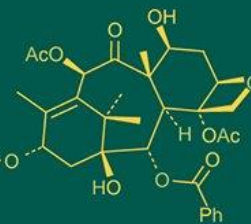
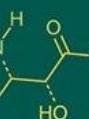
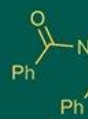


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Boosting soybean yield and economics: Unveiling the power of Biostimulants

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Abstract

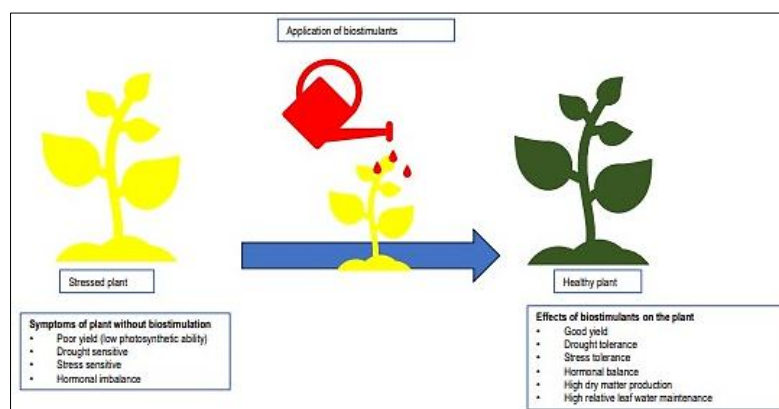
Aim: The present study aimed to augment both yield and economic outcomes by integrating common recommended fertilizer doses (RDF) with the application of biostimulants at varying concentrations.

Methodology: The study was conducted during 2021 & 2022 *Kharif* season at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, India. Randomized complete block design with three replications was employed. Statistical analyses including ANOVA, correlation coefficient, and linear regression were conducted.

Results: Among the different treatments spraying of CropMax @ 750 ml ha⁻¹ at flowering and 625 ml ha⁻¹ at pod formation stage, resulted in significantly taller plants and higher dry matter production. This is also exhibited substantial increases in leaf area and relative leaf water content at 60 DAS. Additionally, it yielded the highest seed yield (2693 kg ha⁻¹) and a benefit-cost ratio of 3.4.

Interpretation: The model aims to optimize soybean yield by considering weather impacts. Significant correlations were found between temperature, rainfall, and yield. While temperature had no impact, rainfall positively affected yield ($R^2 = 0.557$). Regression analysis underscored the importance of biostimulation in enhancing yield attributes, suggesting simultaneous improvement of these traits is achievable.

Graphical abstract



Keywords: CropMax, Biostimulant, Productivity, Returns, Soybean

1. Introduction

The cultivation of soybean presents a promising avenue for Agricultural advancement, with potential yields ranging from 3 to 4 tons per hectare. However, realizing these yields necessitates diligent monitoring and innovative approaches due to challenges such as escalating food prices, climate change, and resource constraints. Biostimulants emerge as a viable solution in this landscape, offering avenues to bolster productivity sustainably. These are also called as metabolic enhancers.

India stands 5th place for soybean production with 126 lakh MT covering on area of 121 lakh ha and productivity being 1042 kg ha⁻¹ (ICAR-IISR, 2023) [13]. In Karnataka the area, production and productivity of soybean is 4.07 lakh ha, 3.80 lakh MT and 950 kg ha⁻¹,

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respectively (SOPA, 2023) ^[42].

Biostimulants are not fertilizers meant to correct a severe nutrient deficiency, but are mixtures of one or more microorganisms, trace elements, enzymes, plant hormones or the seaweed extracts. Biostimulants can be used to supplement and enhance the resource use efficiency, which might lead to slight reductions in quantity of fertilizer (Kalaivanan *et al.*, 2012) ^[14] application of present agricultural practices and crop inputs. Biostimulants are the products that stimulate natural processes in the plant or around the roots to enhance nutrient uptake, increased tolerance to abiotic stress, crop quality, vigour and yield. [European Biostimulants Industry Council (EBIC)] ^[8]. Biostimulants are classified as main 5 categories including Humic substances (which include humic and fulvic acids), Plants and seaweed extracts, Peptides and amino acids and Microbial biostimulant products (fungi and bacteria) “pure strain” fermentation solutions, based on individual isolates; consortia of admixed or co-fermented isolates; or much more complex “natural” communities derived from organic matter processing and lastly some Inorganic compounds that include some chemical elements that promote plant growth (IFA).

The mode of biostimulants is panoramic and includes activation of several complex metabolisms inclusive of nitrogen fixation, photosynthesis, nucleic acid synthesis and ion uptake, respiration or mineralization of unavailable nutrients to absorbable or labile forms by which plant uptake is made easy. These trigger the microbial activity of the soil and tremendous growth of roots and root hair, as a result, the roots are reachable to site of moisture, nutrients which helps the plants to survive under stress condition. These are indirectly involved in acting against pest and diseases, by emphasizing on increasing the plant's physiology to overcome the stress situations. Biostimulants are reported to encourage and improve the plant metabolism by stimulating the germination, root growth, increased photosynthetic activity, improved accumulation and assimilation of photosynthates, enhanced absorption of nutrients and ultimately increased plant's overall productivity. To accomplish these objectives of several studies have been conducted earlier.

In recent years, extensive research has delved into the advantageous effects of natural plant biostimulants on the growth, yield, and quality of various crops. Ramarajan *et al.* (2012) ^[31] investigated the effects of seaweed liquid fertilizer (SWE) on soybean germination, observing significant increases in shoot and leaf length with *ulva lactuca* and improved leaf width, number of leaves, and root length with *sargassum wightii*, attributed to the presence of growth hormones. The concentration 15 mL L⁻¹ biostimulant (*Ascophyllum nodosum*) showed the best results for percentage and index of germination and the lowest mean germination time and increase plant height and fresh and dry mass of shoots, SWE (*A. nodosum*) increased RWC, fresh weight, and dry weight in spinach (*Spinacia oleracea* L.) (Santos *et al.*, 2019) ^[37]. Leaf area is considered to be one of the photosynthetic determinants in crop plants a, application of PEPTO resulted in higher Leaf area index (Baradhan *et al.*, 2019) ^[3]. Kamalovna *et al.* (2020) ^[15] demonstrated the beneficial effects of the VL-77 biostimulator on sunflower growth, resulting in larger plants with increased leaf numbers. Bhuvaneswari and Dhanasekaran (2007) ^[4] explored the impact of soil

application of humic acid (HA) derived from lignite on radish growth, observing significant improvements in leaf, root, and shoot length. Rose *et al.* (2014) ^[34] found significant increases in shoot dry weights and dry matter contents for various crops with the application of humic substances and K-Humate. Tandon and Dubey (2015) ^[43] conducted an experiment to study effect of Biozyme (*Ascophyllum nodosum*) biostimulant on growth and development of soybean observed that with use of Biozyme™ crop-plus spray @ 500ml ha⁻¹ + half recommended NPK recorded significantly higher grain yield, straw yield and biological yield. Meshram (2013) ^[25] discussed the synergistic effects of integrated nutrient management and biostimulants on soybean growth and yield. They found that treatments with Kappaphycus-sap and Gracilaria-sap significantly increased various yield parameters compared to the control, possibly due to the presence of growth-promoting hormones. Sharma (2012) ^[39] studied the effects of different doses of IB4WT Shakambhari on soybean yield attributes, observing increased pod and seed characteristics, grain, and straw yield attributed to rapid nitrogen mineralization from organics. Investigation from Karthikeyan and Shanmugam 2015, revealed the efficacy of SLF (seaweed liquid fertilizer) on peanut and sunflower crops, observing substantial improvements in growth and yield. Also illustrated the stimulatory effects of foliar application of AQUASAP on peanut and sunflower crops. Additionally, application of specific plant biostimulants may reduce fertilizer use and nutrient solution concentrations. Additionally, Sangeetha and Singaram (2007) ^[36] observed significant improvements in onion growth and dry matter with soil application of humic acid compared to foliar spray. Lucini *et al.* (2015) ^[23] observed increased yield and dry weight in lettuce upon the application of plant-derived protein hydrolysate, particularly noteworthy for its role in enhancing tolerance to salinity. Poudineh *et al.* (2015) ^[29] highlighted the potential of humic acid and folic acid application in enhancing soybean yield and its components. Nasreen *et al.* (2012) ^[27] emphasized the significance of soil proteases and urease in enhancing plant nutrition through the breakdown of proteinaceous substances and urea hydrolysis. Singh (2013) ^[41] evaluated the efficacy of seaweed sap on soybean productivity, reporting higher grain yield, straw yield, and harvest index with specific treatments, attributed to the higher concentration of plant hormones. Teli *et al.* (2020) ^[45] investigated the impact of soil and foliar application of humic acid on groundnut productivity, finding notable improvements in several yield parameters linked to enhanced nutrient absorption and physiological processes. Mahmoud *et al.* (2011) ^[24] reported increased soybean growth and yield with humic acid application. Arjumend *et al.* (2015) ^[2] documented enhancements in pepper and wheat growth and yield with humic acid application, also reported enhancements in wheat yield components following humic acid application. Further “Fertileader Gold” biostimulant can be beneficial to improve growth, development and total yield of inoculated sunflower (Glijin *et al.*, 2013) ^[10]. It was observed that the biostimulator Microzym-2 was more effective when used in combination with mineral fertilizers than when used in its pure form (Bozarovich *et al.*, 2021) ^[5].

However, results indicate that application of these biostimulants generally enhances plant growth, yield, and

quality attributes. Optimum doses of biostimulants have been identified for specific crops, with varying responses observed in different growth stages and under different environmental conditions. Additionally, biostimulants have shown potential in improving plant tolerance to stresses (Rouphael and Colla, 2020; Kocira *et al.*, 2019) [35, 21] such as salinity, antioxidant (Teixeira *et al.*, 2017; Kocira *et al.*, 2018) [44, 20], water stress (Mitra *et al.*, 2013) [26] and biotic (e.g., bacterial and fungal pathogens) (Pellegrini *et al.*, 2020) [28]. The studies highlights the multifaceted effects of biostimulants on biochemical, physiological, morphological, and ecological levels, supporting the hypothesis that biostimulants can enhance seed yield.

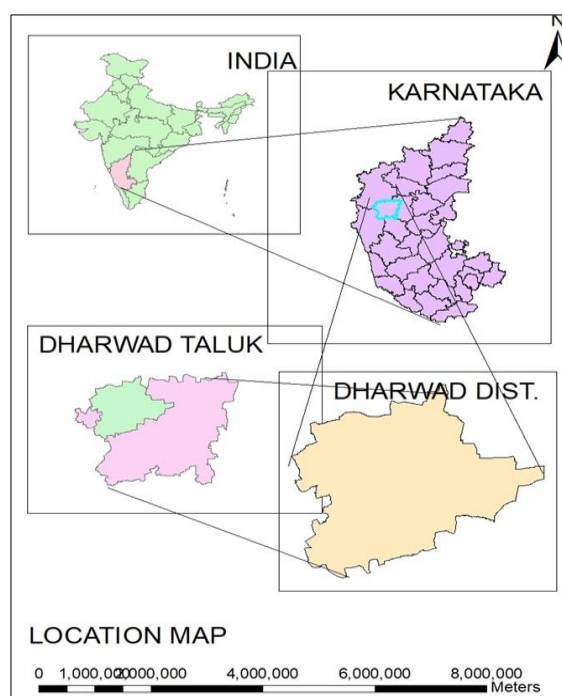
The collective findings highlight the promising potential of natural biostimulants in improving both crop productivity and quality, providing sustainable pathways for agricultural enhancement. Therefore, this study aimed to investigate the significance of biostimulants in enhancing soybean yield and economic returns. The objective was to augment both yield and economic outcomes by integrating common

recommended fertilizer doses (RDF) with the application of biostimulants at varying concentrations.

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2. Materials and Methods

2.1 Experimental site and conditions



The present study was conducted during 2021 & 2022 *Khari* season at the experimental Farm of the Agriculture at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, India. Experiment location was situated at 15° 29' 51.2" N latitude, 74° 59' 19.9" E longitude and at an altitude of 678 m above mean sea level. Soil samples (0 to 30 cm) were taken before sowing and analyzed for soil parameters. The physico-chemical properties of soil are given in the table 1.

2.2 Experimental material

The soybean cultivar used in this study was MACS 1188. This variety was recommended for the South zone across India, which matures in 100-105 days and contains high oil content and yield potential, reaching up to 2,475 kg ha⁻¹. Notably, it is known for its resistance to pod shattering, Rhizoctonia aerial blight, and charcoal rot. The biostimulants used for the trial were CropMax and Biozyme (Confer Fig. 1) and the composition of the CropMax is given in the table 3.

2.3 Experimental design and treatments

The experiments were arranged in a randomized complete block design, comprising three replications. Each experimental replication was consisted of 11 rows, each measuring 3.6m×6 m, covering an area of 21.60m². The plots were separated by ditches with a width of 0.5m. Throughout the growing seasons, the crop received a total of 402 mm of precipitation, distributed uniformly. Every plot received a recommended dose of fertilizer (RDF), including N: P₂O₅: K₂O @ 40:80:25 kg ha⁻¹. Additionally, each plot was treated with FYM @ 6.25 t ha⁻¹, ZnSO₄ @ 12.5 kg ha⁻¹, and biofertilizers, specifically Rhizobium and PSB (Phosphate Solubilizing Bacteria), each applied at a rate of 1250 g ha⁻¹.

2.4 Cultural practices

The seasonal experiment commenced on June, 2021 & 2022. Two seeds were dibbled at each spot to a depth of 2.5 to 3 centimeters, spaced at 30cm×10cm, resulting in a plant

density of 333,333 plants ha⁻¹. Adequate soil moisture facilitated proper germination. Seedlings were thinned out at 21 days after sowing (DAS) to maintain an optimal population and minimize intra-row competition. All cultural operations were conducted uniformly and in accordance with standard practices, except for the application of

biostimulants. The first treatment application commenced at 30 DAS, and the specific concentrations of biostimulant application are provided in Table 2. The initial crop harvest took place on September (gross plot - 101 DAS), followed by the subsequent harvest of plants from the net plot on day 102 after sowing.



Fig 1: The products used in the experimentation

2.5 Statistical analysis

The analysis of individual characteristics' data utilized the mean values from three replications. The collected data underwent a proper statistical analysis of variance (ANOVA) using a randomized complete block design, outlined by Gomez and Gomez (1984) [11]. Assumptions underlying the ANOVA were verified. Data from one year was assessed for homogeneity through randomization and were found to be homogeneous. When the F-test for the effect of biostimulants treatments was significant at a 5% probability level, Duncan's multiple range test (1955) [7] was employed to identify statistical differences among treatment means.

Data from kharif season concerning growth and yield per hectare and its attributes underwent simple correlation coefficient and simple linear regression analysis, in line with the methodology of Sendecor and Cochran (1989) [38]. Stepwise multiple linear regression analysis, as outlined by Draper and Smith (1998) [6], was utilized to identify the most influential variables in explaining the variance in seed yield. The relative contribution was quantified as the coefficient of determination (R²). Finally, statistical evaluation was conducted using the MS Excel statistical program version 2402 and R software version 4.3.3.

3. Results and Discussion

3.1 Growth attributes

Plant height is influenced by hormonal activity such as balance of auxin and cytokinin during the crop's early stages, as well as environmental factors such as rainfall, temperature maximum and minimum, relative humidity and wind speed and wind direction. Statistical analysis of the data showed that biostimulant treatments had significant ($p = 0.01$) as illustrated in Fig. 2 (a). At 60 DAS the taller plants were recorded in the T₈ and it was found on par with T₇, T₆ and T₄. At harvest there was slight increase in the

height of the plant compared to 60 days, and was recorded maximum in the T₈ and found on par with T₇, T₆, T₄ and T₅. The shorter plants were recorded in the control plots at 60 DAS and at harvest. There was no notable impact on plant height observed at 30 days after sowing (DAS). The impact of biostimulants on plant height highlights that the crop reaches its maximum height with higher concentrations during flowering, while Biozyme exhibits the greatest effect during pod formation (T₈) compared to other treatments, Dry matter production is a crucial indicator of a plant's growth and productivity, reflecting its capability to convert resources such as water, nutrients, and sunlight into organic matter. It was clear from the Fig. 2 (b) that effect of biostimulant on the dry matter production of soybean was significantly higher with higher concentration at flower initiation and even at pod formation (T₈) and it was found at par with T₇, T₆, T₄ and T₅ at harvest and at 60 DAS.

Leaf area at 60 DAS was found significant. To contemplate the leaf area, the biostimulated plants exhibited enormous increase unlike in the control plants. In comparison to both the control group and biostimulated plots with lower concentrations, there was 18.2, 11.04, 11.04, 10.4, 7.3 and 4.6% increase to control, T₉, T₁, T₂, T₁₀ and T₃, respectively. Significantly higher plant height, leaf area at 60 DAS and dry matter production (at 60DAS and at harvest) (Fig. 2 a and b) was mainly due to the presence of adequate amount of nutrients provided through biostimulation in the form of droplets (spray solution), the biostimulants contains not only the nutrients (table 3) but also certain amounts of beneficial hormone in a balanced ratio, which aids in better plant growth leaf area expansion and dry matter production. The basic nutrient demand is quenched by the basal application but once those nutrients are subjected to different losses (fixation, runoff, volatilization, leaching below effective crop root zone etc.), might be replaced through biostimulation. The treatment receiving the CropMax

followed by the Biozyme perform better compared to the Biozyme or CropMax alone, which shows that CropMax's performance was incredible at earlier growth stages compared to later stages and Biozyme's effect was least caused during initial stages and same was better after the pod formation.

Foliar nutrition of Cop Max (which is a product containing fermentation metabolites, selected marine algae extracts, protein hydrolysates with complexed or chelated micronutrients) to the growing plants results in increasing the cell division, cell multiplication and elongation ultimately recorded taller plants, due to the presence of phytohormones and growth regulators in different seaweed algae and marine algal extracts in different concentrations viz. Auxins, Gibberellins, Cytokinins (Kalaivanan *et al.*, 2012) ^[14] etc. and the precursors of the phytohormones simulates growth, flower initiation, root length and root biomass when applied externally (Pramanick *et al.*, 2013) ^[30]. These results are also line with the findings of Zhang and Ervin (2008) ^[47], Aslam (2019) and Vishwanatha *et al.* (2022) ^[46].

The increased growth attributed to Crop Max foliar nutrition along with RDF likely stems from enhanced nutrient availability, particularly Mg^{+2} and Mn^{+2} . These nutrients play vital roles in photosynthesis, root growth, nitrogen fixation, and enzyme activation, essential for chlorophyll formation and nitrate reduction. Additionally, trace elements like Fe, Cu, and Zn (Kalaivanan *et al.*, 2012) ^[14] in seaweed extracts reduce redox reactions (Pramanick *et al.*, 2013) ^[30], promoting enzyme activity and facilitating photosynthate transfer. This encourages meristematic cell division and expansion (Hussein *et al.*, 2019) ^[12], positively influencing leaf area. These results are in line with the findings of Shyamrao *et al.* (2015) ^[40], increased leaf area of sunflower seedlings was due to synergistic effect of combined treatment of Mg + Main grain extract (Rehman *et al.*, 2018) ^[33], the combination of Microzyme-2 along with the fertilizers increased the leaf area (Bozarovich *et al.*, 2021) ^[5].

The observed increase in dry matter accumulation may be attributed to augmented leaf area and leaf count, facilitating greater light interception. The plant dry weight increased with the increase the spraying concentrations (Khalaf and Assal, 2021) ^[16]. This sustains chlorophyll levels and boosts photosynthesis, ultimately bolstering dry matter accumulation. Elevated chlorophyll content enhances photosynthetic efficiency, thereby fostering greater dry matter accumulation and providing the foundation for increased yields. Similar findings were reported by Kuttimani and Velayutham (2011) ^[22], Kavipriya *et al.*, (2011) ^[17], Singh (2013) ^[41], and Vishwanatha *et al.*, (2022) ^[46].

3.2 RLWC (%) (relative leaf water content) (Fig. 2 (d))

At 30 days after sowing (DAS), there were no significant differences in relative leaf water content. However, the control-1 (T₁₀) exhibited a numerically higher relative leaf water content of 82.26%. By 60 DAS, T₈, resulted in significantly higher relative leaf water content at 84.77%. This was comparable to the T₇ with 83.49%, T₄ with 83.1%, and control-1 with 82.9%. The T₁₁ exhibited the significantly lowest relative leaf water content of 79.91%.

3.3 Yield attributes

The study revealed a significant impact of biostimulation on test weight. In the control group lacking biostimulation, the seed index measured 8.3 grams. Conversely, in the biostimulated variants, a notable increase in the weight of 100 seeds was observed. Specifically, with a biostimulation treatment of 750 ml ha⁻¹ +625 ml ha⁻¹, the weight of 100 seeds reached 9.23 grams, marking an 11% increase compared to the omitted treatment. To contemplate that the seed plant⁻¹ was observed significant in the biostimulation plants compared to omitted plants and it was noted to be on par with T₄ T₇ and T₆. The biostimulated plants exhibited a 16.4% increase in the seed plant⁻¹ compared with the biostimulation omitted plants. Comparable trend was observed in case of number of pods plant⁻¹, compared with the control there was 40.7% increase of number of pods plant⁻¹ and was found notably at par with T₄ T₇ and T₆.

The spraying of biostimulants might have increased the source capacity of the leaves thus, enhancing the supply of assimilates for pod filling. The synergistic effect of biostimulants might have increased the yield attributes resulting in the hike in the productivity per plant which led to and increased seed and haulm yield. These findings are in conformity with Rawheya *et al.* (2008) ^[32] and Kocira (2017) ^[19].

3.4 Seed yield (kg ha⁻¹)

Seed yield is a complex trait influenced by various factors, including environmental conditions, morphology, and physiological characteristics. Analysis of Variance (ANOVA) reveals that the seed yield of soybean is significantly impacted by biostimulant treatments, as depicted in Figure 2 (d). The 11 treatments exhibited varying effects on soybean seed yield (kg ha⁻¹). The highest seed yield (2693 kg ha⁻¹) was observed for Treatment 8 (750 ml ha⁻¹ of crop max and 625 ml ha⁻¹ of Biozyme), where biostimulants were applied during flowering and pod formation stages. In contrast, the lowest yield (2467 kg ha⁻¹) was recorded for Treatment 11 (no biostimulant), which omitted biostimulants during flower initiation and pod formation. The highest seed yield (2693 kg ha⁻¹) in Treatment 8 was 9.16% higher than that of the control treatment.

Seed yield is greatly affected by growth parameters. Stress during the crop's growth stages can significantly diminish seed yield. The application of nutrients based on the plant's needs influences its growth patterns. If the plant receives an ample amount of nutrients during its vigorous growth phase, it can achieve its maximum yield potential.

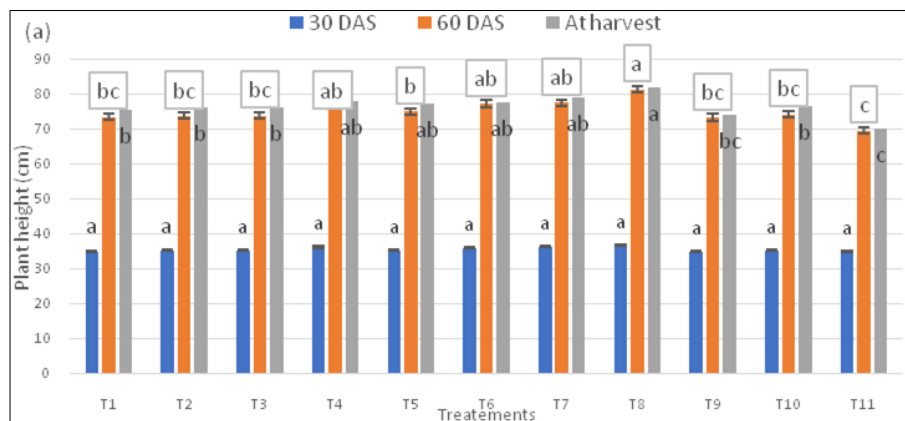
Foliar application of crop max increases the photosynthesis, improves the uptake and absorption of nutrients through the efficient root system which might have led to the increased in the yield attributes, grain yield and haulm yield of soybean. Biostimulants are involved in up-regulation of genes linked to nutrient metabolism, nutrient uptake and photosynthesis (Ertani *et al.*, 2017) ^[9], which are involved in plant response to stresses at crucial stages thus continuous supply of nutrients ultimately, increasing the yield attributes. Similar results were obtained by Meshram (2013) ^[25] in soybean, Pramanick *et al.* (2013) ^[30] in greengram and Kocira (2017) ^[19] in soybean. Seaweed extracts likely promote flowering by stimulating plant growth, with cytokinins contributing to increased yield (Khan *et al.*, 2009) ^[18].

3.5 B:C ratio

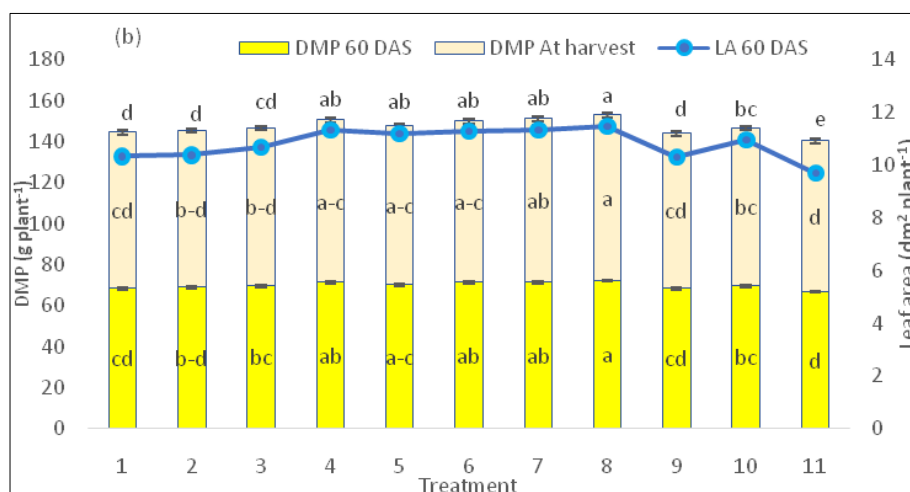
A notably higher B:C ratio of 3.4 was recorded when Crop Max was applied through foliar at 750 ml ha⁻¹ during flower initiation, followed by Biozyme at 625 ml ha⁻¹ during pod formation (T₈). This result was comparable to applying Crop Max at 625 ml ha⁻¹ during flower initiation followed by Biozyme at 625 ml ha⁻¹ during pod formation (T₇) and Crop Max at 500 ml ha⁻¹ during flower initiation followed by Biozyme at 625 ml ha⁻¹ during pod formation (T₆) which

yielded a BC ratio of 3.35.

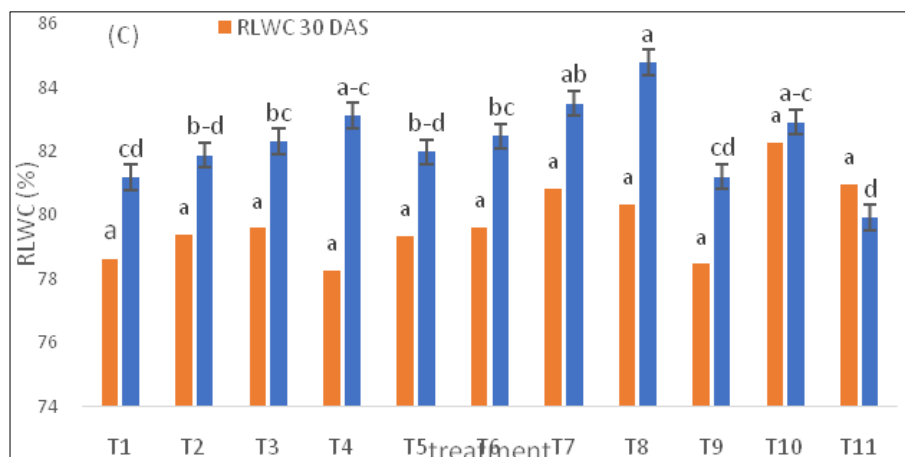
Significantly BC ratio (3.4) was observed with foliar application of Crop Max @ 750 ml ha⁻¹ at flower initiation followed by Biozyme @ 625 ml ha⁻¹ at pod formation stage (T₈) and was on par with the foliar application of Crop Max @ 625 ml ha⁻¹ at flower initiation followed by Biozyme @ 625 ml ha⁻¹ at pod formation (T₇) stage (3.35) (Fig. 9). This was due to higher gross returns and lesser cost incurred in the crop husbandry.



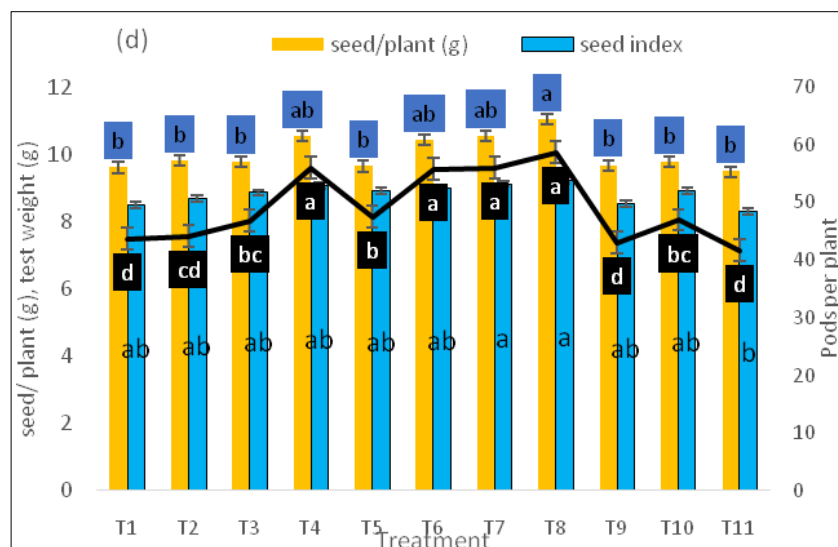
Means followed by common letters are not significantly different from each other according to Duncan's multiple range test (DMRT).



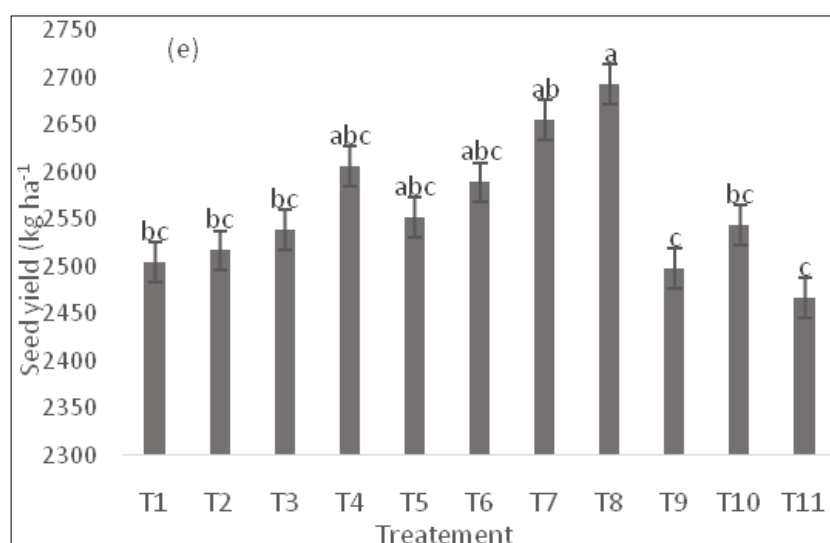
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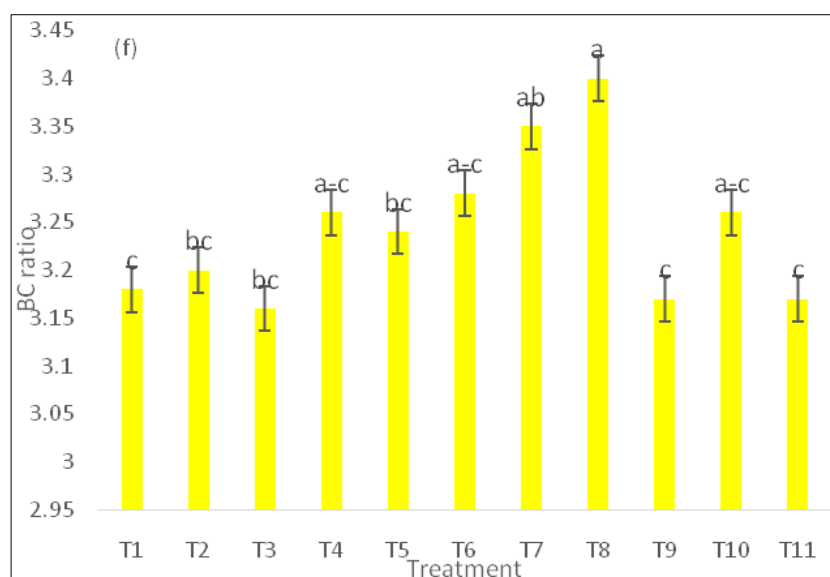
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Fig 2: influence of biostimulants on the (a) plant height (cm), (b) dry matter production (g plant⁻¹), (c) RLWC (%), (d) yield attributes, (e) yield (kg ha⁻¹) and (f) BC ratio of soybean.

Correlation and regression analysis

To assess the model's performance under varying temperature and rainfall conditions, the aim is to prevent critical growth stages from coinciding with adverse weather conditions, thereby minimizing yield losses. The correlation between maximum and minimum temperatures, rainfall (x), and soybean yield (y) was found to be significant at the 0.05 level of significance, as indicated in Table 6. However, the relationship was deemed nonsignificant in some cases.

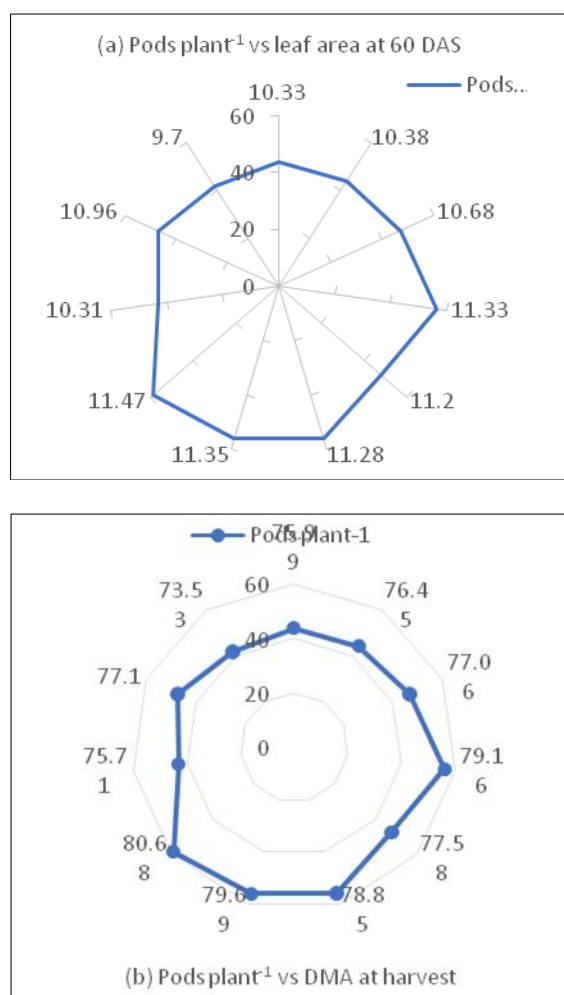
The model's analysis of maximum and minimum temperatures (a and b) reveals a negative correlation with soybean seed yield, indicating that temperature variations did not significantly impact seed yield and there is no significant rate of change of seed yield with every raise of fall in the temperature. Consequently, the null hypothesis was accepted. However, rainfall during the *Kharif* season demonstrated a significant positive correlation with seed yield at $P < 0.05$, leading to the rejection of the null hypothesis concerning rainfall's effect on seed yield and there is significant rate of change of yield with every mm change of the rainfall ($R^2 = 0.557$). It can be noted from the table (4) that the increase or decrease in the temperature coupled with the higher concentration of the biostimulation, that there is linear decline in the seed yield.

Regression analysis (Confer Table 5 and Fig. 4) revealed that the most suitable model for describing the response was a linear regression for both pods per plant and seed yield, influenced by biostimulation, which led to an increase in leaf area and dry matter production.

The relationship between pods per plant and biostimulation effect on leaf area at 60 DAS was described by the equation pods per plant = $9.7207x - 55.969$, with an R^2 value of 0.8026, significant at $p \leq 0.05$. Similarly, the relationship between pods per plant and biostimulation effect on dry matter production at 60 DAS and harvest, respectively was expressed by the equation pods per plant = $3.6907x - 208.38$, $2.8827x - 174.04$, with an R^2 value of 0.930, 0.899, significant at $p \leq 0.05$.

For seed yield, its association with biostimulation effect on leaf area at 60 DAS was described by seed yield = $106.73x + 1406$, with an R^2 value of 0.788, significant at $p \leq 0.05$. Additionally, seed yield's correlation with biostimulation effect on dry matter production at 60 DAS was illustrated by seed yield = $40.548x - 269.3$, with an R^2 value of 0.9145, significant at $p \leq 0.05$. Furthermore, the relationship between seed yield and biostimulation effect on dry matter production at harvest was depicted by seed yield = $32.508x + 43.14$, with an R^2 value of 0.9319, significant at $p \leq 0.05$. The significant linear relationships observed between yield attributes and growth parameters suggest that these traits are reliant on the concentration of biostimulants.

With increase in the leaf area at 60 DAS and dry matter production at harvest there is significant and positive increment escalate in the number of pods per plant in addition to number of pods per plant there is also a positive and significant escalate in the seed yield with increase in the growth attributes at 60 DAS and at harvest. (Figure 3 and 5).



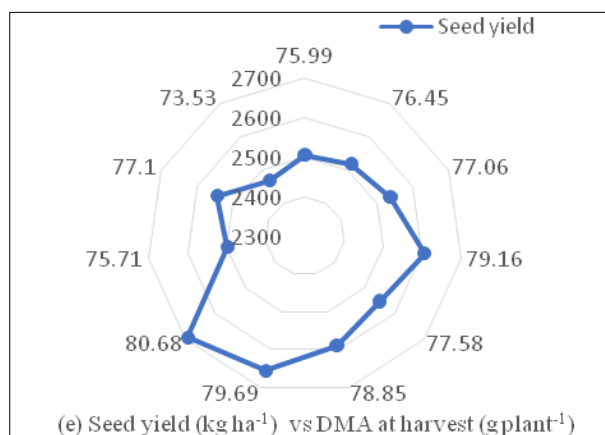
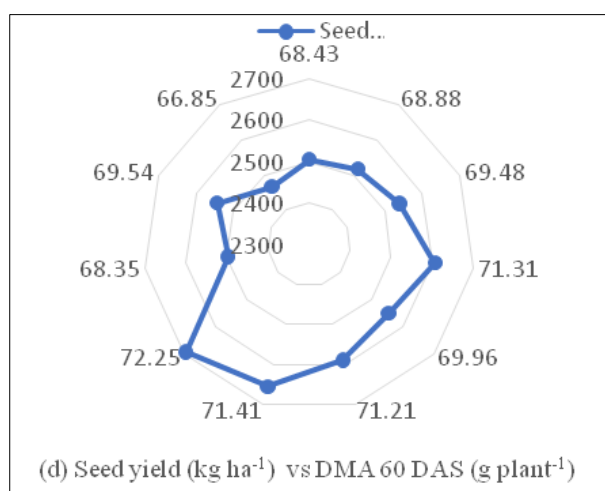
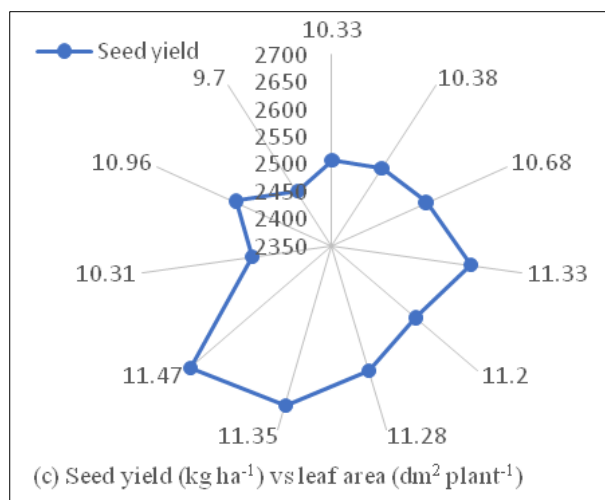


Fig 3: Representing the relationship between yield and yield attributes with growth attributes

Seed yield is substantially affected by a range of yield attributes. Any enhancement in these attributes due to treatment effect of biostimulation consistently results in a proportional increase in yield. This correlation is evident in Figure 5, there is a notable positive correlation, indicating

that any change in yield attributes significantly corresponds to changes in seed yield (kg/ha). Among the yield attributes, pods per plant demonstrate a particularly pronounced effect compared to others.

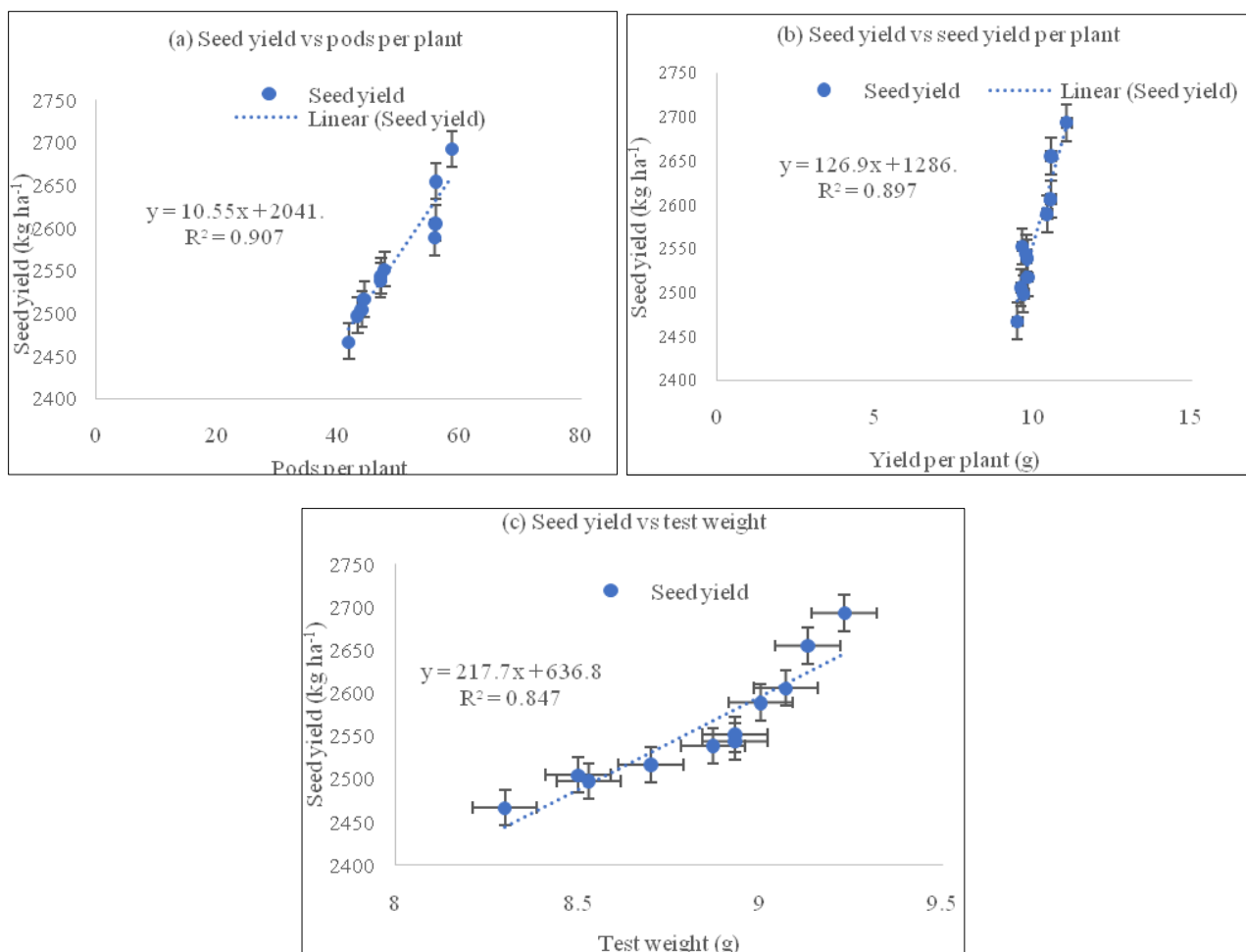


Fig 4: Mathematical model representing the relationship between yield with yield attributes (a) Seed yield vs pods per plant, (b) Seed yield vs seed yield per plant and (c) Seed yield vs test weight

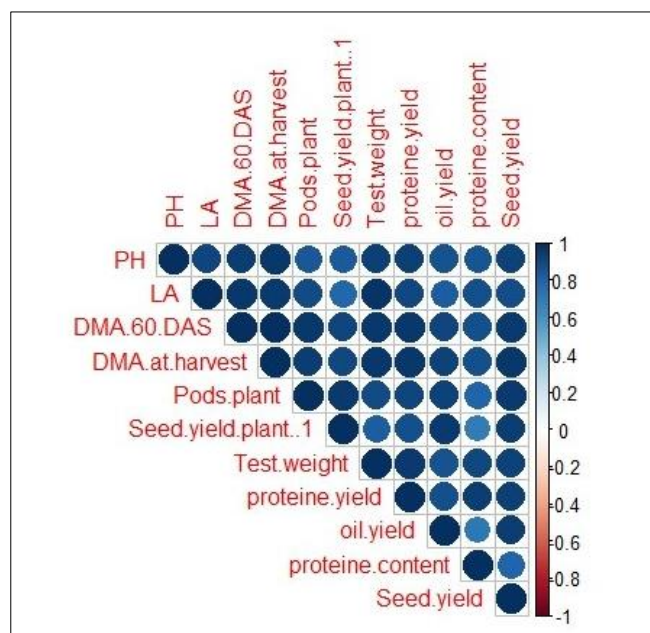


Fig 5: Simple correlation coefficients among seed yield ha⁻¹ and its related growth and yield characters in soybean

In crop plants like soybeans, seed yield is typically influenced by various physiological and morphological characteristics related to yield. Understanding the relationships between grain yield and these attributes is

crucial for identifying selection criteria to enhance soybean yields.

Significant associations were observed among the studied characters under biostimulation, as detailed in Fig. 5. The correlation coefficients provided in Fig. 5 demonstrated that seed yield exhibited positive and significant correlations with several traits: Plant height ($r = 0.921^{**}$), Leaf area (dm² per plant) at 60 DAS ($r = 0.888^{**}$), Dry matter accumulation (g plant⁻¹) at 60 DAS ($r = 0.956^{**}$), Dry matter accumulation (g plant⁻¹) at harvest ($r = 0.965^{**}$), Number of pods per plant ($r = 0.953^{**}$), Seed yield per plant ($r = 0.948^{**}$), 100-seed weight ($r = 0.921^{**}$), Protein yield (kilograms per hectare) ($r = 0.934^{**}$), Oil yield (kilograms per hectare) ($r = 0.941^{**}$) and Protein content (%) ($r = 0.793^{**}$).

The strong correlations between seed yield and these traits suggest that simultaneous improvement of these traits is achievable. These findings imply that selecting for traits such as the number of pods per plant, 100-seed weight, and seed yield per plant would likely result in increased overall yield potential.

From the correlation and regression analysis, the seed yield has exhibited significant and positive correlation with growth and yield attributes these findings are similar with Abd El-Mohsen *et al.*, (2013) [1], however showed negative correlation with temperature and positive with the rainfall. Significant linear relationship between seed yield and growth and yield attributes provides the clue that these traits are dependent upon concentration of biostimulation. From

the above-mentioned results, it could be concluded that the coefficients of determination (R^2) of 0.80, 0.93, 0.89, 0.78, 0.91 and 0.93 indicated that the biostimulation involved in this study affected the total variability of seed yield by 80, 93, 89, 78, 92 and 93%, respectively with growth attributes

(leaf area at 60 DAS and dry matter (at 60 DAS and at harvest)) and seed yield that coefficient of determination (R^2) of 0.90, 0.89 and 0.84 as influenced by the yield attributes (pods per plant seed yield per plant and test weight) by 90, 89 and 84%, respectively.



Fig 6: Crop at 60 DAS comparing with control

The study concluded that biostimulant treatments significantly influenced plant height, dry matter production, leaf area, relative leaf water content, yield attributes, and

seed yield. Treatment 8, comprising Crop Max at flowering and Biozyme at pod formation, resulted in taller plants, higher dry matter production, and increased leaf area.

Biostimulated plants showed notable enhancements in yield attributes, including test weight, seed weight, and number of pods per plant, ultimately leading to higher seed yield. The observed linear relationships between biostimulation, growth parameters, and yield attributes underscored the importance of biostimulation in optimizing soybean yield. Correlation and regression analyses further confirmed significant associations between yield attributes and growth

parameters, suggesting that improvements in these traits are achievable through biostimulation. The findings indicate that biostimulation plays a crucial role in enhancing soybean yield potential by mitigating adverse environmental effects and promoting plant growth and productivity. The study provides valuable insights into the effectiveness of biostimulation as a sustainable approach to improving crop yield and addressing global food security challenges.

Table 1: Initial physico-chemical properties of the soil

Sl. No.	Particulars	Value obtained	Remarks
A.	Physical properties		
1.	Particle size analysis		
a.	Coarse sand (%)	7.01	--
b.	Fine sand (%)	12.21	
c.	Silt (%)	28.83	
d.	Clay (%)	51.95	
2.	Textural class	Clay	
B.	Chemical properties		
1.	pH (1:2.5 soil: water suspension)	7.1	Neutral
2.	EC (1:2.5 soil: water extract) (dS m-1)	0.32	Normal
3.	Organic carbon (%)	0.44	Low
4.	Available nitrogen (kg ha-1)	263	Low
5.	Available phosphorus(P2O5) (kg ha-1)	30.5	Medium
6.	Available potassium (K2O) (kg ha-1)	366	High
7.	Available sulphur (S) (kg ha-1)	5.9	-

Table 2: The details of the treatment at varied concentration and stage of application

Treatment	Concentration of biostimulants	Product information	Stage of application
T ₁	375 ml ha ⁻¹ +375 ml ha ⁻¹	Crop max 100	FI & PF
T ₂	500 ml ha ⁻¹ +500 ml ha ⁻¹	Crop max 100	FI & PF
T ₃	625 ml ha ⁻¹ +625 ml ha ⁻¹	Crop max 100	FI & PF
T ₄	750 ml ha ⁻¹ +750 ml ha ⁻¹	Crop max 100	FI & PF
T ₅	375 ml ha ⁻¹ +625 ml ha ⁻¹	Crop max 100+Biozyme	FI & PF
T ₆	500 ml ha ⁻¹ +625 ml ha ⁻¹	Crop max 100+Biozyme	FI & PF
T ₇	625 ml ha ⁻¹ +625 ml ha ⁻¹	Crop max 100+Biozyme	FI & PF
T ₈	750 ml ha ⁻¹ +625 ml ha ⁻¹	Crop max 100+Biozyme	FI & PF
T ₉	625 ml ha ⁻¹ +625 ml ha ⁻¹	Biozyme+ Biozyme	FI & PF
T ₁₀	Urea @ 2% and KNO ₃ @ 1%)	-	Top dressing
T ₁₁	Water spray	-	FI & PF

Along with the RDF the biostimulants was sprayed at FI & PF, FI- flower initiation, PF-pod formation

Table 3: Composition of CropMax: Liquid nutrient supplement containing 24 per cent organic compounds (fermentation metabolites, selected marine algae and protein hydrolysates) + 18 per cent mineral components

Sl. No.	1. Mineral component	Content (%)
1	S	4.20
2	B	0.08
3	Co	0.054
4	Cu	0.90
5	Fe	0.90
6	Mn	3
7	Mo	0.037
8	Zn	2.70
	2. Organic component	
9	N	1.00
10	P ₂ O ₅	0.14
11	K ₂ O	0.83
12	Mg	0.31

Table 4: Mathematical model representing the relationship between seed yield (y) with change in the environmental parameters viz., Maximum temperature, minimum temperature and rainfall

Sl. No	Independent variable (x)	Regression equation	Coefficient of correlation (r)	Coefficient of determination (r ²)
a	Max. temp.	$y = 4446.5 - 69.341x$	-0.605 ^{ns}	R ² = 0.366
b	Min. temp.	$y = 3097.2 - 26.091x$	-0.109 ^{ns}	R ² = 0.012
c	Rainfall	$y = 2375.3 + 6.6413x$	0.746*	R ² = 0.557

*, ^{ns} means that r is significant at p < 0.05 and non-significant level of probability.

Table 5: Mathematical model representing the relationship between yield attribute and yield with growth parameters viz., leaf area (60 DAS), dry matter production (DMP-g plant-1) at 60 DAS and at harvest.

Sl. No	Independent variable (x)	Dependent value (y)	Regression equation	Coefficient of correlation (r)	Coefficient of determination (r ²)
1	Leaf area at 60 DAS	Pods per plant	$y = 9.7207x - 55.969$	0.896 **	0.8026
2	DMP at 60 DAS		$y = 3.6907x - 208.38$	0.964 **	0.9302
3	DMP at harvest		$Y = 2.8827x - 174.04$	0.948 **	0.8997
4	Leaf area at 60 DAS	Seed yield (kg ha-1)	$y = 106.73x + 1406$	0.888 **	0.788
5	DMP at 60 DAS		$y = 40.548x - 269.3$	0.956 **	0.9145
6	DMP at harvest		$y = 32.508x + 43.14$	0.965 **	0.9319

**, means that r is significant at p < 0.01 level of probability.

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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; R. Channakeshava: Co-PI of the project, supervised the research work and monitored preparation of the manuscript; K. Shivashanakar: helped in preparing the map and also helped in preparing the correlation matrix plot in R.

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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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