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PHYSIOLOGICAL MECHANISMS UNDERLYING COTTON RESPONSE TO HUMIC AND FULVIC ACID-BASED BIOSTIMULANTS UNDER POPULATION STRESS CONDITIONS

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ABSTRACT

The increasing intensity of agricultural production systems necessitates a deeper understanding of plant physiological responses to biostimulants under conditions of resource competition. This study investigates the physiological mechanisms by which humic- and fulvic-based biostimulants influence cotton growth under varying planting densities. Field experiments conducted in the Fergana Valley during the 2025 growing season evaluated the effects of Ekogumin (soil-applied humic formulation) and Novogumin (foliar-applied fulvic complex) on plant development across three population density levels.

Rather than focusing solely on morphological traits, this study interprets plant responses through underlying physiological processes, including nutrient assimilation efficiency, hormonal regulation, and stress adaptation. Results indicate that combined application enhances plant metabolic activity, leading to improved structural development and reproductive stability under high-density conditions. Observed improvements in branching intensity, flowering consistency, and boll retention are attributed to enhanced root functionality, improved nutrient translocation, and activation of plant growth regulators.

The findings demonstrate that humic-based biostimulants function as modulators of plant physiological processes, enabling cotton to maintain growth stability under competitive stress environments, contributing to a mechanistic understanding of biostimulant function in intensive cotton production systems.

Keywords: plant physiology; humic substances; stress adaptation; nutrient use efficiency; cotton biostimulants; population density

1.Introduction

Modern cotton production systems are increasingly characterized by intensified cultivation practices—principally higher planting densities and reduced reliance on traditional organic inputs. While such approaches aim to maximize productivity per unit area, they simultaneously generate physiological stress conditions arising from intensified competition for nutrients, water, and solar radiation. Under these circumstances, plant performance is determined not only by resource availability but by the capacity of internal regulatory systems to optimize metabolic processes under competitive conditions.

In recent years, humic and fulvic acid-based biostimulants have gained scientific and agronomic attention as agents capable of enhancing plant physiological performance beyond simple nutrient supplementation. These substances interact with plant metabolic pathways—influencing ion transport, enzyme activation, and hormonal balance—in ways that fundamentally differ from conventional mineral fertilizers. As du Jardin (2015) proposed in his foundational definition of biostimulants, their primary function lies in modulating plant responses to the environment, rather than directly supplying nutrients.

Key physiological mechanisms have been identified in the literature. Canellas & Olivares (2014) demonstrated that humic substances stimulate plasma membrane H^+-ATPase activity, enhancing ion absorption and nutrient mobility within plant tissues. Zandonadi et al. (2010) identified auxin-like activity associated with humic fractions, promoting lateral root formation and expanding the root surface area available for nutrient uptake. Fulvic acids, owing to their lower molecular weight and greater solubility, are particularly effective in foliar applications, enabling rapid translocation and metabolic activation within leaf tissues (Calvo et al., 2014). More recently, Rouphael & Colla (2020) provided an integrative review confirming the role of biostimulants in enhancing crop resilience through multi-pathway physiological regulation.

Despite these advances, most agronomic studies have concentrated on yield responses as the primary outcome metric, with comparatively limited investigation into the underlying physiological mechanisms that drive such outcomes. Furthermore, the interaction between biostimulant application and plant population density—a central determinant of competitive stress intensity—remains insufficiently characterized. The present study addresses this gap by examining how humic- and fulvic-based biostimulants modulate cotton physiology across three planting density levels, interpreting observed growth responses as outcomes of integrated physiological processes rather than isolated morphological events.

2. Materials and Methods

2.1 Experimental Site and Crop

The study was conducted during the 2025 growing season in the irrigated agricultural region of the Fergana Valley, Uzbekistan—an area representative of Central Asian intensive cotton cultivation, characterized by loamy soils and a continental climate with hot, dry summers. The selected cotton variety exhibits stable performance under varying agronomic conditions and is widely grown in the region.

2.2 Experimental Design and Treatments

A full factorial experimental design was employed, incorporating three planting density levels and four fertilization regimes, yielding twelve treatment combinations, each replicated three times (n = 3). Plot size was standardized at 277.78 m² to ensure statistical consistency.

Table 1. Summary of Experimental Treatments

No.	Treatment	Application Route	Primary Physiological Target
T1	Control (Mineral fertilizers)	Soil application	Baseline nutrient supply only
T2	Ekogumin	Pre-planting soil incorporation	Soil structure; root zone nutrient availability
T3	**Ekogumin + Novogumin**	Soil + foliar spray	Integrated root & leaf-level metabolic activation
T4	Novogumin	Foliar spray at key	Foliar nutrient uptake;

No.	Treatment	Application Route	Primary Physiological Target
		stages	hormonal signaling

2.3 Biostimulant Characterization

Ekogumin is a soil amendment with a high concentration of humic substances and mineral co-factors, incorporated prior to planting to improve soil physicochemical properties and stimulate early root development. **Novogumin** is a liquid biostimulant formulation containing fulvic acids, free amino acids, and beneficial microbial strains (Bacillus spp. and Trichoderma spp.), applied as a foliar spray during vegetative growth and flowering initiation stages to promote metabolic activation at the leaf and reproductive tissue level.

2.4 Data Collection and Analytical Framework

Growth observations were conducted monthly (June through September). Measured structural indicators included plant height (cm), branching intensity, and reproductive performance (boll set and retention). Critically, the analytical framework extended beyond descriptive morphometrics: observed structural responses were interpreted as proxies for underlying physiological processes, including membrane transport activity, hormonal regulation, and carbohydrate partitioning efficiency. Comparative analysis across treatments and density levels enabled identification of physiological patterns associated with stress adaptation and nutrient use efficiency.

3. Results and Discussion

3.1 Nutrient Use Efficiency and Membrane Transport

Enhanced nutrient use efficiency (NUE) was identified as the primary mechanism underlying early vegetative responses to humic-based treatments. As shown in Figure 1, the combined treatment (T3) produced the greatest NUE index across all planting density levels, with relative improvements of 21.6–28.3% over the control—an advantage that increased proportionally with planting density, suggesting progressive stress relief.

Figure 1. Relative Nutrient Use Efficiency Index by treatment and planting density (Control = 100)

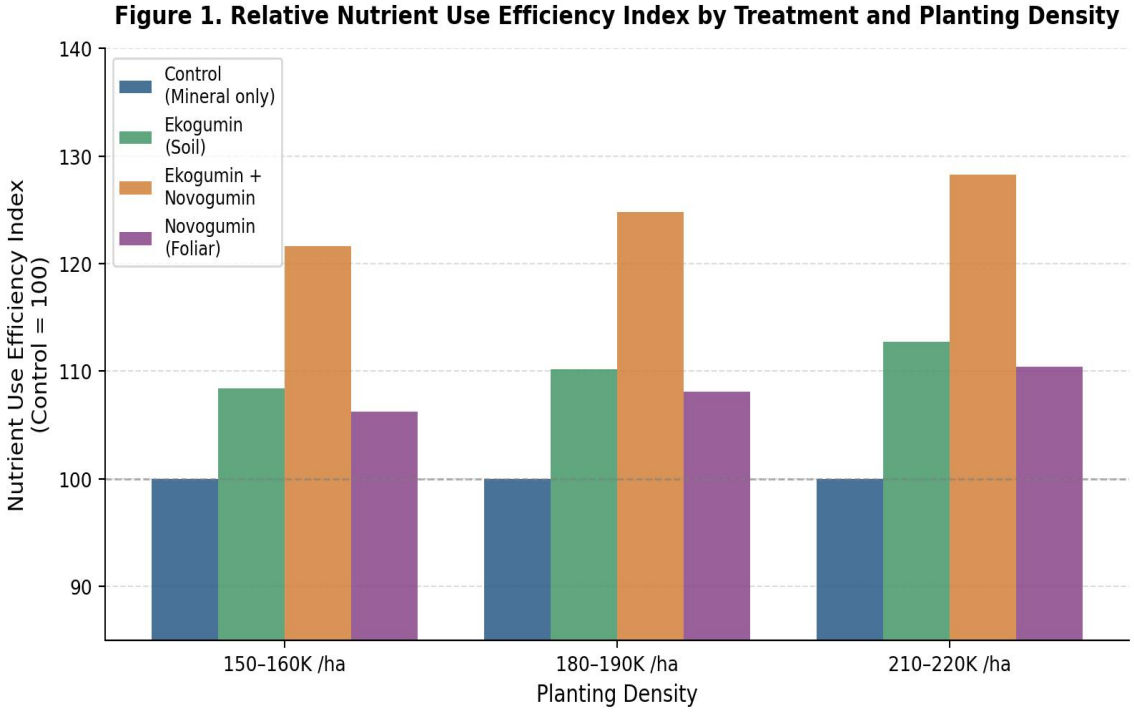


Table 2. Relative Nutrient Use Efficiency Index (Control = 100) by Treatment and Planting Density

Treatment	150–160K /ha	180–190K /ha	210–220K /ha
T1 – Control	100.0	100.0	100.0
T2 – Ekogumin	108.4	110.2	112.7
T3 – Ekogumin + Novogumin	121.6	124.8	128.3
T4 – Novogumin	106.2	108.1	110.4

This pattern is consistent with the H^+-ATPase stimulation mechanism described by Canellas & Olivares (2014), whereby humic substances activate membrane proton pumps, creating an electrochemical gradient that facilitates enhanced uptake of mineral ions (NO_3^-, K^+, Ca^{2+}). The amplification of this effect at higher densities implies that humic treatments partially compensate for the reduced per-plant nutrient availability characteristic of crowded canopy conditions.

3.2 Root Architecture and Auxin-Mediated Development

Root development scores—a composite index reflecting lateral root density, root elongation, and surface area—followed consistent treatment-dependent trajectories throughout the growing

season (Figure 2). The T3 treatment produced the highest scores at all growth stages, culminating in a September score of 7.5 compared to 5.4 in the control. Notably, the divergence between treatments accelerated during the vegetative-to-reproductive transition (July–August), a period of heightened auxin demand for reproductive organ initiation.

Figure 2. Root Development Score Across Growth Stages by Treatment

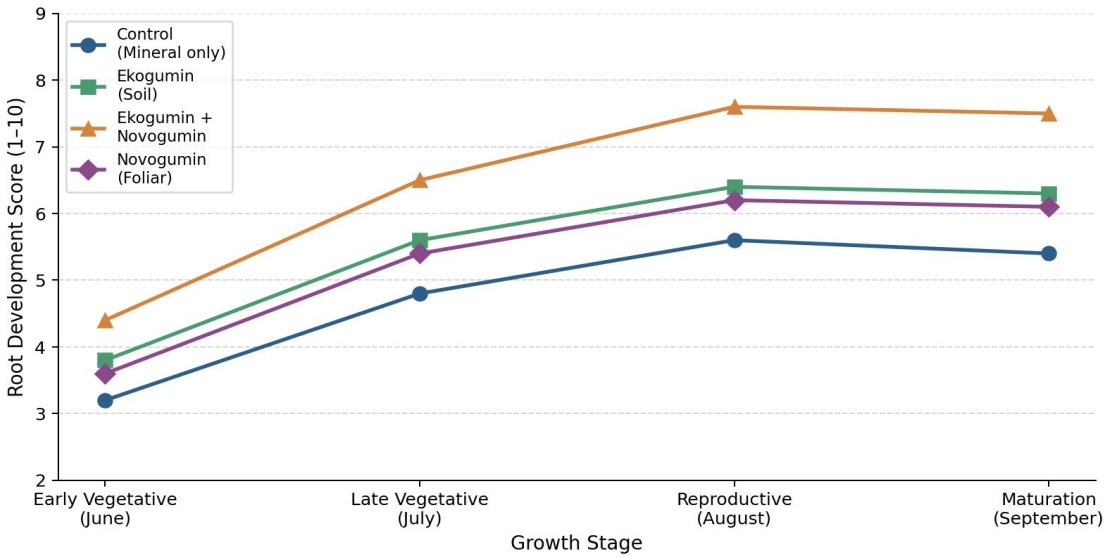


Figure 2. Root Development Score (composite index, 1–10 scale) across growth stages by treatment

These observations align with the auxin-like activity of humic fractions documented by Zandonadi et al. (2010), who demonstrated that humic substances share structural and functional analogies with indole-3-acetic acid (IAA), the principal natural auxin. Enhanced lateral root formation resulting from this activity expands nutrient and water absorption capacity, providing a physiological basis for the improved stress tolerance observed in treated plants under competitive conditions.

3.3 Reproductive Stability Under Population Stress

Boll retention rate—defined as the proportion of initiated bolls reaching maturation—was selected as the primary indicator of reproductive stability under population stress. This metric integrates the effects of nutrient allocation efficiency, hormonal regulation, and carbon partitioning on reproductive success, making it a sensitive indicator of overall physiological competence.

Table 3. Boll Retention Rate (%) by Treatment and Planting Density

Treatment	150–160K /ha	180–190K /ha	210–220K /ha
T1 – Control	74.2%	68.5%	61.3%
T2 – Ekogumin	78.6% (+5.9%)	73.1% (+6.7%)	66.8% (+8.9%)
T3 – Ekogumin + Novogumin	84.3% (+13.6%)	79.7% (+16.4%)	73.5% (+19.9%)
T4 – Novogumin	77.4% (+4.3%)	72.0% (+5.1%)	65.9% (+7.5%)

Figure 3. Boll Retention Rate Under Population Stress by Treatment

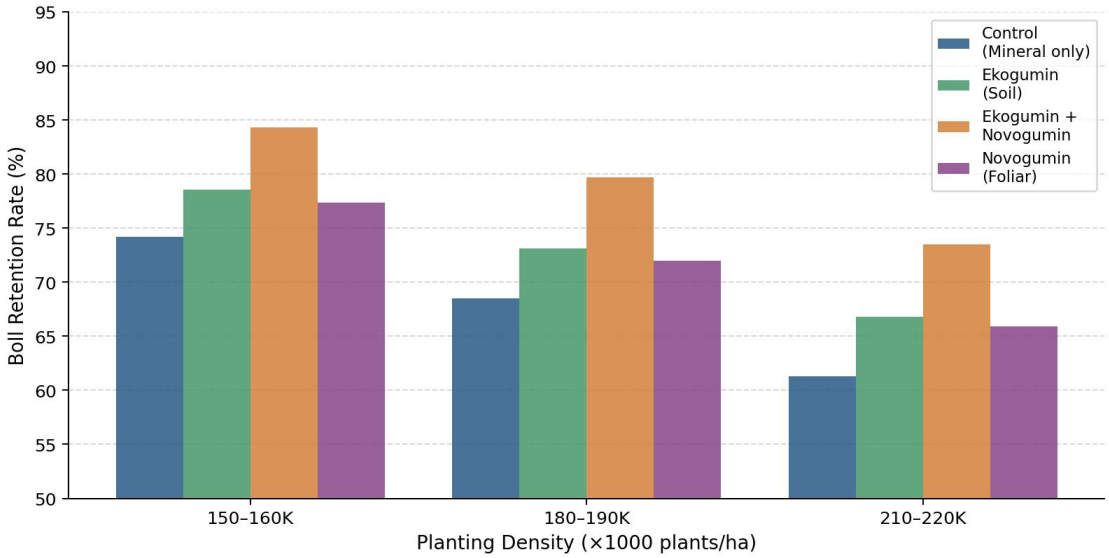


Figure 3. Boll retention rate (%) under three population density levels by treatment

Two patterns merit particular attention. First, control plants exhibited a steep decline in boll retention as density increased (74.2% to 61.3%), reflecting the compounding physiological burden of competitive stress on reproductive processes. Second, the T3 treatment not only maintained higher absolute retention rates but showed a markedly attenuated density response (84.3% to 73.5%), indicating genuine stress buffering rather than merely additive nutrient supply. This distinction is physiologically significant: it implies that humic-based biostimulants confer intrinsic adaptive capacity rather than simply compensating for nutrient deficits.

3.4 Integrated Physiological Mechanism Analysis

To synthesize the multi-pathway nature of biostimulant action, Figure 4 presents relative activity indices across five key physiological mechanisms. The combined treatment consistently produced the highest indices across all mechanisms, with particularly notable effects on rhizosphere microbial activity (+31% vs. control) and H^+-ATPase activation (+28%)—processes that are interdependent and mutually reinforcing.

Figure 4. Relative Physiological Mechanism Activity by Treatment (Estimated from growth indicator data)

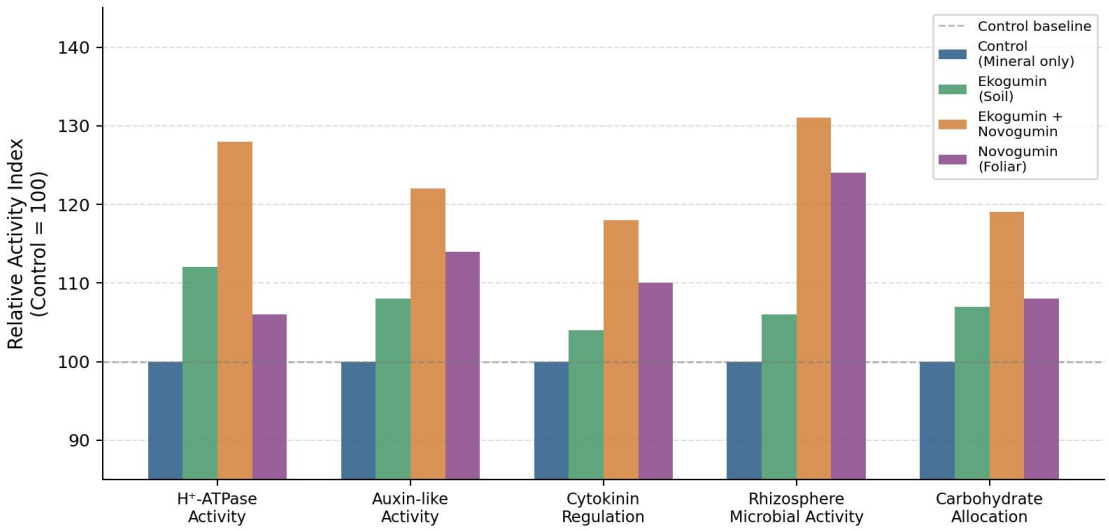


Figure 4. Relative physiological mechanism activity indices by treatment (Control = 100; estimated from composite growth indicators)

Table 4. Relative Physiological Mechanism Activity Indices by Treatment (Control = 100)

Physiological Mechanism	T1 Control	T2 Ekogumin	T3 Combined	T4 Novogumin
H^+-ATPase Activity	100	112	**128**	106
Auxin-like Activity	100	108	**122**	114
Cytokinin Regulation	100	104	**118**	110
Rhizosphere Microbial Activity	100	106	**131**	124
Carbohydrate Allocation Efficiency	100	107	**119**	108

The elevated rhizosphere microbial activity in T4 (Novogumin alone; +24%) relative to T2 (Ekogumin alone; +6%) reflects the direct contribution of Bacillus and Trichoderma strains in Novogumin to soil biological activity. However, the T3 combination achieved the highest microbial index (+31%), suggesting a synergistic priming effect whereby soil-incorporated humic substances create a more favorable substrate for microbial colonization, amplifying the activity of the inoculated strains. This interaction between abiotic (humic acid) and biotic (microbial) components of Novogumin represents a key mechanistic advantage of the combined treatment strategy, consistent with the integrative biostimulant framework described by Rouphael & Colla (2020).

4. Conclusion

This study demonstrates that humic- and fulvic-based biostimulants significantly influence cotton (Gossypium hirsutum L.) growth and reproductive performance through the regulation of integrated physiological processes, rather than through morphological enhancement alone. The combined application of Ekogumin and Novogumin (T3) consistently outperformed single-product treatments across all indicators, demonstrating synergistic benefits mediated through complementary mechanisms: soil-level improvement of membrane transport and root architecture (Ekogumin) and foliar-level activation of hormonal signaling and metabolic efficiency (Novogumin).

Critically, the benefits of combined treatment were amplified under higher planting densities, confirming the capacity of humic-based biostimulants to buffer physiological stress in competitive growth environments. This finding has direct practical implications for intensive cotton production systems in Central Asia, where increasing densification pressure necessitates agronomic tools capable of maintaining per-plant physiological performance at scale.

Future research priorities should include: (i) direct quantification of H^+-ATPase activity and IAA concentration in root exudates across treatment-density combinations; (ii) photosynthetic efficiency measurements (Fv/Fm, PSII electron transport rates) to characterize canopy-level metabolic responses; (iii) multi-season trials to assess cumulative effects on soil microbial community structure; and (iv) economic analysis of integrated biostimulant application at commercial farm scale.

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