



## Effect of BioTriNova on Growth, Yield, and Productivity of Sugarcane

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### Abstract

This study investigated the impact of BioTriNova, which incorporates green seaweed extract, mycorrhizal fungi, and humic substances on the initial growth performance of sugarcane under controlled field conditions. A Completely Randomized Design with two treatments, BioTriNova and a non-treated control was used, and observations were recorded at 15, 30, 45, 60, and 75 days after sowing. Treated plants showed higher values for plant height, root length, and root biomass than the control throughout the study. In the treatment group, plant height varied from 38.14 to 88.00 cm, whereas in the control group, it only reached 28.45 to 69.40 cm. Root length increased substantially during treatment, ranging from 19.43 to 63.62 cm, contrary to 11.14 to 44.23 cm in the control. Root biomass of BioTriNova-treated plants compared with from 0.21 to 1.34 g, surpassing the control range of 0.07 to 1.00 g. The improvements are increased to the combined action of seaweed-derived bioactive substances that encourage shoot and root initiation, humic substances that improve nutrient mobility and soil activity, and mycorrhizal fungi that increase nutrient and water absorption. The results show that the integrated formulation provides greater development benefits than individual bio-stimulants reported in earlier studies. Consequently, BioTriNova emphasizes strong potential as a sustainable alternative for enhancing early sugarcane development and decreasing chemical fertilizer input.

**Keywords:** Sugarcane; BioTriNova; Seaweed Extract; Humic Substances; Mycorrhizal Fungi; Biostimulant; Root Development; Sustainable Agriculture

### Abbreviation

AMF: Arbuscular Mycorrhizal Fungi; CRD: Completely Randomized Design; DAS: Days After Sowing; PSB: Phosphate-Solubilizing Bacteria.

### Introduction

Sugarcane (*Saccharum officinarum* L.) is a major commercial crop cultivated worldwide for sugar, bioethanol, and various value-added products [1]. As a high-biomass, nutrient-demanding crop, sugarcane requires large quantities of nitrogen, phosphorus, and

potassium throughout its growth cycle to sustain vigorous tillering, rapid stem elongation, and sucrose accumulation [2].

However, the continuous dependence on synthetic fertilizers to meet nutrient requirements has led to multiple soil-related and environmental challenges [3]. Over-application of chemical fertilizers frequently results in nutrient losses through leaching and volatilization, causing low nutrient use efficiency and economic burden for farmers [4]. Moreover, long-term use of chemical inputs contributes to soil compaction, reduced organic matter, microbial

imbalance, and contamination of nearby water bodies [5]. These concerns have strengthened the need for alternative nutrient management strategies that support high productivity while maintaining long-term soil health.

In recent years, biostimulants such as seaweed extracts, humic substances, and beneficial fungi have gained considerable attention for their ability to enhance crop growth through soil and plant-mediated mechanisms [6]. Green seaweed extracts, particularly those derived from *Ulva*, *Codium*, or *Enteromorpha* species, contain a rich composition of bioactive compounds including alginic acid, amino acids, cytokinins, betaines, and trace minerals [7]. These compounds stimulate cell division, chlorophyll synthesis, and root development, contributing to improved plant vigor [8]. Furthermore, seaweed derived polysaccharides promote the activity of beneficial rhizosphere microorganisms such as *Bacillus*, *Azotobacter*, *Pseudomonas*, and *Trichoderma*, enhancing nutrient cycling and soil enzymatic activity [9]. Because sugarcane has an extensive root system with high nutrient demand, seaweed extracts offer significant potential to support early establishment and overall plant robustness [10].

Humic compounds, such as humic acid and fulvic acid, are a separate class of biostimulants known for their potential to improve soil structure, cation exchange capacity, and nutrient retention [11]. These substances are formed by the decomposition of organic matter and have a high molecular complexity, which improves the physical, chemical, and biological aspects of soil [12]. Humic acids, when sprayed to sugarcane fields, increase root surface area, improve nutrient uptake, and encourage microbial communities that fix nitrogen and solubilize phosphorus [13]. Many beneficial microbes, such as *Azospirillum brasiliense*, *Paenibacillus polymyxa*, and *Bacillus megaterium*, respond positively to humic compounds, increasing activity and enhancing nutrient transformation [14]. Humic compounds can chelate nutrients and prevent nutrient loss, which helps reduce fertilizer requirements in sugarcane growing systems [15].

Arbuscular mycorrhizal fungi (AMF), such as *Glomus*, *Rhizophagus*, *Acaulopspora*, and *Funneliformis* species, form symbiotic asso-

ciations with sugarcane roots and significantly enhance plant access to phosphorus, micronutrients, and water [16]. The extensive hyphal network created by AMF increases the effective absorptive surface area of roots, enabling the plant to utilize nutrients from soil zones that roots alone cannot reach [17]. Besides improving nutrient uptake, AMF also contribute to soil aggregation, drought tolerance, and resistance to abiotic stress [18]. Their ability to interact synergistically with other beneficial microbes such as phosphate-solubilizing bacteria (PSB) like *Pseudomonas fluorescens* and *Bacillus subtilis* further increases the efficiency of nutrient mobilization in the rhizosphere [19]. Because phosphorus availability is a major limiting factor in sugarcane production, the incorporation of AMF represents an essential component of sustainable nutrient management [20].

Considering these benefits, the combined use of green seaweed extracts, humic substances, and mycorrhizal fungi has emerged as a promising strategy to improve both soil fertility and crop performance [21]. Together, these biostimulants influence plant physiological functions, enhance root-microbe interactions, and increase nutrient use efficiency [22]. Seaweed extracts stimulate root initiation, humic substances promote nutrient mobility and microbial activity, and mycorrhizae enhance nutrient absorption and stress resilience [23]. Their synergistic effects create an active rhizosphere environment rich in beneficial organisms such as *Trichoderma harzianum*, *Aspergillus niger*, *Azotobacter chroococcum*, and various AMF species, which collectively contribute to improved nutrient cycling, soil health, and plant growth [24].

The growing limitations of chemical fertilizers reinforce the need for sustainable alternatives in nutrient management. Continuous use of synthetic fertilizers often results in soil acidification, nutrient imbalances, and a decline in beneficial microbial communities, ultimately compromising long-term soil productivity [25]. In contrast, biostimulants enhance the natural biological processes that regulate nutrient availability by improving soil structure, increasing organic matter content, and stimulating microbial activity [26]. Their capacity to enhance nutrient use efficiency while reducing reliance on chemical inputs makes them a promising tool for environmentally friendly and cost-effective sugarcane production [27].

Given these advantages, integrating green seaweed extracts, humic substances, and mycorrhizal fungi provides a comprehensive biological strategy to improve plant growth, nutrient uptake, and soil health [28]. This study aims to evaluate the combined effects of these biostimulants on sugarcane performance, with the overall goal of developing an efficient and eco-friendly nutrient management approach suitable for sustainable cultivation systems.

Although the individual benefits of seaweed extracts, humic substances, and mycorrhizal fungi have been extensively reported in various crops, their combined effects in sugarcane remains unidentified. Most studies assess these biostimulants separately, providing limited few data into their synergistic effects on soil microbial activity, nutrient-use efficiency, and biochemical processes in the rhizosphere. This gap emphasizes the need for integrated biological approaches suitable for nutrient derived crops such as sugarcane. To address this, the present study investigates BioTriNova, a composite biostimulant containing green seaweed extract, humic substances, and mycorrhizal fungi. Evaluating BioTriNova will assist to determine its ability to improve nutrient uptake, stimulate soil biological activity, and reduce reliance on chemical fertilizers.

## Materials and Methods

### Experimental site and duration

The experiment was conducted during the year 2024 under controlled field conditions to evaluate the effect of BioTriNova, a biostimulant formulation consisting of Green Seaweed Extract, Humic Substances, and Mycorrhizal Fungi, manufactured by Ashirwad Nutrients, on early growth performance of sugarcane (*Saccharum officinarum L.*). The study was carried out under standard agronomic practices suitable for sugarcane cultivation.

### Experimental crop material

Healthy, disease-free single-eye sugarcane clusters from a popular commercial cultivar were chosen for uniform planting. To ensure uniform germination, all setts were surface sterilized and immersed in clean water for 30 minutes before planting.

### Experimental design and treatments

The experiment employed a Completely Randomized Design (CRD) with two treatments and three replications: Treatment T1: BioTriNova (Green Seaweed + Humic + Mycorrhizae) and Treatment T2: Control (no BioTriNova application). To assure consistency across all replicates, each treatment included uniformly sized pots or plots with identical amounts of soil.

BioTriNova, supplied by Ashirwad Nutrients, was applied at the manufacturer **100g/acre**. The product was diluted in water and applied directly to the root zone at planting to ensure uniform distribution and proper establishment.

### Application method

BioTriNova was applied to the soil (Recommendation is dose 100g/acre) while planting, near the root zone, to promote direct microbial colonization. Further treatments were performed at regular intervals to sustain microbial activity and ensure consistent nutrient absorption. The control plots did not receive any biostimulants but were kept under the same environmental and irrigation conditions during the trial.

### Cultural practices

Standard agronomic practices for sugarcane cultivation were followed throughout the experiment. Irrigation was applied uniformly across all treatments to avoid moisture stress. No chemical fertilizers or growth-promoting substances were applied.

### Data collection

Observations were recorded at 15, 30, 45, 60, and 75 days after sowing. Plant height was measured from the base of the plant to the tip of the tallest leaf. Root length was determined by carefully uprooting the plants and measuring from the stem base to the longest root. For root biomass, roots were washed to remove soil and their fresh weight was recorded using a digital balance.

## Data analysis

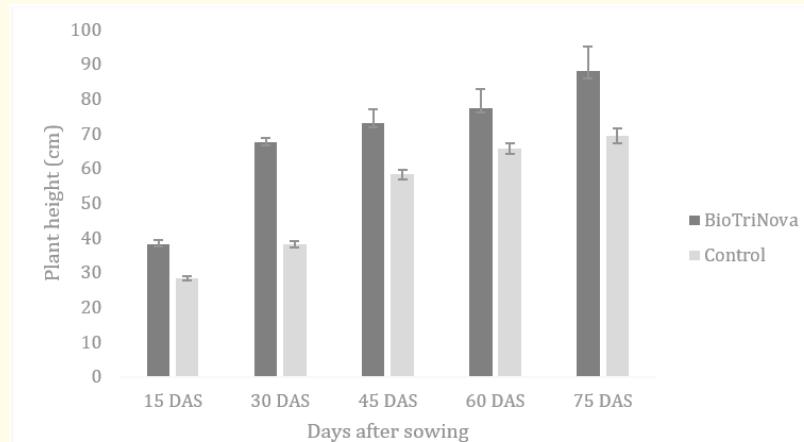
All collected data were subjected to a comparison of means between BioTriNova and Control treatments. Mean values and standard deviations (mean  $\pm$  SD) were calculated for each growth parameter at all observation periods. The difference in plant height, root length, and root biomass between treatments was interpreted to evaluate the efficacy of BioTriNova on early sugarcane growth.

## Result and Discussion

### Plant height

Plant height was progressively increased under both treatments as the crop developed. However, plants treated with BioTriNova consistently exhibited greater growth as shown in Figure 1. At the initial stage of 15 DAS, treated plants measured 38.14 cm, whereas the control reached 28.45 cm. By 30 DAS, this difference increased,

with heights of 67.44 cm and 38.11 cm, respectively. Mid-season assessments showed similar trends; at 45 and 60 DAS, BioTriNova-treated plants recorded 73.10 cm and 77.44 cm, respectively, compared with 58.23 cm and 65.75 cm in the control. At 75 DAS, the tallest plants (88.00 cm) were consistently associated with BioTriNova, while the control remained lower at 69.40 cm. Demonstrating superior vertical growth throughout the observation period. Similar observation was reported in a sugarcane with the highest plant height of sugarcane at 8 MAP was recorded in the biostimulant treatment, which consisted of a consortium of humic substances, phytohormones, and arbuscular mycorrhizal fungi, reaching 137.8 cm compared with 111.6 cm in the control [29]. In another study the greatest plant height in both seasons was obtained with the combined application of *G. macrocarpum*, humic acid, and algal extract (Mix treatment), which reached 115 cm in the first season and 127 cm in the second season [30].

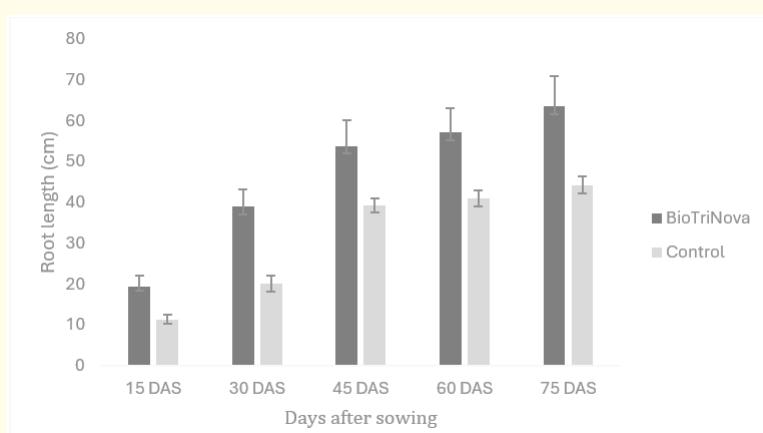


**Figure 1:** Effect of BioTriNova on plant height recorded at 15, 30, 45, 60 and 75 days after sowing (DAS).

### Root length

Root elongation exhibited a different response pattern, with BioTriNova stimulating faster and more extended root growth than untreated plants. At early stages of growth (15 DAS), root length of the treated plants was already 19.43 cm, a substantial increase over the control value (11.14 cm). This early stimulation sustained during successive stages. By 30 DAS, roots under the BioTriNova treatment had grown to 39.01 cm, nearly tripling the length measured in the control (20.13 cm). At 45 DAS, treated plants recorded 53.74 cm, while the control measured 39.21 cm. At 60 DAS, BioTri-

Nova generated 57.20 cm root length, which was greater than the control group (41.0 cm). The treated plants resulted in the longest roots (63.62 cm) at 75 DAS. These results as shown in Figure 2 show that the product improves root penetration and mid season growth. In a comparative research study root length in hemp plants was significantly improved by all treatments compared with the control. The maximum increase (44.91%) was observed with the combined application of AMF (10 g/pot) and seaweed extract (SWE, 3 g/L) along with biochar, showing a synergistic effect of these treatments in enhancing root growth [31].

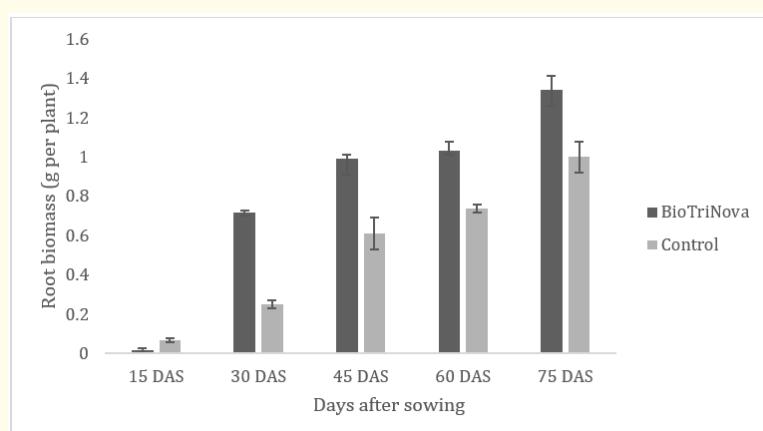


**Figure 2:** Effect of BioTriNova on root length at different growth stages (15, 30, 45, 60 and 75 days after sowing).

### Root biomass

In contrast to height and root length, root biomass increased gradually but remained consistently higher in treated plants (Figure 3). At 15 DAS, root biomass of the treated group was 0.21 g, approximately three times greater than the control (0.07 g). This trend persisted over time, reaching 0.72 g at 30 DAS compared with 0.25 g in the control. At 45 DAS, treated plants recorded 0.99 g of root biomass, while the control measured 0.61 g. By 60 DAS, the difference remained evident (1.03 g vs 0.74 g). The highest biomass was observed at 75 DAS, with treated plants attaining 1.34 g, surpassing the control (1.00 g). These results indicate that the inte-

grated biostimulant effectively enhances root system development and promotes greater biomass accumulation during early growth. Similarly Leite, *et al.* found that sugarcane the highest biomass of sugarcane plants was recorded with the combined application of urea and humic substance (U+HS) at 192 hours after foliar application, reaching 18.6 mg/ plant. Recent studies show that the highest root biomass at 75 DAS was recorded with the AlgaBest treatment, a formulation containing 20% humic acids, 20% fulvic acids, 10% amino acids, 10% seaweed extract, 2% alfalfa extract, 1% carbohydrates, and 37% diluents, which reaching 1.36 g, and exceeding the values observed in than the humic acid and control treatments [33].



**Figure 3:** Effect of BioTriNova on root biomass of plants at 15, 30, 45, 60 and 75 days after sowing.

## Conclusion

The study showed that the combined application of green seaweed extract, humic substances, and mycorrhizal fungi improved early growth of sugarcane compared with the untreated control. Increases in plant height, root length, and biomass indicate that the synergistic action of bioactive compounds, humic materials, and root fungal interactions promotes stronger shoots, deeper roots, and more efficient nutrient uptake. Previous studies mostly evaluated these biostimulants separately, this research demonstrates that their integrated use produces greater growth effects. The experiment focused on the early growth stage of sugarcane and did not assess long-term parameters such as yield, soil nutrient dynamics, or economic outcomes. Nevertheless, the results indicate that multi-component biostimulants can effectively enhance early plant vigor and reduce reliance on chemical fertilizers. These results also emphasize possible long-term benefits for soil health and crop output, suggesting the need for further research to evaluate potential agronomic and environmental implications.

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