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Effect of Biopolymer-based Seed Biopriming on Growth and Yield of Rice (*Oryza sativa* L.)

E. P. Deepna[1], P. S. Panchami*[2], K. Surendra Gopal[3], D. Girija[4] and Raji Namboodiri Vasudevan[5]

[1-4] Department of Agricultural Microbiology, College of Agriculture, Kerala Agricultural University, Thrissur - 680 656, Kerala, India
[5] Department of Plant Breeding and Genetics, College of Agriculture, Kerala Agricultural University, Thrissur - 680 656, Kerala, India

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Abstract

Seed biopriming, a pre-sowing treatment utilizing beneficial microorganisms, has emerged as an environmentally sustainable approach to enhance plant growth and productivity. This study evaluates the impact of biopolymer-based seed biopriming on the growth promotion of rice. Rice seeds were treated with 0.5% sodium alginate and 1% lignosulfonate, in combination with the bioagents *Bacillus subtilis* and *Azospirillum zeae*, and assessed through a pot culture experiment. Monthly observations revealed that population of *Bacillus subtilis* and *Azospirillum zeae* were significantly higher in treatments with bioprimed seeds. Plant growth parameters, such as plant height and the number of tillers, were comparable in sodium alginate and lignosulfonate treated seeds. However, yield parameters, including grain weight and panicle number, were highest in seeds treated with lignosulfonate. These results confirm that biopolymer-based seed biopriming, particularly with lignosulfonate, effectively promotes rice plant growth and yield compared to control and talc-based consortia treatments, offering a promising alternative for sustainable agriculture.

***Key words*:** Biopriming, Biopolymers, Consortium, Rice, Plant growth, Lignosulfonate, Sodium alginate

Plant performance plays a crucial role in agricultural productivity and ecosystem restoration, as primary productivity is vital for environmental sustainability and global food security [1]. However, in these controlled systems, various biotic and abiotic factors often hinder yields and productivity, while also negatively impacting seed quality.

Plant-beneficial microbes are an environmentally safe and effective alternative to high-cost chemical fertilizers. They promote plant growth and productivity through various mechanisms. Though the demand for plant beneficial microbes is increasing steadily, their application is hampered due to poor shelf life and effective delivery to crop niches. Therefore, there is an urgent necessity for an efficient and effective inoculation method to apply plant-beneficial microbes. Biopriming is an effective way to introduce microbial inocula into the soil where they will be well positioned to colonize seedling roots [2]. Biopriming involves physical seed improvement as well as biological seed enhancement utilizing various bacteria and compounds mediated by microorganisms. The ability of seed priming to improve seed germination and seedling establishment even in less-than-ideal environments is its most crucial component. Biopriming seeds with plant growth-promoting rhizobacteria (PGPR) is a cost-effective and environmentally friendly method to enhance growth during the early stages of development [3]. Biopriming enhances seed germination, promotes uniform seedling emergence, improves stand establishment, and increases seed viability, plant vigor, growth, and yield [4]. Additionally, the use of biopolymers as seed coatings is a growing trend, as they help to protect and deliver bioinoculants more effectively, further enhancing the success of biopriming. Biopolymers, including sodium alginate, carboxymethylcellulose, gum Arabic, chitosan, and xanthan gum, are highly effective as seed coating materials. Biopolymer-based seed coatings are particularly valuable in sustainable agriculture, where they can replace synthetic polymers and chemicals. They are versatile and can be customized with additional nutrients, growth regulators, or protective agents to suit specific crop and soil needs.

This study focused on evaluating the impact of biopolymer based biopriming on plant growth and yield in rice. The experiment involved assessing growth and yield parameters in bioprimed seeds compared to untreated controls under pot culture conditions. Additionally, nutrient content and total soil microbial populations were analyzed to determine the effectiveness of biopriming in a non-sterile environment.

MATERIALS AND METHODS

The experiment was conducted at the Department of Agricultural Microbiology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur, Kerala from June to September 2024. The Kerala Agricultural University (KAU) Manu Ratna rice variety was used for the pot culture study.

Seed biopriming

For the preparation of biopolymer solutions, biopolymer concentrations of 0.5% sodium alginate and 1% lignosulfonate

***Correspondence to:** P. S. Panchami, E-mail: panchami.ps@kau.in; Tel: +91 989577874

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were taken and UV sterilized for 1 hour. The sterilized biopolymer powders were dissolved in 100 ml distilled water. For biopriming, surface sterilized rice seeds were soaked in 0.5% sodium alginate or 1% lignosulfonate solutions containing bioagent consortia (*Bacillus subtilis* and *Azospirillum zeae*) for 1 hour, followed by air drying before sowing [5].

Treatment details

The experiment was conducted using a completely randomized design (CRD) with four treatments and five replications. The four treatments included: T_1 - seeds bioprimed with 0.5% sodium alginate and bioagent consortia, T_2 - seeds bioprimed with 1% lignosulfonate and bioagent consortia, T_3 - seeds treated with talc-based *Bacillus subtilis* and *Azospirillum zeae*, and T_4 - control with uninoculated seeds.

Preparation of potting mixture

A potting mixture was prepared by combining soil and farmyard manure in a 2:1 ratio. Bioprimed rice seeds, seeds treated with a talc-based consortium, and control seeds were initially raised in a pot tray for 15 days and then transplanted into labeled 11 x 14 inch pots filled with potting mixture. N, P, and K fertilizers were applied during the panicle emergence stage following the recommendations outlined in the KAU package of practices.

Physico-chemical analysis of soil

Soil physico-chemical analysis including parameters such, soil pH [6], EC [6], available nitrogen [7]; phosphorus [8]; potassium [6], and organic carbon [9] were analyzed before and after pot culture experiment.

Enumeration of total microbial population of soil during pot culture

Total soil microbial population such as, *Bacillus* sp. *Azospirillum* sp., bacteria, fungi and actinobacteria count was analyzed by serial dilution followed by pour plate method [10] during 30, 60, 90 days after sowing.

Evaluation of plant growth and yield parameters

Plant growth and yield parameters such as, plant height, number tillers per plant, number of panicles per plant, number of grains per panicle and grain yield was observed during monthly intervals up to 90 days.

RESULTS AND DISCUSSION

In general, the microbial population in the soil was significantly higher in treatments involving seeds bioprimed with bioagent consortia and biopolymers compared to those treated with talc-based formulations and the untreated control across all intervals. After 30 days, the highest *Bacillus* population of 34.67 x 10^7 cfu/g was recorded in T_2 (bioagent consortia + lignosulfonate followed by T_3 (talc-based consortia) with 30 x 10^7 cfu/g, and T_1 (bioagent consortia + sodium alginate) having 27 x 10^7 cfu/g, all three were onpar with each other. Similarly, after 90 days, the *Bacillus* population remained significantly higher in the bioagent consortia and sodium alginate treatment (19.67 x 10^7 cfu/g) whereas the lowest *Bacillus* population of 0.161 x 10^7 cfu/g was observed in the control treatment. A similar pattern was observed in the *Azospirillum* population at all intervals, with the highest population count recorded in T_1 (bioagent consortia + sodium alginate) at 20.33 x 10^7cfu/g, followed by T_2 (bioagent consortia + lignosulfonate) with 16.33 x 10^7 cfu/g after 90 days. The most significant effects of biopriming were observed during the early stages (Fig 1-2). Since the experiment was conducted with native soil, the microbial load also includes the native microbial population.

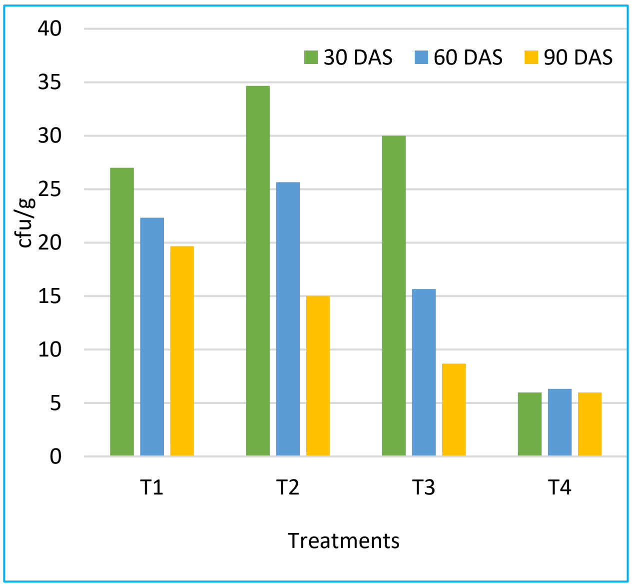

Fig 1 Total *Bacillus* sp. population in soil at monthly intervals

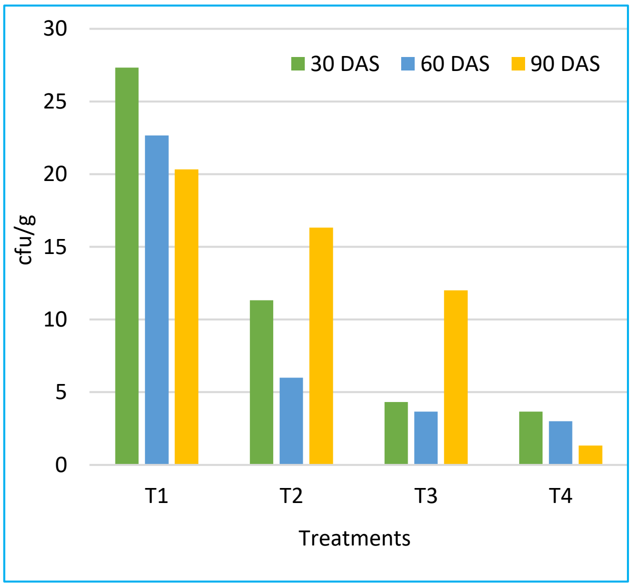

Fig 2 Total *Azospirillum* sp. population on soil at monthly intervals

After 30 days of sowing, significantly higher bacterial population of 89.33 x 10^7cfu/g was observed in T_1(bioagent consortia + 0.5 % sodium alginate) followed by T_2 (bioagent consortia + 1% lignosulfonate) with a population of 75.33 x 10^7cfu/g. Even after 90 days, T_1 maintained significantly higher bacterial population of 23 x 10^7cfu/g. Across all time intervals, lowest bacterial count was recorded in T_4 (control). Similarly total fungal count was highest in T_1 (119.67 x 10^4 cfu/g) followed by T_2 (13.33 x 10^4cfu/g) at 30 days after sowing (Table 1). After 90 days, fungal population in treatments involving bioprimed seeds remained higher compared to talc-based treatments and control. The actinobacterial count present in the soil was found to be non-significant in nature across all the intervals of observation (Table 2).

Table 1 Effect of seed biopriming on bacterial and fungal population in soil at monthly intervals during pot culture

Treatments	Total bacterial count(x10^7cfu/g)			Total fungal count (x 10^4cfu/g)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1: Bioagent consortia + 0.5% sodium alginate	89.33 (1.95)^{a}	65.33 (1.82)^{a}	23.67 (1.37)^{a}	119.67 (2.08)^{a}	46.00 (1.66)^{a}	14.33 (1.15)^{a}
T_2: Bioagent consortia + 1% lignosulfonate)	75.33 (1.88)^{b}	30.67 (1.50)^{b}	13.33 (1.12)^{b}	101.67 (2.01)^{b}	36.33 (1.56)^{b}	12.33 (1.09)^{a}
T_3: Talc-based consortia of *B.subtilis* + *A. zeae*)	65.67 (1.81)^{c}	18.33 (1.28)^{c}	6.67 (0.82)^{c}	83.67 (1.92)^{c}	18.00 (1.25)^{c}	6.33 (0.80)^{b}
T_4: Control	62.33 (1.80)^{c}	16.67 (1.24)^{c}	6.00 (0.76)^{c}	82.67 (1.92)^{c}	14.00 (1.14)^{d}	11.00 (1.03)^{a}
CD (0.05)	0.052	0.091	0.143	0.017	0.076	0.139

Values given in the parenthesis are log transformed value

Table 2 Effect of seed biopriming on actinobacterial population in soil at monthly intervals during pot culture

Treatments	Total actinobacterial count (x 10^5cfu/g)		
	30 DAS	60 DAS	90 DAS
T_1: Bioagent consortia + 0.5% sodium alginate	1.33 (0.32)	1.00 (0.26)	0.33 (0.10)
T_2: Bioagent consortia + 1% lignosulfonate)	1.33 (0.30)	1.00 (0.26)	0.67 (0.20)
T_3: Talc-based consortia of *B.subtilis* + *A. zeae*)	1.00 (0.26)	1.00 (0.30)	0.67 (0.16)
T_4: Control	1.33 (0.30)	0.67 (0.20)	0.67 (0.20)
CD (0.05)	NS	NS	NS

Values given in the parenthesis are log transformed value

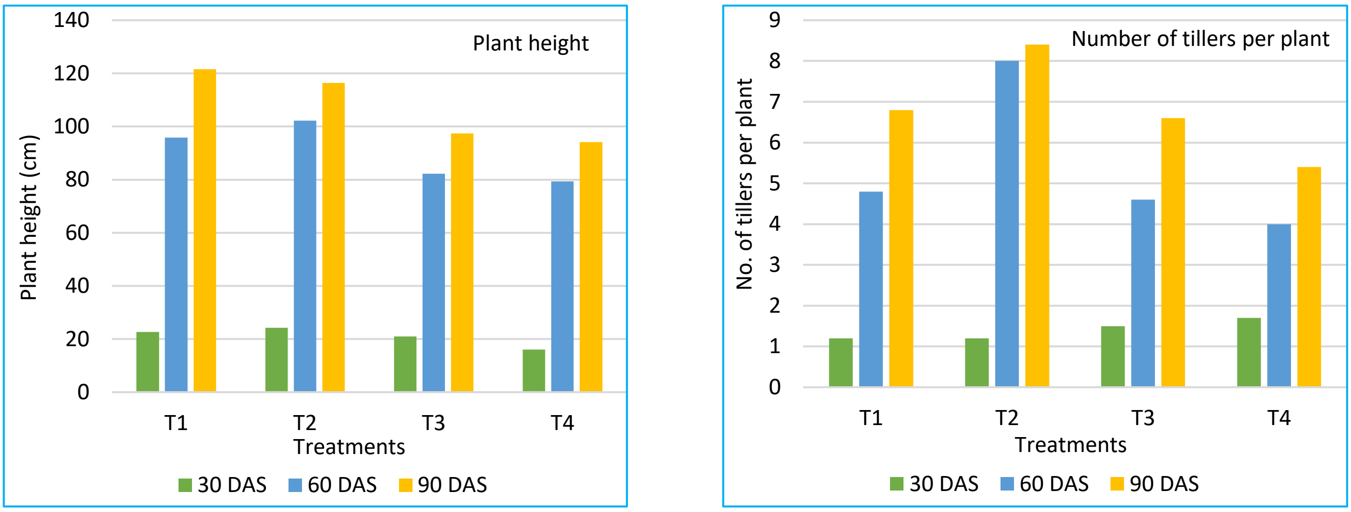

Fig 3 Plant growth parameters at monthly intervals

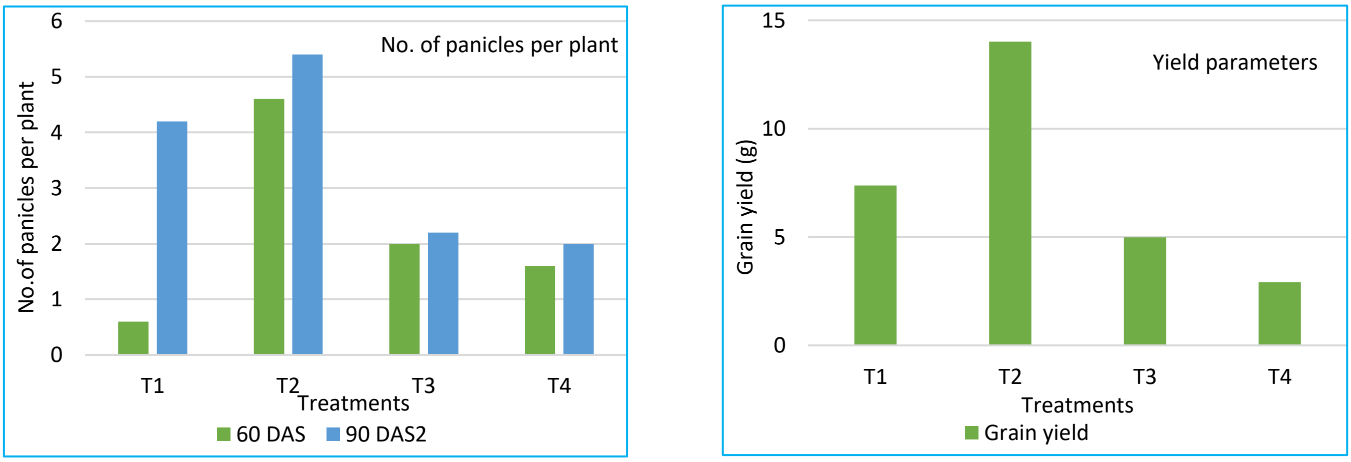

Fig 4 Yield parameters at monthly intervals

At the harvest stage, the observed plant growth parameters indicated comparable performance between seeds treated with lignosulfonate and sodium alginate (Fig 3). Among the treatments, plant height was highest in sodium alginate treated seeds (121.6 cm), closely followed by lignosulfonate-treated seeds (116.4 cm). The number of tillers per plant was highest in lignosulfonate treated seeds (8.4), followed sodium alginate treated seeds (6.8). The yield parameters were significantly higher in lignosulfonate treated seeds (Fig 4). While the number of panicles per plant showed no significant differences among treatments, grain yield per plant was highest in lignosulfonate treated seeds (14.01 g), followed by sodium alginate treated seeds (7.38 g), and talc-based consortia-treated seeds (2.92 g). The number of grains per panicle and straw yield was found to be high in lignosulfonate treated seeds (Fig 4). The enhanced growth parameters observed in rice during the present study might be attributed to the synergistic effect of the bioagent consortia (*Bacillus subtilis* and *Azospirillum zeae*) in combination with the biopolymers. These bioagents have shown to produce phytohormones like IAA which may be one of the reasons for increased plant height. The observed increase in plant height and vigor can likely be attributed to the production of indole-3-acetic acid (IAA) by bioagents. IAA, the main auxin in plants, is crucial for regulating various growth and developmental processes, such as cell division and elongation, tissue differentiation, apical dominance, and responses to environmental factors like light, gravity, and pathogens [11]. Similar results were obtained in the investigation of Srinivasan *et al.* [12], reported that applying liquid formulations of *Azospirillum* and phosphobacteria significantly boosted the rhizosphere microbial population, enhanced plant growth metrics, and increased the yield of maize and tomato under field conditions. Similarly, Zorita and Canigia [13], demonstrated that inoculating wheat seeds with a liquid formulation of *Azospirillum brasilense* promoted more vigorous vegetative growth, improved shoot and root development, and increased dry matter accumulation along with increased yield parameters.

Table 3 Effect of biopriming on no. of grains and straw yield of rice

Treatments	90 DAS	
	No.of grains per panicle	Straw yield per plant (g)
T_1: Bioagent consortia + 0.5% sodium alginate	100.00^{ab}	20.00^{b}
T_2: Bioagent consortia + 1% lignosulfonate)	102.26^{a}	26.00^{a}
T_3: Talc-based consortia of *B.subtilis* + *A. zeae*)	87.61^{bc}	12.00^{d}
T_4: Control	82.22^{c}	14.82^{c}
CD (0.05)	13.528	2.243

Table 4 Physico-chemical analysis of soil under pot culture study

Treatments	pH	EC (dS m^{-1})	Organic carbon (%)	Available N (Kg ha^{-1})	Available P (Kg ha^{-1})	Available K (Kg ha^{-1})
T_1: Bioagent consortia + 0.5% sodium alginate	6.11	0.35	3.88 (H)	872.00 (H)	41.62 (H)	825.43 (H)
T_2: Bioagent consortia + 1% lignosulfonate)	5.79	0.23	1.79 (H)	732.00 (H)	51.51 (H)	938.67 (H)
T_3: Talc-based consortia of *B.subtilis* + *A. zeae*)	5.96	0.28	3.64 (H)	718.00 (H)	97.05 (H)	780.00 (H)
T_4: Control	6.22	0.28	2.20 (H)	708.00 (H)	35.55 (H)	776.22 (H)
Initial soil physico-chemical analysis	6.60	0.38	1.89 (H)	861 (H)	29.59 (H)	155.23 (H)

The physico-chemical analysis of soil conducted before the pot culture experiment revealed a neutral pH (6.6) and electrical conductivity within the normal range (0.38 dS m^{-1}). The soil was found to have high levels of available nitrogen (861 kg ha^{-1}), phosphorus (29.59 kg ha^{-1}), potassium (155.23 kg ha^{-1}), and organic carbon (1.89%). Post experiment analysis indicated an increase in available nitrogen content, particularly in T_1 (bioagent consortia + 0.5% sodium alginate). Organic carbon content showed a remarkable increase of 105.29% in the sodium alginate-treated seeds, along with a significant rise in available phosphorus and potassium levels (Table 4).

The study demonstrates that, throughout the crop period, treatments involving biopolymers combined with bioagent consortia consistently exhibited higher microbial populations, improved growth parameters, and enhanced yield parameters compared to uninoculated seeds. These findings highlight the synergistic effect of biopolymers and bioagent consortia in promoting plant growth. Podile and Kishore [14] observed that plant growth-promoting rhizobacteria (PGPR) enhance plant growth by stimulating root hair branching, improving seed germination, and increasing leaf area. They also facilitate nutrient and water uptake, contributing to greater biomass production, improved carbohydrate storage, and enhanced phosphorus solubilization. Additionally, plant growth-promoting rhizobacteria (PGPR) release plant hormones such as gibberellic acid and produce ACC deaminase, which lowers ethylene levels and supports root development. Seed biopriming is enhanced by using adjuvants such as biopolymers, which boost the efficiency of microbial inoculants and optimize seed performance [15-17]. Biopolymers aid in dispersing bioagents on seed surfaces, enhancing their adhesion and prolonging their viability, which leads to improved seedling emergence, better performance, and increased survival of the bioagents. Gel forming ability of sodium alginate improves the adhesion of bioagents to the seeds which helps in uniform sprouting and plant vigor [18]. Chelation ability of lignosulfonate helps to bind micronutrients to the seeds and make a favourable environment for the survival and activity of inoculated bioagents that could be the reason for increased plant growth and yield parameters [19-20].

CONCLUSION

The study demonstrates that biopolymer-based seed biopriming is an effective, eco-friendly, and farmer-friendly strategy for enhancing crop productivity. Pot culture experiments under soil conditions with diverse native microorganisms showed comparable plant growth parameters for sodium alginate and lignosulfonate-treated seeds, with optimal yield in lignosulfonate treated seeds. Higher soil microbial populations during early growth stages indicated enhanced microbial activity. Cost-effective and easy to implement, lignosulfonate emerges as a practical biopolymer for improving seed vigor, germination, root development, nutrient uptake, and yield. This sustainable technique reduces reliance on synthetic chemicals, aligning with modern agricultural practices focused on productivity and environmental stewardship.

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