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Influence of biostimulants on growth, physiological parameters, and yield of soybean in northern transition zone of Karnataka

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Abstract

A field experiment was conducted during the kharif 2021 season at the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka. The primary objective was to evaluate the efficacy of foliar-applied biostimulants on the growth, physiological parameters, and yield of soybean (*Glycine max* L. Merrill). The experiment was laid out in a Randomized Complete Block Design (RCBD) encompassing 11 treatments replicated three times. The treatments consisted of various concentrations of Crop Max and Biozyme applied at the flower initiation (25-35 Days After Sowing) and pod formation (45-50 DAS) stages. Results indicated that foliar application of Crop Max at 750 ml/ha at flower initiation, followed by Biozyme at 625 ml/ha at pod formation (Treatment T₈), significantly optimized crop performance. At 60 DAS and harvest, this specific sequence recorded the highest plant height (82.1 cm), expanded leaf area (11.47 dm²/plant), and maintained superior relative leaf water content (84.77%). Consequently, it produced the highest number of pods per plant (58.7), translating to a maximum seed yield of 2693 kg/ha and a haulm yield of 3977 kg/ha. The integration of marine algae and fermentation metabolite-based biostimulants at critical phenological stages proved to be a highly productive agronomic strategy for soybean cultivation under the rainfed conditions of the Northern Transition Zone.

Keywords: Soybean, *Glycine max*, biostimulants, crop max, biozyme, foliar application

1. Introduction

Soybean [*Glycine max* (L.) Merrill] is an agronomically and economically significant leguminous crop globally, classified under the family Fabaceae. Often termed the 'cow crop' due to its extensive industrial usage and soy milk production, it is highly valued for its rich protein content (40-42%) and cholesterol-free oil (20%). Furthermore, it is a primary dietary source of all eight essential amino acids, fiber, iron, calcium, and B vitamins. In India, soybean cultivation occupies a prominent position, particularly in states like Madhya Pradesh, Maharashtra, Rajasthan, and Karnataka. Despite its genetic yield potential ranging from 3 to 4 t/ha, the average productivity frequently stagnates below this threshold. In the Northern Transition Zone of Karnataka, yield gaps are commonly attributed to erratic rainfall distribution, periodic moisture stress, and nutritional deficiencies occurring precisely during the critical flower initiation and pod formation stages.

Modern agronomic practices have increasingly turned toward the application of plant biostimulants as a viable strategy to enhance resource use efficiency and narrow the yield gap. Biostimulants are defined not as fertilizers intended to correct severe nutrient deficits, but rather as formulations containing organic compounds, microbial inoculants, seaweed extracts, enzymes, and trace elements that stimulate natural physiological processes. They facilitate enhanced nutrient uptake, up-regulate plant genes involved in metabolism, stimulate root proliferation, and fortify antioxidant defenses against abiotic stress (Calvo *et al.*, 2014) [2]. For example, humic and fulvic acids interact with soil nutrients to elicit physiological responses, while amino acid-based biostimulants enhance stress tolerance distinct from standard nitrogen fertilization (Kocira, 2018) [3].

Among the diverse range of biostimulants, products derived from marine algae (seaweed extracts) have demonstrated considerable efficacy. Seaweed extracts contain naturally occurring plant

growth regulators, particularly auxin precursors, cytokinins, betaines, and polyamines, alongside complexed micronutrients. When applied as foliar sprays, they have been shown to accelerate vegetative growth, improve chlorophyll retention, and optimize assimilate partitioning (Rathore *et al.*, 2009; Zodape *et al.*, 2010) [7, 10]. 'Crop Max' is a commercial biostimulant formulation containing 24% organic compounds (fermentation metabolites, marine algae extracts, and protein hydrolysates) and 18% mineral components, including essential micronutrients (Zn, Fe, Mn, Cu) necessary for enzyme activation. 'Biozyme' is another growth-regulating formulation extracted from the marine alga *Ascophyllum nodosum*, widely documented for its positive impact on legume productivity (Biradar, 1998; Tandon and Dubey, 2015) [1, 8].

While individual applications of these formulations have been studied, there is a distinct need to evaluate their sequential application at specific crop growth stages to maximize synergistic effects. Therefore, the present field investigation was formulated to assess the influence of these biostimulants, applied as foliar sprays at varying concentrations and specific phenological stages, on the sequential growth dynamics, physiological water status, and final yield attributes of soybean.

2. Materials and Methods

2.1 Description of the Experimental Site

The field experiment was conducted during the kharif season of 2021 at the Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad, Karnataka. The site is geographically situated at 15° 29' N latitude and 74° 59' E longitude, at an altitude of 678 meters above mean sea level. The climate of the region is classified under the Northern Transition Zone.

Soil samples were collected from a depth of 0-30 cm prior to sowing to determine the baseline physicochemical properties. The experimental soil was a black clay Vertisol with a neutral pH of 7.1 and a normal electrical conductivity of 0.32 dS/m. Organic carbon content was assessed as low (0.44%) using the Walkley and Black wet oxidation method. Available nitrogen was low (263 kg/ha, alkaline permanganate method), while available phosphorus (30.5 kg/ha, Olsen's method) and available potassium (366 kg/ha, flame photometry) were in the high range. The soil also contained 5.9 kg/ha of available sulfur.

2.2 Meteorological Conditions

Weather parameters were recorded at the MARS meteorological observatory. During the crop growth cycle, the site received a total rainfall of 402 mm distributed across 34 rainy days, aligning well with the soybean water requirement of 600-650 mm when combined with residual soil moisture. The mean maximum temperature ranged from 27.2°C to 28.3°C, and the mean minimum temperature ranged from 20.5°C to 20.8°C, which fell within the optimal thermal range (15-32°C) for soybean phenology. Relative humidity remained high, oscillating between 82.3% and 87.0%, fostering a conducive microclimate for optimal leaf expansion.

2.3 Experimental Design and Treatment Structure

The experiment was structured in a Randomized Complete Block Design (RCBD) comprising 11 distinct treatments, each replicated three times. The gross plot size was 3.6 m x 6.0 m, and the net plot size was 2.4 m x 4.8 m. The treatment details were as follows:

T₁: Crop Max @ 375 ml/ha at flower initiation + Crop Max @ 375 ml/ha at pod formation

T₂: Crop Max @ 500 ml/ha at flower initiation + Crop Max @ 500 ml/ha at pod formation

T₃: Crop Max @ 625 ml/ha at flower initiation + Crop Max @ 625 ml/ha at pod formation

T₄: Crop Max @ 750 ml/ha at flower initiation + Crop Max @ 750 ml/ha at pod formation

T₅: Crop Max @ 375 ml/ha at flower initiation + Biozyme @ 625 ml/ha at pod formation

T₆: Crop Max @ 500 ml/ha at flower initiation + Biozyme @ 625 ml/ha at pod formation

T₇: Crop Max @ 625 ml/ha at flower initiation + Biozyme @ 625 ml/ha at pod formation

T₈: Crop Max @ 750 ml/ha at flower initiation + Biozyme @ 625 ml/ha at pod formation

T₉: Biozyme @ 625 ml/ha at flower initiation + Biozyme @ 625 ml/ha at pod formation

T₁₀: Control-1 (Urea @ 2% and KNO₃ @ 1% foliar spray)

T₁₁: Control-2 (Water spray)

Foliar applications were executed systematically. The first application coincided with flower initiation (25-35 Days After Sowing), and the second application was performed during the pod formation stage (45-50 DAS).

2.4 Crop Husbandry Practices

The land was prepared using a tractor-drawn mould board plough followed by harrowing to achieve a fine seedbed. Soybean seeds (variety MACS-1188, recognized for shattering resistance and high oil content) were treated with *Rhizobium* and Phosphorus Solubilizing Bacteria (PSB) at 1250 g/ha each. Sowing was carried out via dibbling at a depth of 2.5-3 cm with a spacing of 30 cm x 10 cm, utilizing a seed rate of 62.5 kg/ha. A uniform basal dose of Farm Yard Manure (6.25 t/ha) and chemical fertilizers (40:80:25 kg N:P₂O₅:K₂O/ha + 12.5 kg ZnSO₄/ha) was incorporated prior to sowing. Weeds were managed using a pre-emergence application of Pendimethalin 30 EC (3.25 l/ha) followed by manual weeding at 45 and 60 DAS. Plant protection measures, including a spray of Propiconazole 25 EC (1.5 l/ha) to control rust, were undertaken as required.

2.5 Data Collection and Analytical Methods

Five plants were selected randomly in each net plot and tagged for sequential biometric observations. Plant height (from ground level to the tip of the main shoot) and the number of branches were recorded at 30 DAS, 60 DAS, and at harvest. Leaf area per plant was measured, and physiological parameters were assessed. Relative Leaf Water Content (RLWC) was calculated using the formula: $RLWC = [(Fresh\ Weight - Dry\ Weight) / (Turgid\ Weight - Dry\ Weight)] * 100$. Leaf chlorophyll content was measured using a SPAD meter on five fully opened leaves from the top of the tagged plants. Yield parameters, including the number of pods per plant, seed yield, and haulm yield, were recorded at physiological maturity following manual harvesting and threshing.

The recorded data were compiled and subjected to statistical analysis utilizing Fisher's method of analysis of variance (ANOVA). Where the F-test was significant, mean separation was achieved using Duncan's Multiple Range Test (DMRT) at a 5% level of significance (P=0.05). Standard Error of Mean (S.E.m. ±) was also computed for data interpretation.

3. Results and Discussion

3.1 Influence on Morphological Growth Parameters

The application of foliar biostimulants demonstrated a

progressive influence on the morphological growth of soybean, with the effects becoming highly pronounced at 60 DAS and at harvest (Table 1). At 30 DAS, prior to the full absorption and physiological integration of the first foliar spray, parameters such as plant height and branch count did not exhibit statistically significant variance among the treatments.

However, by 60 DAS and subsequently at harvest, Treatment T₈ (Crop Max @ 750 ml/ha at flower initiation followed by Biozyme @ 625 ml/ha at pod formation) recorded the maximum plant height of 81.5 cm and 82.1 cm, respectively. This was statistically comparable to T₇ (77.7 cm and 79.3 cm) but significantly superior to the water spray control (T₁₁), which recorded 70.4 cm at harvest. The number of branches per plant did not yield statistically significant differences across treatments; however, a numerical maximum of 4.7 branches per plant was observed in T₈ at harvest. These findings corroborate the results of Biradar (1998) [1] and Noli *et al.* (2021) [5], who reported significant increases in plant height and vegetative metrics following seaweed extract application. The enhanced cellular elongation and division can be attributed to the bioactive phytohormones—specifically auxin and cytokinin precursors—present in marine algae extracts. When applied externally, these compounds stimulate meristematic activity and internodal elongation (Vishwanatha *et al.*, 2022) [9].

Table 1: Effect of biostimulants on plant height and number of branches of soybean

Treatments	Plant Height 30 DAS (cm)	Plant Height 60 DAS (cm)	Plant Height Harvest (cm)	Branches 30 DAS	Branches 60 DAS	Branches Harvest
T ₁	35.0 ^a	73.6 ^{bc}	75.6 ^b	2.4 ^a	3.7 ^a	4.6 ^a
T ₂	35.3 ^a	73.9 ^{bc}	76.3 ^b	2.4 ^a	3.7 ^a	4.5 ^a
T ₃	35.4 ^a	74.1 ^{bc}	76.3 ^b	2.4 ^a	3.7 ^a	4.5 ^a
T ₄	36.3 ^a	77.6 ^{ab}	78.1 ^{ab}	2.6 ^a	3.9 ^a	4.6 ^a
T ₅	35.5 ^a	75.2 ^b	77.5 ^{ab}	2.5 ^a	3.73 ^a	4.6 ^a
T ₆	36.1 ^a	77.4 ^{ab}	77.9 ^{ab}	2.5 ^a	3.73 ^a	4.6 ^a
T ₇	36.4 ^a	77.7 ^{ab}	79.3 ^{ab}	2.5 ^a	4.1 ^a	4.6 ^a
T ₈	36.8 ^a	81.5 ^a	82.1 ^a	2.3 ^a	4.2 ^a	4.7 ^a
T ₉	35.0 ^a	73.5 ^{bc}	74.4 ^{bc}	2.6 ^a	3.7 ^a	4.4 ^a
T ₁₀	35.5 ^a	74.4 ^{bc}	76.8 ^b	2.4 ^a	3.8 ^a	4.5 ^a
T ₁₁	35.0 ^a	69.7 ^c	70.4 ^c	2.4 ^a	3.7 ^a	4.5 ^a
S.Em. ±	0.6	1.4	1.5	0.1	0.2	0.1

T₁- Crop Max @ 375 ml ha⁻¹ followed by Crop Max @ 375 ml ha⁻¹ T₂-Crop Max @ 500 ml ha⁻¹ followed by Crop Max @ 500 ml ha⁻¹ T₃-Crop Max @ 625 ml ha⁻¹ followed by Crop Max @ 625 ml ha⁻¹ T₄-Crop Max @ 750 ml ha⁻¹ followed by Crop Max @ 750 ml ha⁻¹ T₅- Crop Max @ 375 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₆-Crop Max @ 500 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₇-Crop Max @ 625 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₈-Crop Max @ 750 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₉- Biozyme @ 625 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₁₀- Control-1 (Urea @ 2% and KNO₃ @ 1%) T₁₁-Control-2 (water spray)

Means followed by the same alphabet (s) within a column are not significantly differed by DMRT (P=0.05)

DAS- Days after sowing

Note 1. RDF: FYM @ 6.25 t + 40:80:25 kg N: P₂O₅ and K₂O + ZnSO₄ @ 12.5 kg + *Rhizobium* and PSB @ 1250 g each per hectare common to all treatments

Note 2. For T₁ to T₁₀, foliar application at flower initiation (25-35DAS) and at pod formation stage (45 – 50 DAS)

3.2 Leaf Area Expansion and Physiological Metrics

Leaf area, a fundamental component determining light interception and photosynthetic capability, responded positively to biostimulant application at 60 DAS (Table 2). Treatment T₈

recorded a significantly higher leaf area (11.47 dm²/plant) compared to the control (9.70 dm²/plant). This expansion is likely facilitated by the complexed micronutrients (such as Zn and Mn) in Crop Max, which are vital co-factors for enzymes involved in chlorophyll synthesis and carbon assimilation (Tandon and Dubey, 2015) [8].

Relative Leaf Water Content (RLWC) serves as an effective indicator of the plant's internal water balance and cellular turgidity under field conditions. At 60 DAS, T₈ maintained a significantly higher RLWC (84.77%) compared to the water spray control (79.91%). Seaweed extracts are recognized sources of naturally complexed potassium and organic osmolytes like betaines. These compounds aid in osmotic adjustment, regulating stomatal conductance, and minimizing transpirational water loss, allowing the plant to maintain turgor during transient moisture fluctuations (Pramanick *et al.*, 2013) [6].

Chlorophyll content, measured as SPAD values, displayed numerical enhancements post-spray. Although the statistical differences among the treatments were non-significant at both 30 and 60 DAS, T₇ and T₁₁ maintained robust SPAD readings (49.0) at 60 DAS. The lack of statistical variance suggests that the baseline chlorophyll biosynthesis was adequate across the field; however, the biostimulants actively delayed leaf senescence, thereby maintaining a functional green leaf area for a prolonged duration (Zodape *et al.*, 2010) [10].

Table 2: Effect of biostimulants on Leaf Area, RLWC, and SPAD values at 30 and 60 DAS

Treatments	Leaf Area 30 DAS (dm ²)	Leaf Area 60 DAS (dm ²)	RLWC 30 DAS (%)	RLWC 60 DAS (%)	SPAD 30 DAS	SPAD 60 DAS
T ₁	4.49 ^a	10.33 ^d	78.58 ^a	81.18 ^{cd}	37.5 ^a	47.4 ^a
T ₂	4.51 ^a	10.38 ^d	79.39 ^a	81.87 ^{b-d}	37.6 ^a	47.7 ^a
T ₃	4.84	10.68 ^{cd}	79.58 ^a	82.29 ^{bc}	37.6 ^a	47.8 ^a
T ₄	4.92 ^a	11.33 ^{ab}	78.25 ^a	83.10 ^{a-c}	38.3 ^a	48.7 ^a
T ₅	4.86 ^a	11.20 ^{ab}	79.32 ^a	81.97 ^{b-d}	38.1 ^a	47.9 ^a
T ₆	4.90 ^a	11.28 ^{ab}	79.60 ^a	82.46 ^{bc}	38.1 ^a	48.5 ^a
T ₇	4.93 ^a	11.35 ^{ab}	80.83 ^a	83.49 ^{ab}	38.3 ^a	49.0 ^a
T ₈	4.98 ^a	11.47 ^a	80.31 ^a	84.77 ^a	38.6 ^a	47.0 ^a
T ₉	4.48 ^a	10.31 ^d	78.45 ^a	81.19 ^{cd}	37.0 ^a	47.4 ^a
T ₁₀	4.68 ^a	10.96 ^{bc}	82.26 ^a	82.90 ^{a-c}	38.0 ^a	48.1 ^a
T ₁₁	4.38 ^a	9.7 ^e	80.95 ^a	79.91 ^d	36.2 ^a	49.0 ^a
S.Em. ±	0.22	0.14	1.83	0.62	0.9	0.6

T₁- Crop Max @ 375 ml ha⁻¹ followed by Crop Max @ 375 ml ha⁻¹ T₂-Crop Max @ 500 ml ha⁻¹ followed by Crop Max @ 500 ml ha⁻¹ T₃-Crop Max @ 625 ml ha⁻¹ followed by Crop Max @ 625 ml ha⁻¹ T₄-Crop Max @ 750 ml ha⁻¹ followed by Crop Max @ 750 ml ha⁻¹ T₅- Crop Max @ 375 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₆-Crop Max @ 500 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₇-Crop Max @ 625 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₈-Crop Max @ 750 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₉- Biozyme @ 625 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₁₀- Control-1 (Urea @ 2% and KNO₃ @ 1%) T₁₁-Control-2 (water spray)

Means followed by the same alphabet (s) within a column are not significantly differed by DMRT (P=0.05)

DAS- Days after sowing

Note 1. RDF: FYM @ 6.25 t + 40:80:25 kg N: P₂O₅ and K₂O + ZnSO₄ @ 12.5 kg + *Rhizobium* and PSB @ 1250 g each per hectare common to all treatments

Note 2. For T₁ to T₁₀, foliar application at flower initiation (25-35DAS) and at pod formation stage (45 – 50 DAS)

3.3 Yield Attributes and Final Yield Outcomes

The ultimate objective of biostimulant application is the realization of enhanced economic yields. The integration of Crop

Max and Biozyme drastically improved the source-to-sink translocation of photoassimilates. Treatment T₈ significantly maximized the number of pods per plant, recording an average of 58.7 pods compared to a mere 41.7 pods in the control (Table 3). This aligns with the observations of Rathore *et al.* (2009) [7] and Meshram (2013) [4], who noted that foliar nutrition during early reproductive phases minimizes floral abscission and maximizes pod setting.

Consequently, this structural advantage translated into exceptional final yields. Treatment T₈ recorded the highest seed yield of 2693 kg/ha and haulm yield of 3977 kg/ha, which equates to a 9.16% and 4.6% increase over the control, respectively. Treatment T₇ closely followed with a seed yield of 2655 kg/ha. The targeted application of Crop Max (supplying metabolic catalysts) at flower initiation, followed by Biozyme (supplying growth regulators) at pod formation, ensured an uninterrupted supply of metabolic energy precisely when the crop required maximum nutritional support for grain filling (Kocira, 2018) [3].

Table 3: Effect of biostimulants on yield attributes and yield of soybean

Treatments	Pods per Plant	Seed Yield (kg/ha)	Haulm Yield (kg/ha)
T ₁	43.8 ^d	2505 ^{bc}	3875 ^{b-d}
T ₂	44.2 ^{cd}	2517 ^{bc}	3900 ^{a-d}
T ₃	46.9 ^{bc}	2539 ^{bc}	3901 ^{a-c}
T ₄	56.0 ^a	2606 ^{abc}	3954 ^{a-c}
T ₅	47.6 ^b	2552 ^{abc}	3907 ^{a-c}
T ₆	55.9 ^a	2589 ^{abc}	3933 ^{a-c}
T ₇	56.1 ^a	2655 ^{ab}	3970 ^{ab}
T ₈	58.7 ^a	2693 ^a	3977 ^a
T ₉	43.1 ^d	2498 ^c	3868 ^{cd}
T ₁₀	47.0 ^{bc}	2544 ^{bc}	3906 ^{a-c}
T ₁₁	41.7 ^d	2467 ^c	3799 ^d
S.Em. ±	0.97	45	30

T₁- Crop Max @ 375 ml ha⁻¹ followed by Crop Max @ 375 ml ha⁻¹ T₂-Crop Max @ 500 ml ha⁻¹ followed by Crop Max @ 500 ml ha⁻¹ T₃-Crop Max @ 625 ml ha⁻¹ followed by Crop Max @ 625 ml ha⁻¹ T₄-Crop Max @ 750 ml ha⁻¹ followed by Crop Max @ 750 ml ha⁻¹ T₅- Crop Max @ 375 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₆-Crop Max @ 500 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₇-Crop Max @ 625 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₈-Crop Max @ 750 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₉- Biozyme @ 625 ml ha⁻¹ followed by Biozyme @ 625 ml ha⁻¹ T₁₀- Control-1 (Urea @ 2% and KNO₃ @ 1%) T₁₁-Control-2 (water spray)

Means followed by the same alphabet (s) within a column are not significantly differed by DMRT (P=0.05)

DAS- Days after sowing

Note 1. RDF: FYM @ 6.25 t + 40:80:25 kg N: P₂O₅ and K₂O + ZnSO₄ @ 12.5 kg + *Rhizobium* and PSB @ 1250 g each per hectare common to all treatments

Note 2. For T₁ to T₁₀, foliar application at flower initiation (25-35DAS) and at pod formation stage (45 – 50 DAS).

4. Conclusion

The field investigation establishes that the strategic foliar application of biostimulants effectively mitigates physiological constraints and enhances the yield potential of soybean. Applying Crop Max at 750 ml/ha during flower initiation, followed by Biozyme at 625 ml/ha during pod formation (Treatment T₈), proved to be the most optimal regimen. This specific combination significantly improved vegetative parameters (plant height, leaf area), maintained superior cellular turgidity (RLWC), and decisively augmented the final seed and haulm yields by optimizing source-to-sink assimilate partitioning. The adoption of this biostimulant schedule presents a highly productive and sustainable strategy for soybean farmers operating in the Northern Transition Zone of Karnataka.

Authors' contribution

G. Somanagouda: Principal Investigator of the project, supervised the research work and monitored preparation of the manuscript; Aishwarya Golshetti: Carried out the study including collection of samples and analysis of relevant properties of soil and water, structured the manuscript; Ruksar: helped in statistical analysis of the data.

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Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: The authors have followed and complied with the National/International Guidelines of relevant and appropriate authority for the use of plants or animals or humans in the experimental study.

Conflict of Interest: The authors declare that there is no conflict of interest.

Data availability: Available from the author as well as the report of the project

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