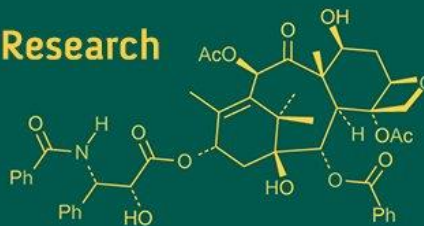


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Aishwarya Golshetti
Department of Agronomy,
College of Agriculture V. C.
Farm Mandya, Karnataka,
India

G Somanagouda
All India Coordinated
Research Project on Soybean,
MARS, University of
Agriculture Sciences, Dharwad,
Karnataka, India

Corresponding Author:
Aishwarya Golshetti
Department of Agronomy,
College of Agriculture V. C.
Farm Mandya, Karnataka,
India

Impact of foliar biostimulants on yield dynamics, quality traits, nutrient uptake, and post-harvest soil status in soybean

Aishwarya Golshetti and G Somanagouda

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Abstract

A field experiment was conducted during the kharif 2021 season in the Northern Transition Zone of Karnataka to investigate the effect of foliar biostimulants on yield parameters, nutritional quality, plant nutrient uptake, and post-harvest soil properties of soybean (*Glycine max* L.). The study utilized a Randomized Complete Block Design (RCBD) with 11 treatments replicated three times, focusing on sequential applications of 'Crop Max' and 'Biozyme' at flower initiation and pod formation stages. The results demonstrated that the sequential 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 maximized productivity. This treatment recorded the highest number of pods per plant (58.7), test weight (9.23 g), and final seed yield (2693 kg/ha). Furthermore, it yielded the maximum protein (1010 kg/ha) and oil (528 kg/ha) outputs, supported by significantly higher crop uptake of nitrogen (209.2 kg/ha), phosphorus (19.77 kg/ha), potassium (73.36 kg/ha), and sulfur (4.07 kg/ha). Post-harvest soil analysis revealed lower residual available nutrients in T₈ plots, corroborating the enhanced extraction efficiency of the crop. These findings confirm that targeted biostimulant application optimizes nutrient assimilation, thereby improving the yield and quality of soybean under rainfed conditions.

Keywords: Soybean, biostimulants, crop max, biozyme, nutrient uptake, yield

1. Introduction

Soybean [*Glycine max* (L.) Merrill] is globally recognized for its exceptional nutritional profile, simultaneously serving as a premier pulse and oilseed crop. The grain typically contains 40-42% high-quality protein and 20% cholesterol-free oil, making it indispensable for human nutrition, livestock feed, and industrial processing. However, realizing the maximum genetic yield and nutritional potential of soybean in field conditions is frequently hindered by nutritional imbalances and environmental stresses during critical reproductive stages.

While conventional macro-fertilization supplies foundational nutrients, the crop's ability to partition these elements efficiently into the developing pods often becomes a limiting factor. This limitation has driven research into plant biostimulants—formulations comprising marine algae extracts, protein hydrolysates, humic substances, and fermentation metabolites. Unlike synthetic fertilizers, biostimulants function by modulating plant metabolism, enhancing root exudation, and up-regulating genes associated with nutrient transport and assimilation (Calvo *et al.*, 2014; Ertani *et al.*, 2013) [3, 5].

Marine algae-based biostimulants, in particular, are rich sources of naturally complexed trace elements (Zn, Fe, Mn, Cu) and auxin/cytokinin precursors. Evidence suggests that foliar application of these extracts during flowering and pod development minimizes floral abscission, enhances sink capacity, and improves the biochemical synthesis of proteins and lipids within the seeds (Rathore *et al.*, 2009; Zodape *et al.*, 2010) [12, 15]. 'Crop Max' (a composite of fermentation metabolites and marine algae) and 'Biozyme' (an *Ascomyces* nodosum extract) are commercial biostimulants noted for their metabolic enhancement properties.

While previous studies have largely focused on the vegetative and physiological impacts of these products, comprehensive assessments linking yield dynamics with plant nutrient uptake, seed quality (protein/oil), and the corresponding post-harvest soil nutrient status remain limited.

Therefore, the present investigation was designed to critically evaluate the influence of sequential biostimulant applications on the yield attributes, nutritional quality, elemental uptake, and soil chemical properties in a soybean cropping system.

2. Materials and Methods

2.1 Experimental Site Details

The experiment was conducted at the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, during the kharif 2021 season. The soil was a black clay Vertisol. Initial soil chemical analysis indicated a neutral pH (7.1), normal electrical conductivity (0.32 dS/m), and low organic carbon (0.44%). The baseline available nutrient status was 263 kg/ha for nitrogen, 30.5 kg/ha for phosphorus, 366 kg/ha for potassium, and 5.9 kg/ha for sulfur.

2.2 Treatments and Crop Husbandry

The study adopted a Randomized Complete Block Design (RCBD) with 11 treatments and three replications. The treatments evaluated single and combined sequential applications of Crop Max and Biozyme at flower initiation (25-35 Days After Sowing) and pod formation (45-50 DAS). Key treatments included optimal concentrations of Crop Max (375 to 750 ml/ha) followed by either Crop Max or Biozyme (625 ml/ha), alongside a urea/KNO₃ foliar control and a water spray control.

Soybean seeds (MACS-1188) were dibbled at a spacing of 30 cm x 10 cm. A uniform basal application 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 into all plots. Seeds were inoculated with Rhizobium and PSB before sowing.

2.3 Analytical Procedures and Data Collection

At harvest, five randomly selected plants per plot were assessed for yield parameters (pods per plant, test weight, seed yield). Quality parameters were determined from the harvested seeds. Seed protein content was calculated by estimating nitrogen via the micro-Kjeldahl method and

multiplying by a factor of 6.25. Seed oil content was quantified non-destructively using a Nuclear Magnetic Resonance (NMR) spectrophotometer. Protein and oil yields (kg/ha) were subsequently derived.

For nutrient uptake, plant samples (grain and stover) were digested in a di-acid mixture. Total nitrogen was determined by the micro-Kjeldahl method, phosphorus by the Vanado-Molybdo-Phosphoric yellow color method (spectrophotometry), potassium via flame photometry, and sulfur by the turbidimetric method. Post-harvest soil samples (0-30 cm depth) were analyzed using standard procedures: pH and EC (1:2.5 soil-water suspension), organic carbon (Walkley and Black), available nitrogen (alkaline permanganate), available phosphorus (Olsen's), available potassium (neutral normal ammonium acetate), and available sulfur (turbidimetry). Data were analyzed using Fisher's ANOVA, with mean separation via Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1 Yield Attributes and Final Productivity

The application of biostimulants profoundly influenced the reproductive phase and final yield of soybean (Table 1). Treatment T₈ (Crop Max @ 750 ml/ha at flower initiation followed by Biozyme @ 625 ml/ha at pod formation) recorded the highest number of pods per plant (58.7) and test weight (9.23 g), which were significantly superior to the water spray control (T₁₁) which recorded 41.7 pods and an 8.30 g test weight. The exogenous supply of complexed trace elements and phytohormone precursors during peak reproductive phases minimizes flower drop and facilitates efficient photosynthate mobilization into the developing pods (Aslam, 2019; Pal *et al.*, 2015) [1, 10].

Consequently, T₈ registered the maximum seed yield (2693 kg/ha) and haulm yield (3977 kg/ha), marking a substantial improvement over the control (2467 kg/ha and 3799 kg/ha, respectively). This yield enhancement is attributed to the synchronized application of metabolic enhancers that match the crop's heightened nutritional demand during grain filling (Singh, 2013) [13].

Table 1: Effect of biostimulants on yield attributes and yield of soybean

Treatments	Pods/Plant	Test Wt. (g)	Seed Yield (kg/ha)	Haulm Yield (kg/ha)	Biological Yield (kg/ha)
T ₁	43.8 ^d	8.50 ^{ab}	2505 ^{bc}	3875 ^{b-d}	6380 ^{cd}
T ₂	44.2 ^{cd}	8.70 ^{ab}	2517 ^{bc}	3900 ^{a-d}	6417 ^{cd}
T ₃	46.9 ^{bc}	8.87 ^{ab}	2539 ^{bc}	3901 ^{a-c}	6440 ^{b-d}
T ₄	56.0 ^a	9.07 ^{ab}	2606 ^{abc}	3954 ^{a-c}	6559 ^{a-c}
T ₅	47.6 ^b	8.93 ^{ab}	2552 ^{abc}	3907 ^{a-c}	6459 ^{b-d}
T ₆	55.9 ^a	9.00 ^{ab}	2589 ^{abc}	3933 ^{a-c}	6522 ^{a-c}
T ₇	56.1 ^a	9.13 ^a	2655 ^{ab}	3970 ^{ab}	6625 ^{ab}
T ₈	58.7 ^a	9.23 ^a	2693 ^a	3977 ^a	6670 ^a
T ₉	43.1 ^d	8.53 ^{ab}	2498 ^c	3868 ^{cd}	6366 ^{cd}
T ₁₀	47.0 ^{bc}	8.93 ^{ab}	2544 ^{bc}	3906 ^{a-c}	6450 ^{b-d}
T ₁₁	41.7 ^d	8.30 ^b	2467 ^c	3799 ^d	6266 ^d
S.Em.±	0.97	0.23	45	30	61

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 Nutritional Quality Parameters

Biostimulants influenced the biochemical composition of the seeds, reflecting in the protein and oil yields (Table 2). While the percentage of protein and oil content showed numerical variations (peaking at 37.44% and 19.6% respectively in T₈), the differences in absolute percentages were non-significant. However, due to the significant variations in total seed yield, the calculated protein and oil yields per hectare demonstrated significant differences.

Treatment T₈ achieved the highest protein yield (1010 kg/ha) and oil yield (528 kg/ha). The application of marine algae extracts supplies readily available amino acids and sulfur, which act as precursors in lipid biosynthesis and protein assimilation. These findings parallel the observations of Mahajan *et al.* (2017) [8] and Kocira (2018) [7], highlighting the role of biostimulants in upgrading the nutritional output of leguminous crops.

Table 2: Quality parameters of soybean as influenced by biostimulants

Treatments	Protein (%)	Protein Yield (kg/ha)	Oil (%)	Oil Yield (kg/ha)
T ₁	35.17 ^a	879 ^{a-c}	18.4 ^a	459 ^{ab}
T ₂	36.33 ^a	925 ^{a-c}	18.2 ^a	464 ^{ab}
T ₃	36.81 ^a	935 ^{a-c}	18.5 ^a	472 ^{ab}
T ₄	37.31 ^a	974 ^{ab}	19.7 ^a	513 ^{ab}
T ₅	37.06 ^a	928 ^{a-c}	18.6 ^a	466 ^{ab}
T ₆	37.13 ^a	962 ^{a-c}	18.6 ^a	483 ^{ab}
T ₇	37.25 ^a	990 ^a	18.8 ^a	499 ^{ab}
T ₈	37.44 ^a	1010 ^a	19.6 ^a	528 ^a
T ₉	33.67 ^a	847 ^{bc}	18.5 ^a	466 ^{ab}
T ₁₀	35.75 ^a	908 ^{a-c}	18.6 ^a	472 ^{ab}
T ₁₁	33.57 ^a	828 ^c	18 ^a	444 ^b
S.E.m.±	1.38	46	0.7	21

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 Nutrient Uptake by the Crop

The total uptake of macronutrients (N, P, K, S) at harvest was significantly augmented by the biostimulant treatments (Table 3). Treatment T₈ recorded the maximum extraction of nitrogen (209.2 kg/ha), phosphorus (19.77 kg/ha), potassium

(73.36 kg/ha), and sulfur (4.07 kg/ha). The control (T₁₁) exhibited the lowest uptake for all elements. Although the biostimulants are applied in small quantities and do not serve as bulk nutrient sources, they exert a profound influence on root architecture and root hair proliferation. This expanded root surface area, coupled with increased metabolic activity, drastically improves the crop's ability to forage and assimilate nutrients from the soil matrix (Rathore *et al.*, 2009) [12].

Table 3: Total nutrient uptake (kg/ha) at harvest of soybean as influenced by the biostimulants

Treatments	Nutrient uptake (kg ha ⁻¹)			
	Nitrogen	Phosphorus	Potassium	Sulfur
T ₁	184.8 ^{abc}	15.64 ^{ef}	58.12 ^f	3.21 ^e
T ₂	188.5 ^{abc}	15.75 ^{ef}	59.55 ^f	3.41 ^d
T ₃	193.0 ^{ab}	16.23 ^{de}	62.77 ^e	3.43 ^d
T ₄	203.0 ^a	18.49 ^b	69.16 ^{bc}	3.77 ^b
T ₅	197.5 ^a	17.41 ^c	64.98 ^{de}	3.56 ^{cd}
T ₆	200.7 ^a	17.63 ^c	67.28 ^{cd}	3.64 ^{bc}
T ₇	205.8 ^a	19.16 ^{ab}	71.76 ^{ab}	3.79 ^b
T ₈	209.2 ^a	19.77 ^a	73.36 ^a	4.07 ^a
T ₉	168.6 ^{bc}	15.27 ^{fg}	55.04 ^g	3.09 ^{ef}
T ₁₀	195.7 ^{ab}	16.63 ^d	62.85 ^e	3.47 ^d
T ₁₁	162.6 ^c	14.70 ^g	54.44 ^g	2.98 ^f
S.E.m.±	8.6	0.25	0.93	0.05
Initial values	263	30.5	366	5.9

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).

3.4 Post-Harvest Soil Parameters

Post-harvest analysis of the soil chemical properties indicated no significant shifts in soil pH, electrical conductivity (EC), or organic carbon (OC) percentage among the treatments, highlighting that foliar biostimulant application does not aggressively alter the basic physicochemical structure of the soil over a single season (Table 4).

However, the available nutrient status in the soil exhibited an inverse relationship with crop uptake. The plots treated with T₈ recorded significantly lower residual availability of nitrogen (203 kg/ha), phosphorus (24.0 kg/ha), potassium (322 kg/ha), and sulfur (5.20 kg/ha). Conversely, the highest residual soil nutrients were found in the water-sprayed control plots (T₁₁). This clearly demonstrates that biostimulant-treated plants utilize soil reserves much more efficiently, leaving fewer unutilized nutrients in the soil post-harvest (Aslam, 2019) [1].

Table 4: Post-harvest soil chemical parameters and available nutrient status

Treatments	pH	EC (dS/m)	OC (%)	N (kg/ha)	P (kg/ha)	K (kg/ha)	S (kg/ha)
T ₁	7.11 ^a	0.32 ^a	0.44 ^a	258 ^{ab}	27.1 ^{bc}	338 ^{ab}	5.86 ^{ab}
T ₂	7.17 ^a	0.31 ^a	0.45 ^a	246 ^{bc}	26.8 ^{bc}	333 ^{ab}	5.69 ^{ab}
T ₃	7.22 ^a	0.31 ^a	0.47 ^a	245 ^{bc}	26.2 ^{bcd}	333 ^{ab}	5.56 ^{ab}
T ₄	7.14 ^a	0.30 ^a	0.50 ^a	229 ^c	25.8 ^{bcd}	325 ^{ab}	5.30 ^{ab}
T ₅	7.36 ^a	0.31 ^a	0.48 ^a	239 ^{bc}	25.9 ^{bcd}	327 ^{ab}	5.43 ^{ab}
T ₆	7.20 ^a	0.30 ^a	0.48 ^a	232 ^c	25.8 ^{bcd}	326 ^{ab}	5.37 ^{ab}
T ₇	7.12 ^a	0.30 ^a	0.48 ^a	225 ^c	25.1 ^{cd}	325 ^{ab}	5.27 ^b
T ₈	7.20 ^a	0.32 ^a	0.49 ^a	203 ^d	24.0 ^d	322 ^b	5.20 ^b
T ₉	7.12 ^a	0.30 ^a	0.44 ^a	272 ^a	27.8 ^b	339 ^{ab}	6.02 ^{ab}
T ₁₀	7.23 ^a	0.31 ^a	0.47 ^a	243 ^{bc}	26.1 ^{bcd}	329 ^{ab}	5.45 ^{ab}
T ₁₁	7.21 ^a	0.32 ^a	0.40 ^a	275 ^a	30.0 ^a	345 ^a	6.24 ^a
S.E.m.±	0.14	0.01	0.04	6	0.7	8	0.3
Initial values	7.1	0.32	0.44	263	30.5	366	5.9

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)

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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 integration of marine algae and fermentation metabolite-based biostimulants as foliar sprays is a highly effective agronomic strategy for soybean cultivation. The sequential application of Crop Max at 750 ml/ha at flower initiation, followed by Biozyme at 625 ml/ha at pod formation (Treatment T₈), fundamentally optimized the source-sink dynamics. This led to a maximized seed yield of 2693 kg/ha alongside exceptional protein and oil outputs. Furthermore, the enhanced metabolic activity drove superior extraction of soil nutrients, demonstrating that biostimulants significantly improve fertilizer use efficiency. This practice proves to be a sustainable and highly productive approach for soybean farmers.

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