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Research Paper

Synergistic Effects of Algal Biofertilizers and *Rhizobium* on Growth and Nodulation in Soybean(*Glycine max* L.) Merr.

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Abstract

Blue-green algae (cyanobacteria), play a vital role in enhancing soil fertility and plant growth through nitrogen fixation and the release of bioactive substances. The present research work was carried out to evaluate the effect of algal biofertilizer (*Nostoc commune*) alone and in combination with *Rhizobium japonicum* on the growth, and nodulation of soybean (*Glycine max* L.) under pot conditions. Results shows that algal inoculation significantly improved various growth parameters such as root length, shoot length, total seedling length, and dry weight compared to the control. The combined application showed a more pronounced effect than individual treatments, indicating a synergistic interaction between cyanobacteria and rhizobia. Increase in nodulation was also observed in combined treatments. The findings suggest that the use of algal biofertilizers, either alone or in combination with rhizobial inoculants, can serve as an effective and eco-friendly alternative to chemical fertilizers.

Introduction

Sustainable agriculture has become a global priority in response to the adverse effects of excessive chemical fertilizer use on soil health, environmental quality, and crop productivity. In recent years, there has been increasing emphasis on eco-friendly and cost-effective alternatives such as biofertilizers (Vessey, 2003). Among these, algae—particularly cyanobacteria (blue-green algae)—have gained considerable attention due to their ability to improve soil fertility and promote plant growth through natural processes (Singh et al., 2016).

Algae are photosynthetic microorganisms that play a significant role in nutrient cycling within soil ecosystems. Certain genera such as *Nostoc*, *Anabaena*, and *Cylindrospermum* are well-known for their nitrogen-fixing ability, converting atmospheric nitrogen into forms readily available to plants (Venkataraman, 1972). In addition to nitrogen fixation, these organisms release various bioactive substances, including vitamins, amino acids, and plant growth regulators like auxins, gibberellins, and cytokinins, which enhance seed germination, root development, and overall plant vigor (Prasanna et al., 2010). Their application also improves soil structure, water-holding capacity, and microbial activity, contributing to long-term soil health (Rai et al., 2000).

Soybean (*Glycine max* L.) is an important leguminous crop valued for its high protein and oil content. It plays a crucial role in agricultural economies and soil fertility through its symbiotic association with nitrogen-fixing bacteria such as *Rhizobium japonicum* (Hungria et al., 2006). However, the increasing demand for higher yields has led to reliance on chemical fertilizers, which may negatively impact soil sustainability. Integrating algal biofertilizers with traditional practices offers a promising approach to enhance soybean productivity while maintaining ecological balance (Singh, 2014). The use of algae as a biofertilizer in soybean cultivation can potentially improve nutrient availability, stimulate plant growth, and enhance yield quality. Moreover, algal inoculation may act synergistically with rhizobial associations, further boosting biological nitrogen fixation (Prasanna et al., 2013).

Effect of inoculation of fresh and dry forms of blue-green algae (Bisoyi and Singh, 1986), their culture filtrates (Mohan and Mukerjee, 1980), cell suspensions (Jain and Sharma, 1984) on the growth and yield of paddy have been already been reported. In rice-wheat cropping system, application of 10 kg/ha BGA in rice could save fertilizer nitrogen needs of rice by 40 kg/ha besides leaving significant residual effect on the succeeding crop of wheat (Rathore et al., 1995).

Pachpande and Kottawar (1992) reported increase in yield of fresh fruits of banana and shoot yield by the inoculation of mixture of *Anabaena variabilis, Aulosira fertilissima, Nostoc muscorum* and *Oscillatoria* species. Singh, 1961 reported beneficial effect of algal inoculation on the yield of sugarcane and grass. Dadhich et al., 1969 obtained increased yield of *Lactuca sativa* and *Capsicum* annum due to the inoculation with *Tolypothrix tenuis*. Kerni et al., 1981 revealed that algal inoculation significantly promoted the growth of guava seedling (*Psidium guajava* L.). Stimulatory effect of inoculation of fresh form of *Aulosira fertilissima* and *Spirulina subsalsa* were reported by Rizvi and Sharma, 1994 on vegetative growth of tomato plant.

Blue green algae characteristically liberate substantial quantities of extracellular nitrogenous compounds into the medium irrespective of whether they are growing on elemental nitrogen or on combined nitrogen (Fogg, 1952). Kabli, et.al.,1997 has reported supply of atmospheric nitrogen to wheat plant by cyanobacteria. In vitro conditions, transfer of fixed nitrogen from diazotrophic filamentous cyanobacteria, *Microcoleus chthonoplastes* to black mangrove seedlings were observed by Bashan et al., (1998). The beneficial effect of the nitrogen fixing BGA and *Azotobacter* on the growth of crop plants has been demonstrated by many workers (Allen, 1956; De and Sulaiman, 1950; Singh, 1961; Rewari et al., 1961; Watanabe et al., 1951).

From the persual of above literature, it is clear that algalization gave very enhancing results in paddy as well as in other crops also. It is also evident that certain Cyanobacteria shows enhanced nitrogen fixation in presence of bacteria. Hence, in the present investigation attempt has been made to study the effect of fresh form of *Nostoc commune* alone and along with *Rhizobium japonicum* on growth, development and nodules of soybean, in order to evaluate efficiency of Cyanobacteria as biofertilizer for Soybean.

MATERIALS AND METHODS

Site description

The pot experiment was conducted in the Botany Department in Nagpur (Maharashtra, India) during the kharif season. The experiment was carried out under natural photoperiod with temperatures ranging from 25–35 °C.

Preparation and Application of Biofertilizers

Algal Biofertilizer:

A culture of cyanobacteria (*Nostoc*) was grown in BG-11 medium under laboratory conditions. The algal biomass was harvested and applied to soil and mixed thoroughly into the soil before sowing.

Rhizobium Inoculation:

A suitable strain of *Rhizobium japonicum* was procured from a PKV, Nagpur laboratory. Soybean seeds (*Glycine max* L.) were surface sterilized with 0.1% mercuric chloride ($HgCl_2$)

for 1–2 minutes and washed thoroughly with sterile distilled water. Seeds were then coated with *Rhizobium* culture and shade-dried before sowing. The observation was taken after 15, 30 and 45 days of sowing.

Treatments:

T1 – Control
T2 – Rhizobium
T3 - Alga 100mg dry wt.
T4 - Alga 200mg dry wt.
T5 - Alga 300mg dry wt.
T6 - Rhizo+Alga 100mg
T7 - Rhizo+Alga 200mg
T8 - Rhizo+Alga 300mg

Morphological characteristics

Different growth characteristics of Soyabean plant like root length, shoot length, total length of seedling, dry weight of seedling were studied. Plants were separated into shoot and root by cutting, in the laboratory. Plant height and root length was measured with the help of a thread. Seedling dry weight of each plant was taken after oven drying at 70°C till constant weight. Number of nodules per plant and Dry weight of nodules was observed. The experiment was laid out in a Completely Randomized Design (CRD) with eight treatments and three replications.

Result and Discussion

The results revealed that seed germination percentage varied across different treatments involving algal biofertilizer and *Rhizobium* inoculation. The control treatment (T1) recorded a germination of 75%, while *Rhizobium* alone (T2) showed an increase to 80.33%, indicating its positive effect on seed viability and early growth(Table 1). The observation after 15 DAS shows that application of algal biofertilizer alone resulted in a gradual increase in root length with increasing concentration. Root length improved from 8.82 cm (T3) at 100 mg to 9.35 cm (T4) at 200 mg and reached 10.21 cm (T5) at 300 mg, indicating a positive dose-dependent response(Table 2). The combined treatments of *Rhizobium* and algae showed the most pronounced effect. After 30 days of growth application of algal biofertilizer alone also enhanced seedling length in a concentration-dependent manner. The values increased from 38.86 cm (T3) at 100 mg to 39.19 cm (T4) at 200 mg and reached 43.33 cm (T5) at 300 mg, showing improvement over the control(Table 3). The combined application of *Rhizobium* and algae resulted in the highest seedling growth. Seedling length increased progressively from 44.88 cm (T6) at 100 mg to 45.57 cm (T7) at 200 mg, and the maximum value of 47.68 cm was observed in T8 (Rhizobium + algae 300 mg).

After 30 DAS inoculation with *Rhizobium* alone (T2) markedly increased nodulation to 70.66, indicating its effectiveness in promoting symbiotic nitrogen fixation(Table 4). Application of algal biofertilizer alone showed a gradual increase in nodule number with increasing concentration, from 53.33 (T3) to 55.00 (T4) and 58.33 (T5), though the effect was moderate compared to *Rhizobium* alone. The combined treatments of *Rhizobium* and algal biofertilizer exhibited a pronounced synergistic effect. The number of nodules increased from 76.66 (T6) to 84.00 (T7) and reached the highest value of 88.33 (T8). Overall, the results indicate that the combined application significantly enhances nodulation compared to individual treatments, suggesting improved symbiotic activity and nitrogen fixation in soybean plants.

The present study clearly demonstrates that both algal biofertilizer and *Rhizobium* inoculation positively influence the growth and development of soybean. Among all treatments, the combined application of algal biofertilizer and *Rhizobium* (particularly at higher concentration) produced the most significant improvements in germination percentage, root length, total seedling length, and number of nodules. The results obtained from fresh algal inoculation in present investigation are mostly in tune with findings of previous workers (Chaudhari et al., 1977; Dadhich et al., 1969; Rizvi and Sharma, 1994; Aiyer et al., 1964; Pachpande and Kottawar, 1992).

Individual and combined effects of the nitrogen fixing BGA, *Tolypothrix tenuis* and *Azotobacter chroococcum* on the vitamin C content of the tomato fruits under pot culture conditions shows that the vitamin C contents of the tomato fruit was increased in the combined effects than in the individuals. This suggests the mutual beneficial effect of the biocoenosis of algal and bacteria population in the soils (Aiyer et al., 1964). The effect of individual and combined application of biofertilizers viz. *Azospirillum*, *Azolla* and BGA with and without inorganic nitrogen on rice was investigated at research station and farmers field by Gopalaswamy et al., 1997. Triple combination responded well. There is evidence that BGA have a positive effect on various saprophytic bacteria. These algae intensify the multiplication and biochemical activity of many bacteria, including *Azotobacter, Clostridium pasteurianum* and *Rhizobium* (Verner, 1935; Shtina, 1963; Perminova, 1964).

In cluster beans a well marked increase in leaf length (70%) and root length (98%) was noticed due to inoculation of *Hapalosiphon stuhlmanni, Anabaena fertilissima* and *Nostoc entophytum* (Giriyappanavar, 1989). Beneficial effect of BGA on height of plant, root and fruit length in tomato and chilly was also observed. Banerjee et al., (1997) points out the utility of *Aulosira fertilissima* and algal biomass of the lake of Bhopal as a chief and effective green manure for rice and mustard. Dadhich et al., 1969 observed that inoculation of the soil with the nitrogen fixing blue-green algae *Calothrix anomala* had a positive effect on *Capsiccum annum* and *Lactuca sativa* in terms of yield and percentage nitrogen.

Besides nutritional effects, applications of BGA helped in improvement of soil physical environment as indexed by aggregation, bulk density, soil strength, hydraulic conductivity and infiltration rate (Khan et al., 1997). Favourable soil physical environment stimulated root growth and thus increased the capacity of plant for efficient utilization of nutrients and water. This resulted in better shoot growth and consequently higher grain yield. It has been claimed that considerably larger amounts of nitrogen can be fixed when the nitrogen fixers grew in association with other micro-organisms (Jensen and Holm, 1975).

It is observed in the present investigation that association of rhizobium with BGA proved to be beneficial to crop plant in terms of vegetative growth and yield of soybean plant. Similar results were obtained by Aiyer et al., (1964) with tomato by application of nitrogen fixing BGA and *Azotobacter*. Perminova (1964) used cultures of BGA (*Nostoc muscorum*) and found increase in harvest of barley by 4.5 to 10%, while gain in yield was 10.3 to 15% with algae and *Azotobacter chroococcum*.

Kaushik and Venkataraman, 1968 reported that the addition of whole algal cells of *Chlorella vulgaris* proved to be significantly stimulatory to the growth of *Azotobacter chroococcum* and *Rhizobium leguminosarum*. Like growth, nitrogen fixation by *Azotobacter chroococcum* was also found to be stimulated by the addition of whole algal cells. Velam and Guerra (1966) reported the bacterial proliferation as a function of algal growth and attributed this to the excretion of organic substances by the alga.

While *Rhizobium* alone enhanced nodulation and growth due to its nitrogen-fixing ability, and algal biofertilizer alone showed a dose-dependent improvement, their combined application resulted in a strong synergistic effect. The highest values observed in combined treatments indicate improved biological nitrogen fixation and better nutrient availability, leading to enhanced plant growth and vigor. Increased nodulation further supports the role of *Rhizobium* in establishing effective symbiosis, which is further stimulated by algal biofertilizers.

These findings are in agreement with earlier reports that cyanobacteria and other algal biofertilizers enhance soil fertility, produce growth-promoting substances, and improve crop productivity (Venkataraman, 1972; Rai et al., 2000; Prasanna et al., 2013). Similarly, the synergistic interaction between *Rhizobium* and other biofertilizers in legumes has been reported to significantly improve nodulation, nitrogen fixation, and yield (Hungria et al., 2006; Singh et al., 2016). In conclusion, the integrated use of algal biofertilizer and *Rhizobium* represents a promising, eco-friendly, and sustainable strategy for improving soybean growth and early establishment. This approach can reduce dependence on chemical fertilizers and contribute to sustainable agricultural practices.

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Tables and Figures:

Treatment	Germination percentage	Percent increase over control
T1 - Control	75	-
T2 - Rhizobium	80.33	7.1
T3 - Alga 100mg dry wt.	74	-1.33
T4 - Alga 200mg dry wt.	76.66	2.21
T5 - Alga 300mg dry wt.	78	4.00
T6 - Rhizo+Alga 100mg	82.33	9.77
T7 - Rhizo+Alga 200mg	82.66	10.21
T8 - Rhizo+Alga 300mg	85.33	13.77

Table 1 : Effect of *Nostoc commune* and *Rhizobium* on germination percentage of Soybean.

Treatment	Root length (cm)	Shoot length (cm)	Total length of seedling (cm)	Dry wt. of seedling (g)
T1	8.26	7.21	15.47	0.283
T2	10.5	9.75	20.25	0.383
T3	8.82	9.15	17.97	0.330
T4	9.35	9.5	18.85	0.344
T5	10.21	9.45	19.66	0.380
T6	10.75	10.26	21.01	0.402
T7	12.25	11.83	24.08	0.423
T8	13.24	12.34	25.58	0.438

Table 2 : Effect of *Nostoc commune* and *Rhizobium* on length and dry wt. of seedling after 15 days of sowing.

Treatment	Root length (cm)	Shoot length (cm)	Total length of seedling (cm)	Dry wt. of seedling (g)	No. of Nodules
T1	20.82	13.43	34.25	2.220	50
T2	22.81	20.72	43.53	2.950	70.66
T3	22.23	16.63	38.86	2.502	53.33
T4	22.48	16.69	39.19	2.642	55.00
T5	22.95	20.38	43.33	2.941	58.33
T6	24.20	20.68	44.88	3.123	76.66
T7	24.84	20.73	45.57	3.194	84.00
T8	25.14	22.54	47.68	3.205	88.33

Table 3 : Effect of *Nostoc commune* and *Rhizobium* on length

Treat ment	Root length (cm)	Shoot length (cm)	Total length of seedling (cm)	Dry wt. of seedlin g (g)	No. of Nodule s
T1	**32.13**	**21.32**	**53.45**	**6.432**	**205**
T2	35.24	30.00	65.24	8.253	365
T3	33.10	26.19	59.29	7.035	230
T4	33.32	26.56	59.88	7.385	262
T5	33.85	25.72	59.57	7.268	263
T6	35.82	32.01	67.83	8.894	390
T7	36.78	34.04	70.82	9.124	423
T8	36.48	33.00	69.48	9.085	429

and dry wt. of seedling after 30 days of sowing.

Treat ment	No. of Nodules	Dry wt. of Nodules	No. of Nodules	Dry wt. of Nodules
	After 30 days		After 45 days	
T1	50	0.101	205	0.520
T2	70.66	0.148	365	0.942
T3	53.33	0.107	230	0.582
T4	55.00	0.113	262	0.655
T5	58.33	0.116	263	0.653
T6	76.66	0.158	390	1.018
T7	84.00	0.170	423	1.081
T8	88.33	0.180	429	1.094

Table 4 : Effect of *Nostoc commune* and *Rhizobium* on Number of Nodules after 30 after 45 days of sowing.

Table 4 : Effect of *Nostoc commune* and *Rhizobium* on length and dry wt. of seedling after 45 days of sowing.