* suspension with varying concentrations, there were 6 pods. Similar result was observed by Khaing Khaing.[15]

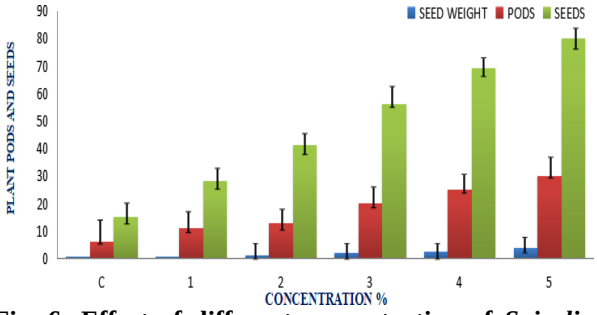

Fig. 6: Effect of different concentration of *Spirulina* on plant pods and seeds.

Effect of different concentration of *Spirulina* on leaf area index

Higher *Spirulina* concentrations enhance plant growth by increasing nutrients, bio-stimulation, stress relief, chlorophyll production, and inducing metabolic changes, impacting leaf area index through improved photo synthesis and altered plant metabolism. The leaf area index was measured to be 30±5 cm² in plants treated with *Spirulina plantesis* at a concentration of T5. Following T5, the leaf area index decreased in the order of T4 (27±2), T3 (22±4), T2 (20±4), T1 (19±1), and the control, as illustrated in Fig. 7 and Fig. 8. Earlier report was observed by Jufri and Sulistyono.[16] This cost-effective and straight forward method can be employed to estimate plant leaf area, regardless of leaf shape or size.[17]

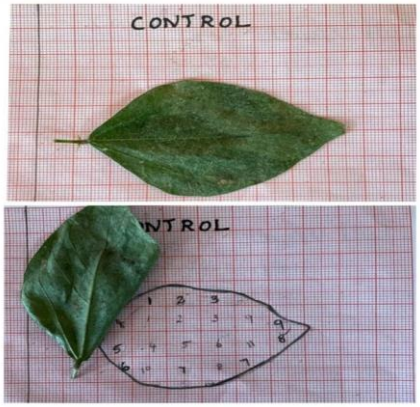

Fig. 7 Effect of different concentration of *Spirulina* on Leaf Area Index.

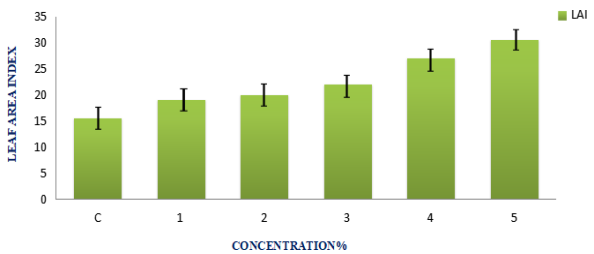

Fig. 8: Effect of different concentration of *Spirulina* on leaf area index.

Effect of different concentration of *Spirulina* on foliar spray application

The effects of foliar spray treatments on *Vigna radiata* plant were assessed and the experiment was conducted with number of replicates for each treatment. The results revealed substantial improvements in various aspect in which treated plants exhibited significant enhancements in growth parameters, including increased plant height, larger leaf area and greater biomass, in comparison to the control group. In that individual application of *Spirulina* is an efficient bio-fertilizer, increasing the productivity of *Vigna radiata*. From the findings, farmers can easily apply *Spirulina* in mung bean fields to enhance production. Furthermore, both applications of *Spirulina* prove more effective in increasing yield compared to individual applications. In this regard, the combined use of *Spirulina* triggers a higher yield than their individual applications. Consequently, it can be concluded that the recommended approach for organic crop production in mung beans involves the use of *Spirulina*.[18] Similar work was carried out by Murugan *et al.*[19]

Quality analysis of soil after foliar spray application

The quality analysis results give information about the physico-chemical characteristics of the soil as shown in table 2. The results revealed that NPK content was increased when compared to normal soil.[20] NPK content in soil could promote the growth which in turn increased the yield of *Vigna radiata*.

Table 2: Quality analysis of soil after foliar spray application.

CHARACTERISTICS	VALUES
Type	Sandy clay loam
EC	0.30ds/m
pH	8.5
O.C %	0.15
Nitrogen(N)	14%
Phosphorus(P)	5ppm
Potassium(K)	218ppm
Iron (Fe)	0.17ppm
Manganese (Mn)	1.26ppm
Zinc (Zn)	0.26ppm
Copper (Cu)	1.55ppm

CONCLUSION

In conclusion, micro algal biofertilizers, particularly *Spirulina platensis*, offer eco-friendly alternatives to chemical fertilizers, avoiding environmental toxicity and associated health risks. The study demonstrates *Spirulina platensis* as an effective and natural biofertilizer for *Vigna radiata*, showing positive effects on growth parameters.

Micro algal biofertilizers, being cost-effective, provide farmers with an economically viable option, enhancing yield and promoting soil quality improvement. Notably, a 5% *Spirulina* suspension (T5) proved most effective for *Vigna radiata*, displaying superior germination, seedling growth, and various growth parameters compared to the control group.

In light of these findings, *Spirulina platensis*, as a biofertilizer, significantly boosts the growth and yield of *Vigna radiata*, with T5 identified as the optimal concentration. This underscores *Spirulina's* efficacy in sustainable agriculture, contributing to environmental health and economic well-being for farmers.

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