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# Nitrogen, Phosphorus, and Potassium Concentrations in Cotton Organs under Humic-Based Biostimulants across Different Irrigation and Fertilization Regimes

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# Nitrogen, Phosphorus, and Potassium Concentrations in Cotton Organs under Humic-Based Biostimulants across Different Irrigation and Fertilization Regimes

*Running title: NPK in cotton organs under humic biostimulants*

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**Ethics Approval**
Not applicable. This study involved field trials on cotton (*Gossypium hirsutum* L.) and soil sampling only; it did not involve human participants or animals.

**Consent to Participate / Consent to Publish**
Not applicable.

**Data Availability**
Data generated and/or analyzed during this study are available from the corresponding author upon reasonable request.

**Materials and Code Availability**
Not applicable (no code or unique materials beyond the commercial biostimulant products named in the manuscript).

**Author Contributions**
Sh. Abdualimov: Methodology, Investigation, Writing – original draft, Supervision. F. Abdullaev: Conceptualization, Investigation, Writing – review and editing. Sh. Khamidullayev: Investigation, Formal analysis. Kh. Khasanov: Formal analysis, Data

curation, Writing – review and editing.

**Statement on the Use of Generative AI**

In preparing this work, the authors used Claude (Anthropic) to organize and tabulate raw experimental data from the underlying dissertation, and to assist in drafting and formatting the manuscript in English and Uzbek. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

# Abstract

How humic-based biostimulants affect cotton nutrition under different irrigation and reduced-fertilization regimes remains poorly documented on the irrigated serozem soils of Uzbekistan. This study evaluated the biostimulants Reflect and Geohumate in field trials conducted on typical serozem soil in Tashkent province. In the first experiment, the UzPITI-103 cultivar received seed and foliar applications of Reflect (200–400 mL/t) under two irrigation regimes (65–70–65% and 70–75–65% relative to field water capacity, FWC). In the second experiment, Geohumate, Bactofert, and their combination were evaluated on the Andijon-37 cultivar against a 40%-reduced N120P84K60 fertilizer background relative to a full N200P140K100 control. Nitrogen, phosphorus, and potassium concentrations in plant organs were determined at the budding, flowering, and boll-opening stages. Changes in NPK concentration under Reflect depended on organ, growth stage, application rate, and irrigation regime, with the most consistent positive responses observed in certain reproductive organs. Under the reduced-fertilizer background, the Geohumate and Geohumate+Bactofert packages matched or exceeded the fully-fertilized control across numerous tissue indicators. Yield exceeded the control by 3.1–5.3 c/ha for Reflect treatments and by 3.4–6.6 c/ha for Geohumate/Bactofert treatments, with corresponding gains in net profit and profitability. However, the tissue analyses are based on treatment means, and no biostimulant-free control was included at the reduced-fertilizer level; the results therefore reflect the performance of the tested technological packages rather than the isolated contributions of the biostimulant and the fertilizer reduction.



**Keywords** humic substances; biostimulant; cotton; NPK concentration; irrigation regime; reduced mineral fertilizer

# Introduction

Nitrogen, phosphorus, and potassium are the principal mineral nutrients governing the growth, boll formation, and fiber development of cotton (Gossypium hirsutum L.), and their balanced uptake affects yield, fertilizer-use efficiency, and production cost (Khan et al. 2017). The effects of individual elements are not independent: potassium nutrition, for example, can alter dry-matter partitioning among organs and shift the concentration of other nutrients in tissue (Pettigrew and Meredith 1997). Assessing cotton's nutritional status therefore requires attention to how nutrient concentrations change across developmental stages and organs, not only to single-point values.

On Uzbekistan's irrigated typical serozem soils, humus and available phosphorus are often low and potassium supply moderate, so cotton's nutrient requirements are met largely through annual mineral-fertilizer applications (Khashimov et al. 2024; Zhumanova et al. 2015). Rising fertilizer prices and the need for resource-saving technologies call for more efficient use of applied nutrients. Biostimulants based on humic and fulvic acids are among the means proposed for this purpose (Abdullaev et al. 2023): they help retain and convert soil nutrients into plant-available forms, improve rhizosphere cation-exchange properties, and stimulate root and root-hair development, potentially expanding the plant's nutrient-absorptive capacity and affecting rhizosphere microbial activity (Geng et al. 2020a, 2020b; Hu et al. 2021). A meta-analysis by Ma et al. (2024) found that humic-acid fertilizers raised crop yield by 12%, nitrogen uptake by 17%, and nitrogen-use efficiency by 27% on average, though efficacy varied considerably with soil pH, initial nitrogen and organic-carbon content, climate, and nitrogen rate — underscoring the need for local field verification.

In cotton specifically, fulvic acid combined with slow-release fertilizer has raised soil available nitrogen and delayed leaf senescence (Geng et al. 2020a), while its combination with potassium fertilizer raised soil available potassium and yield (Geng et al. 2020b); humic-acid preparations have also allowed nitrogen rates to be reduced without yield loss (Zhou et al. 2025). Fulvic acid combined with phosphorus fertilizer has improved root activity, photosynthetic capacity, and yield, with a moderate phosphorus rate sometimes outperforming a higher one (Li et al. 2025a), and has raised boll number and phosphorus-use efficiency without sig-

nificantly altering total biomass or phosphorus uptake (Zhang et al. 2025) — indicating that biostimulant-associated yield gains need not be mediated by a uniform rise in tissue nutrient concentration. Foliar potassium with macro- and micronutrients has likewise raised yield and economic returns (Agac and Haliloglu 2025), and sowing method and irrigation strategy alter soil nutrient dynamics (Wang et al. 2025).

Irrigation regime is a further major factor: soil moisture governs root activity, nutrient movement in soil solution, and nutrient delivery, so biostimulant and fertilizer effectiveness depends on water availability. Irrigation timing interacts with nitrogen rate to affect cotton dry matter and tissue NPK (Ibrahim et al. 1997), and increasing irrigation volume can raise total nitrogen accumulation while lowering percentage concentration in reproductive organs (Li et al. 2025b) — showing that tissue concentration and whole-plant accumulation do not always move together. Biopreparations delivered via fertigation have improved cotton yield and water/fertilizer-use efficiency under semi-arid conditions (Jayakumar et al. 2014, 2015), organic-mineral combinations have improved nutrient partitioning and use efficiency (Hao et al. 2022), and organic or reduced-fertilizer packages have raised soil available N and P and yield (Tewolde et al. 2018; Li et al. 2020; Shi et al. 2023). However, biostimulant efficacy is not necessarily stable as water deficit intensifies: fulvic acid improved soil and yield indicators under good-to-moderate water supply but not under severe deficit (He et al. 2026), while biopreparations under saline-water drip irrigation and reduced-nitrogen-plus-biofertilizer packages have each maintained yield under specific water/salinity conditions (Ding et al. 2024; Lv et al. 2026) — indicating that humic-based products should be evaluated as an agrotechnical factor bounded by soil moisture rather than a universal remedy for water deficit.

Because tissue NPK concentration varies with developmental stage, organ, and biomass — including a "dilution effect" whereby fast biomass gain lowers percentage concentration even when total uptake rises (Pettigrew and Meredith 1997) — analyzing multiple organs across several stages captures whole-plant nutrient status more completely than single-point leaf diagnostics, complementing soil analysis (Sahu et al. 2012; Sabbe and Zelinski 2018). Most existing studies address germination, growth, photosynthesis, yield, and fiber quality; how N, P, and K concentrations change across cotton organs and stages under commercial humic-based

biostimulants — particularly under different irrigation regimes and a reduced mineral-fertilizer background on Uzbekistan's serozem soils — has received far less attention, despite its relevance to yield and quality results already reported for these same field trials (Abdualimov et al. in preparation).

Accordingly, this study aimed: (i) to determine the effect of the Reflect biostimulant on N, P, and K concentrations in UzPITI-103 cotton organs under two soil-moisture thresholds; (ii) to determine the effect of Geohumate, Bactofert, and their combination against a 40%-reduced mineral-fertilizer background on the nutritional status of Andijon-37; and (iii) to assess residual soil agrochemical status and the corresponding agronomic and economic outcomes. We hypothesized that (H1) Reflect treatment would be associated with altered organ NPK concentrations relative to a water-treated control, with direction and magnitude depending on irrigation regime, rate, stage, and organ; and (H2) that, against the 40%-reduced N120P84K60 background, Geohumate- and/or Bactofert-based packages could maintain organ NPK concentrations and yield at or above the fully-fertilized N200P140K100 control. The study's novelty lies in evaluating these commercial biostimulants at the level of individual organs and developmental stages on Uzbekistan's irrigated typical serozem soils, considering tissue nutrient results alongside yield and economic indicators.

# Materials and Methods

## Study Site and Experimental Design

The field trials were conducted at the experimental farm of the Research Institute of Cotton Breeding, Seed Production and Cultivation Agrotechnologies, Kibray district, Tashkent province, Uzbekistan, approximately 7–8 km from the Chirchiq River on the right bank of the Bozsuv canal (500–750 m a.s.l.; typical serozem soil; groundwater at 18–20 m) (Fig. 1).

At the start of each trial year, composite soil samples (five-point "envelope" scheme) were collected from the 0–30 and 30–50 cm layers. Humus, total N, and total P in the 0–30 cm layer ranged from 0.720–0.860%, 0.076–0.078%, and 0.159–0.176% across 2020–2022, with available $NO_3^-$–N, $P_2O_5$, and $K_2O$ of 11.9–12.9, 10.1–21.2, and 226.4–276.0 mg $kg^{-1}$, respectively;

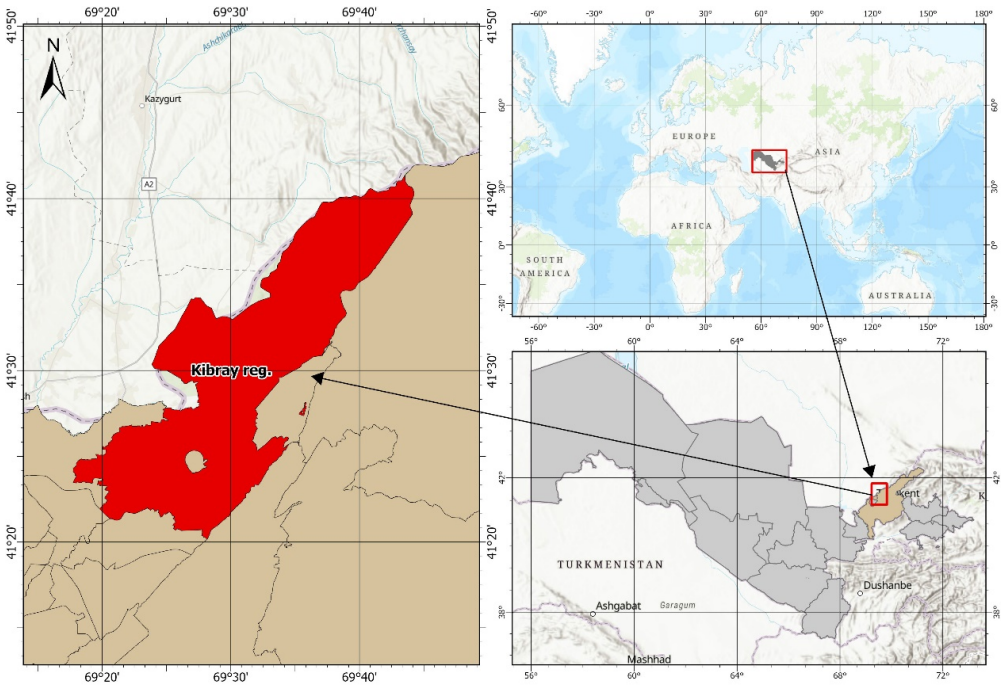



**Fig. 1.** Location of the experimental site, Research Institute of Cotton Breeding, Seed Production and Cultivation Agrotechnologies, Kibray district, Tashkent province, Uzbekistan (inset maps show the site's location within Central Asia and the broader region).

the 30–50 cm layer showed correspondingly lower values (Fig. 2; full values in Supplementary Table S1). Humus and $NO_3^-$–N were consistently higher in the 0–30 cm layer, available $P_2O_5$ declined from 21.2 to 10.1 mg $kg^{-1}$ over the three years, and the soil was classified as low in available phosphorus and moderately supplied with potassium; soil pH, electrical conductivity, bulk density, and particle-size data were not available.

Meteorological data (Oqqovoq station; Table 1) showed mean annual air temperature of 14.4–17.6 °C and annual precipitation of 263.9–590.1 mm across 2020–2022, with 2021 notably warmer and drier than the long-term mean.

**Table 1.** Growing-season meteorological conditions, Oqqovoq agrometeorological station, Tashkent province, 2020–2022, relative to the long-term mean.

| Year | Mean annual air temperature, °C | Long-term mean, °C | Annual precipitation, mm | Long-term mean, mm | April–October effective temperature sum, °C | Long-term mean, °C |
|---|---|---|---|---|---|---|
| 2020 | 14.4 | 14.0 | 404.0 | 515.3 | 2450 | 2356 |
| 2021 | 17.6 | 14.0 | 263.9 | 515.3 | 2742 | 2358 |
| 2022 | 15.5 | 14.0 | 590.1 | 515.3 | 2739 | 2358 |

Source: Oqqovoq agrometeorological station records (dissertation appendices 5–6).

First experiment (Reflect, two irrigation regimes). UzPITI-103 cotton was grown in 2020–2022 under two soil-moisture regimes maintained relative to field water capacity (FWC): 65–

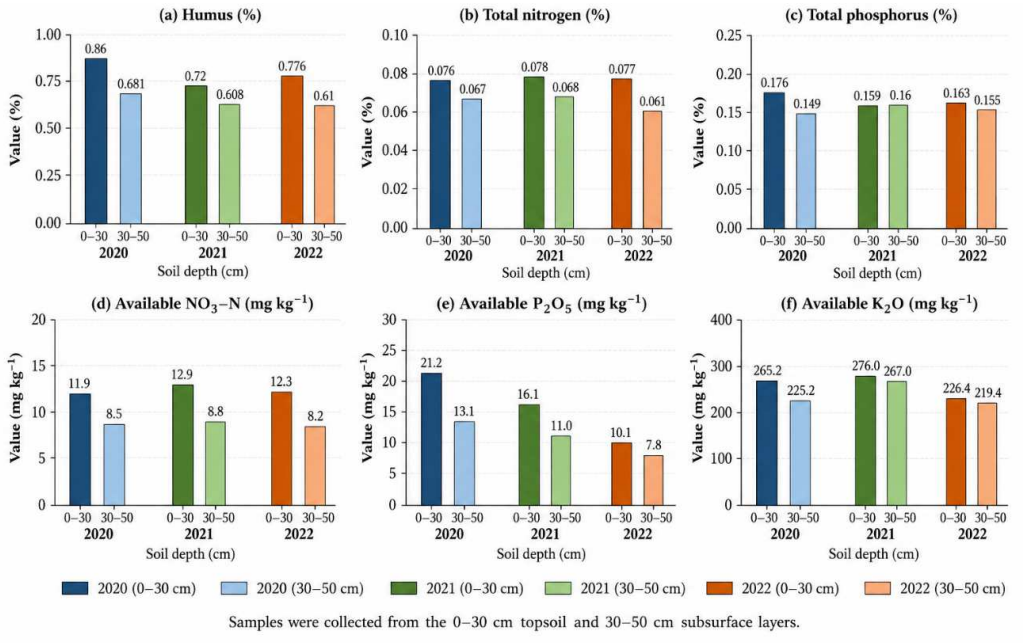



**Fig. 2.** Pre-sowing soil agrochemical indicators (humus, total nitrogen, total phosphorus, available $NO_3^-$-N, available $P_2O_5$, and exchangeable $K_2O$) at 0–30 cm and 30–50 cm depth, Tashkent province, 2020–2022 (principal indicators recorded before sowing in each trial year). Full tabulated values are given in Supplementary Table S1.

70–65% and 70–75–65% across the pre-budding, budding–flowering, and boll formation–maturation stages. The 65–70–65% regime allowed soil moisture to decline to a lower threshold between fewer, larger irrigations (four per season in every year: e.g., 25–26 June, 6–7 August, and 9–10 September in 2020, for a seasonal total of 4181.1 $m^3$/ha); the 70–75–65% regime used more frequent, smaller irrigations (four in 2020, five in 2021 and 2022: e.g., 23–24 June, 15–18 July, 4–6 August, and 9–10 September in 2020, totaling 3920.1 $m^3$/ha). Seasonal irrigation volume was, in fact, slightly higher under 65–70–65% (4046.9–4363.6 $m^3$/ha across the three years) than under 70–75–65% (3766.7–3920.1 $m^3$/ha) (Fig. 3; exact dates for all years are given in Supplementary Table S2) — so the 65–70–65% regime should be read as the regime with a lower moisture threshold between irrigations, not as "lower-water."

Five treatments were tested under each regime: an untreated water-only control; Uzgumi (0.7 L/t seed treatment plus a budding-stage 0.4 L/ha foliar spray); and Reflect applied to seed at 200, 300, and 400 mL/t, combined with foliar sprays at the 1–2- and 4–5-true-leaf stages (200 mL/ha each) and at budding (400 mL/ha) — ten regime × treatment combinations in total (Table 2).

Second experiment (Geohumate/Bactofert, reduced fertilizer). Andijon-37 was grown in

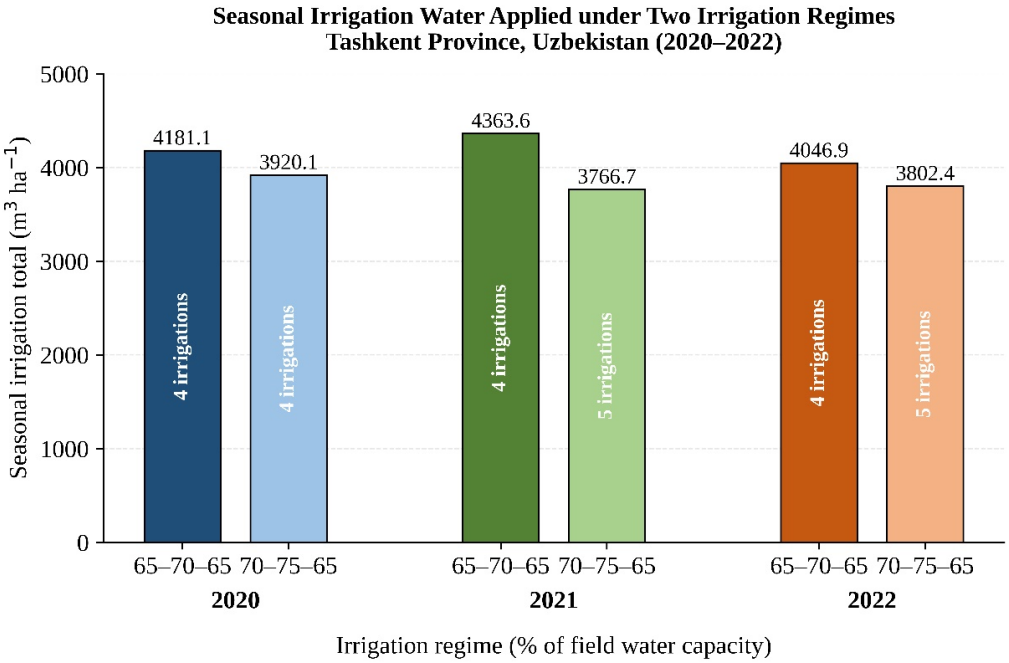



**Fig. 3.** Seasonal irrigation-water volume applied under the two irrigation regimes, Reflect biostimulant experiment (Experiment 1), Tashkent province, 2020–2022; the number of irrigations per season is indicated above each bar. Exact irrigation dates are given in Supplementary Table S2.

**Table 2.** Scheme of Experiment 1: Reflect biostimulant under two irrigation regimes (Tashkent, UzPITI-103 cultivar, 2020–2022).

| No. | Irrigation regime (% FWC) | Treatment | Pre-sowing seed treatment | 1–2 / 4–5 true-leaf stage (mL/ha) | Budding (mL or L/ha) |
|---|---|---|---|---|---|
| 1 | 65–70–65 | Control | untreated | – | – |
| 2 | 65–70–65 | Uzgumi | 0.7 L/t | – | 0.4 L/ha |
| 3 | 65–70–65 | Reflect | 200 mL/t | 200 / 200 | 400 mL/ha |
| 4 | 65–70–65 | Reflect | 300 mL/t | 200 / 200 | 400 mL/ha |
| 5 | 65–70–65 | Reflect | 400 mL/t | 200 / 200 | 400 mL/ha |
| 6 | 70–75–65 | Control | untreated | – | – |
| 7 | 70–75–65 | Uzgumi | 0.7 L/t | – | 0.4 L/ha |
| 8 | 70–75–65 | Reflect | 200 mL/t | 200 / 200 | 400 mL/ha |
| 9 | 70–75–65 | Reflect | 300 mL/t | 200 / 200 | 400 mL/ha |
| 10 | 70–75–65 | Reflect | 400 mL/t | 200 / 200 | 400 mL/ha |

2019–2021 (tissue sampling in 2020–2021) under five treatments (Table 3): a water-only control and an Uzgumi treatment against a full N200P140K100 kg/ha background, and Geohumate (1.0 L/t seed treatment plus foliar sprays at 1.6 L/ha at the 2–3-true-leaf, budding, and flowering stages), Bactofert (500 kg/ha soil-applied before sowing), and their combination against a 40%-reduced N120P84K60 kg/ha background, adopted here as an initial test level rather than an optimized recommendation.

**Table 3.** Scheme of Experiment 2: Geohumate biostimulant and Bactofert biopreparation against a reduced mineral-fertilizer background (Tashkent, Andijon-37 cultivar, 2019–2021).

| No. | Treatment | Fertilizer background | Pre-sowing | 2–3 true-leaf / Budding / Flowering (L/ha) |
|---|---|---|---|---|
| 1 | Control | 100% (N200P140K100) | untreated | – |
| 2 | Uzgumi | 100% (N200P140K100) | 0.7 L/t | – / 0.3 / 0.4 |
| 3 | Geohumate | 60% (N120P84K60) | 1.0 L/t | 1.6 / 1.6 / 1.6 |
| 4 | Bactofert | 60% (N120P84K60) | 500 kg/ha | – / – / – |
| 5 | Geohumate+Bactofert | 60% (N120P84K60) | 1.0 L/t + 500 kg | 1.6 / 1.6 / 1.6 |

Both experiments used three field replications following the standard field-trial methodology for Uzbekistan's cotton-growing zone (Dospekhov 1985; 1981, 2007), with plots of four rows (60 cm spacing, 25 m length; 60 $m^2$ total, 30 $m^2$ harvest area); in Experiment 1, five biostimulant treatments were arranged within each 900 $m^2$ irrigation block. Because full randomization documentation was not available, the trials are described as replicated field-block designs rather than fully randomized designs. Seed in control plots was moistened with plain water; because seed treatment was combined with foliar application in the Reflect, Geohumate, and Uzgumi treatments, their separate contributions cannot be evaluated. Standard agrotechnical operations were applied identically across all treatments.

## Plant Material

The trials used the medium-staple Gossypium hirsutum L. cultivars UzPITI-103 (Reflect/irrigation-regime experiment) and Andijon-37 (Geohumate/Bactofert experiment).

## Biostimulant Products

Reflect is a liquid peat-humic biostimulant (Russia) containing potassium and sodium humates, humic and fulvic acids, microelements, and amino acids (exact concentrations and pH not re-

ported). Geohumate is a liquid humic-fulvic biostimulant (Geofert) containing 34% humic acids, 25% fulvic and other organic acids, N (1.2%), P (0.55%), K (16.5%), Ca (0.56%), S (2.1%), Mg (0.32%), Fe (0.5%), and unspecified Cu, Mn, Co, Mo, B, and Cl. Bactofert is a powder biopreparation containing beneficial microorganisms, humic and fulvic acids, NPK, and microelements. Uzgumi, a liquid humic preparation from Angren coal-mining residues (Uzbekistan), served as a comparison treatment in both experiments. Seed treatment used 600 L water per tonne of seed (three 200 L applications at two-hour intervals) with the biostimulant added at the specified rate; foliar applications used a tractor-mounted sprayer.

## Plant and Soil Sampling and Analysis

Plant samples were collected at budding (root, stem, leaf, square), flowering (stem, leaf, square/flower), and boll opening (stem, leaf, boll wall, seed cotton). Total nitrogen was determined by the Kjeldahl method, phosphorus colorimetrically after wet digestion, and potassium by flame photometry, expressed as % of dry mass; because organ dry-biomass data were unavailable, mass-based nutrient uptake could not be calculated, so results are interpreted as tissue concentrations rather than total nutrient uptake. Soil samples (0–30 and 30–50 cm, two replications, five-point "envelope" composites) were collected at the start and end of each season; humus was determined by the Tyurin method, total N and P by the Maltseva–Gritsenko method, $NO_3^-$–N ionometrically, and available $P_2O_5$ and exchangeable $K_2O$ by the Machigin–Protasov method (Machigin and Protasov 1963), following standard regional agrochemical analytical protocols ( 1963).

## Statistical Analysis

Field trials used three replications. Yield was analyzed by two-factor ANOVA (irrigation regime $\times$ biostimulant treatment) in the Reflect experiment (2020–2022) and by one-way ANOVA in the Geohumate/Bactofert experiment (2019–2021, five treatments), since fertilizer rate and biostimulant product were not fully crossed; treatment differences were compared using $LSD_{0.05}$ in both experiments. Plant-tissue and soil results represent treatment means from composite samples; because individual-replicate laboratory data were unavailable, ANOVA could not be performed for these indicators, and they are interpreted as descriptive differences rather than

statistically confirmed effects. Economic performance was calculated from field-trial yields and 2024 prices: conditional net profit = gross revenue − total production cost; profitability = (conditional net profit / total production cost) × 100.

# Results

## Tissue N, P, and K Concentrations under Reflect across Two Irrigation Regimes

Because laboratory results for individual field replications were not retained for plant tissue, all values below (2021) represent treatment means and are interpreted descriptively rather than as statistically confirmed effects.

At the budding stage, Reflect-associated changes in N, P, and K concentration varied by irrigation regime, organ, nutrient, and rate (Fig. 4). Under the 65–70–65% FWC regime, root nitrogen rose from a 1.34% control to 1.44–1.54% at the 200 and 300 mL/t rates, while root phosphorus (0.43–0.50%) did not exceed the 0.53% control; root potassium exceeded the control (1.500%) only at 400 mL/t (1.755%). Stem nitrogen rose to 1.84–1.94% (versus 1.76% control) at the 300–400 mL/t rates, and leaf nitrogen and phosphorus were elevated across most Reflect treatments (leaf phosphorus 0.82–1.00% versus a 0.74% control). The most numerically consistent difference occurred in square-tissue potassium, which rose from 1.155% (control) to 1.500–2.010% across the Reflect rates. Under the 70–75–65% FWC regime, root nitrogen exceeded the 1.15% control at all rates (1.24–1.54%); stem nitrogen rose from 2.14% to 2.54–2.94% at the higher rates; and leaf nitrogen (3.00–3.55% versus 2.96%) and leaf phosphorus (0.86–0.95% versus 0.78%) were consistently above the control. Square potassium again showed the largest numerical gain, reaching 2.265% at 200 mL/t against a 1.265% control. Root, stem, and square phosphorus and potassium, however, did not follow a consistent dose–response pattern under either regime (full values in Fig. 4).

At flowering, Reflect's effect was most consistent in stem and square/flower nitrogen and in square/flower phosphorus, both of which exceeded the corresponding control at nearly every rate and regime (Fig. 5). Under the 65–70–65% FWC regime, stem nitrogen ranged from 1.76–

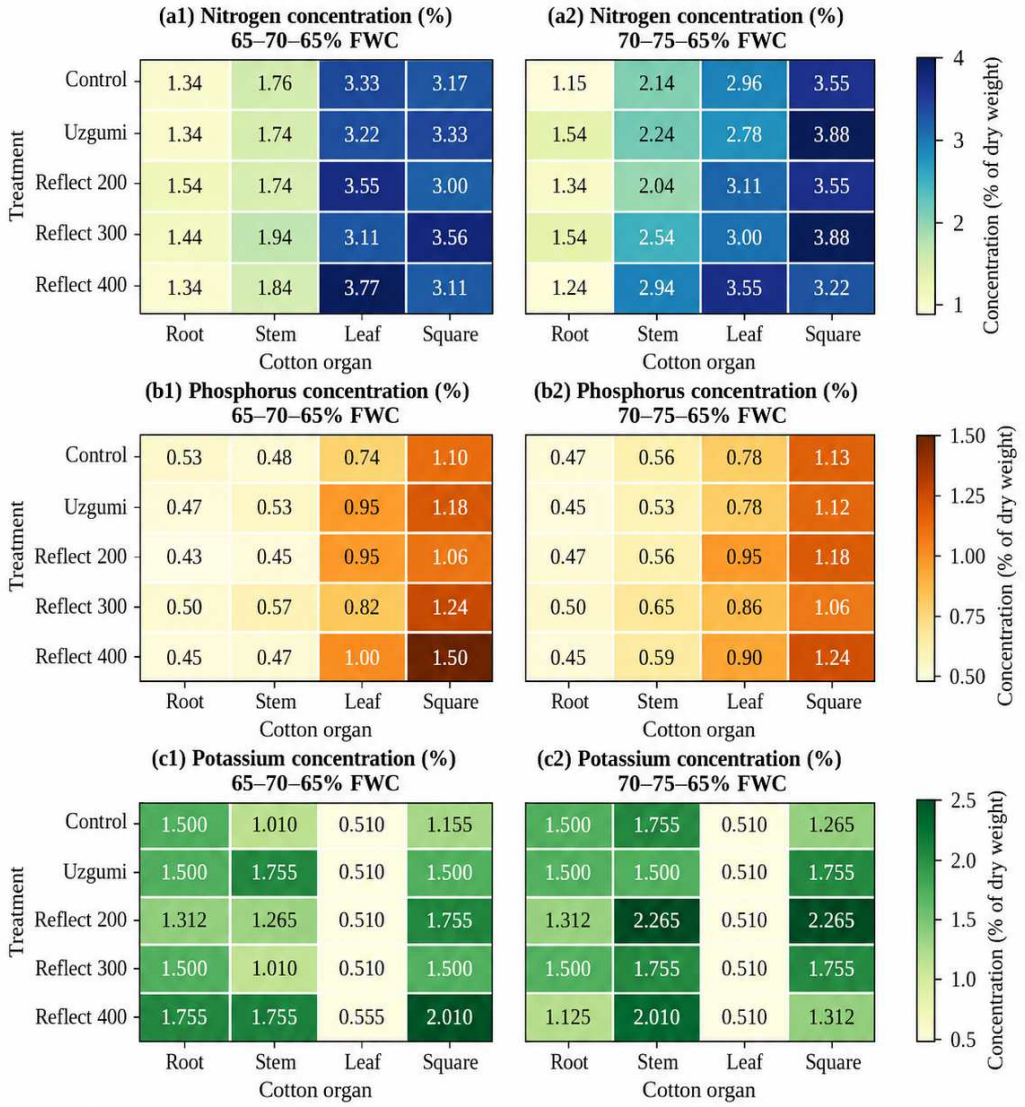



**Fig. 4.** Nitrogen, phosphorus, and potassium concentration (% of dry mass) in cotton root, stem, leaf, and square tissue at the budding stage under Reflect, under two irrigation regimes (Tashkent, UzPITI-103, 2021; treatment means).

1.94% (versus a 1.27% control) and stem phosphorus rose to 1.85–1.90% at the 300–400 mL/t rates; square/flower nitrogen rose from 1.16% to 1.67–1.81%, and square/flower phosphorus reached 1.84–2.00% at the higher rates. Leaf phosphorus (0.64–0.81%) and leaf potassium (1.24–1.50%) were likewise above their respective 0.61% and 0.75% controls. Under the 70–75–65% regime, stem nitrogen rose from 1.16% to 1.94–2.05%, and square/flower nitrogen from 1.05% to 1.81–1.88%, with square/flower phosphorus rising from 0.82% to 1.02–1.23%. Stem potassium and some phosphorus values, in contrast, did not change consistently with dose under either regime.

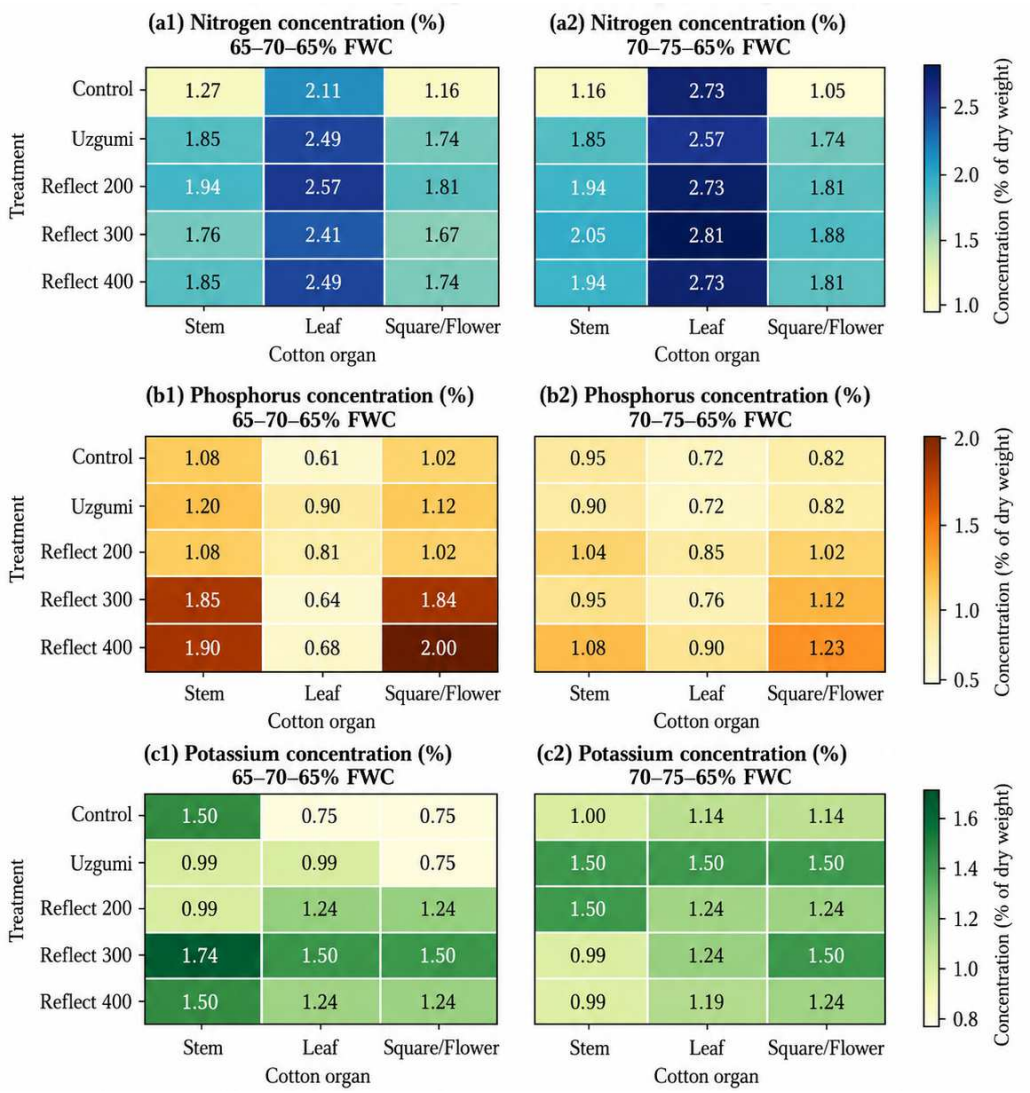



**Fig. 5.** Nitrogen, phosphorus, and potassium concentration (% of dry mass) in cotton stem, leaf, and square/flower tissue at the flowering stage, under three Reflect rates and two irrigation regimes, UzPITI-103 cultivar, Tashkent province, 2021. Values in each cell represent treatment means rather than individual replicate observations.

At boll opening, the clearest differences occurred in boll-wall nitrogen and in seed-cotton

phosphorus and potassium, which exceeded the control at nearly all Reflect rates and both regimes (Fig. 6). Under the 65–70–65% regime, boll-wall nitrogen (1.08–1.19% versus 1.02%) and phosphorus (0.42–0.47% versus 0.40%) were consistently elevated, as was seed-cotton phosphorus (1.25–1.46% versus 1.19%). Under the 70–75–65% regime, seed-cotton phosphorus (1.32–1.60% versus 1.17%) and potassium (1.00–1.18% versus 0.91%) showed the clearest numerical gains, and boll-wall nitrogen was uniformly higher than the control (1.13% versus 1.02%) across all Reflect rates. Leaf nitrogen, by contrast, tended to fall slightly below the control at this stage under both regimes, while stem and seed-cotton nitrogen changed in different directions depending on rate and regime.

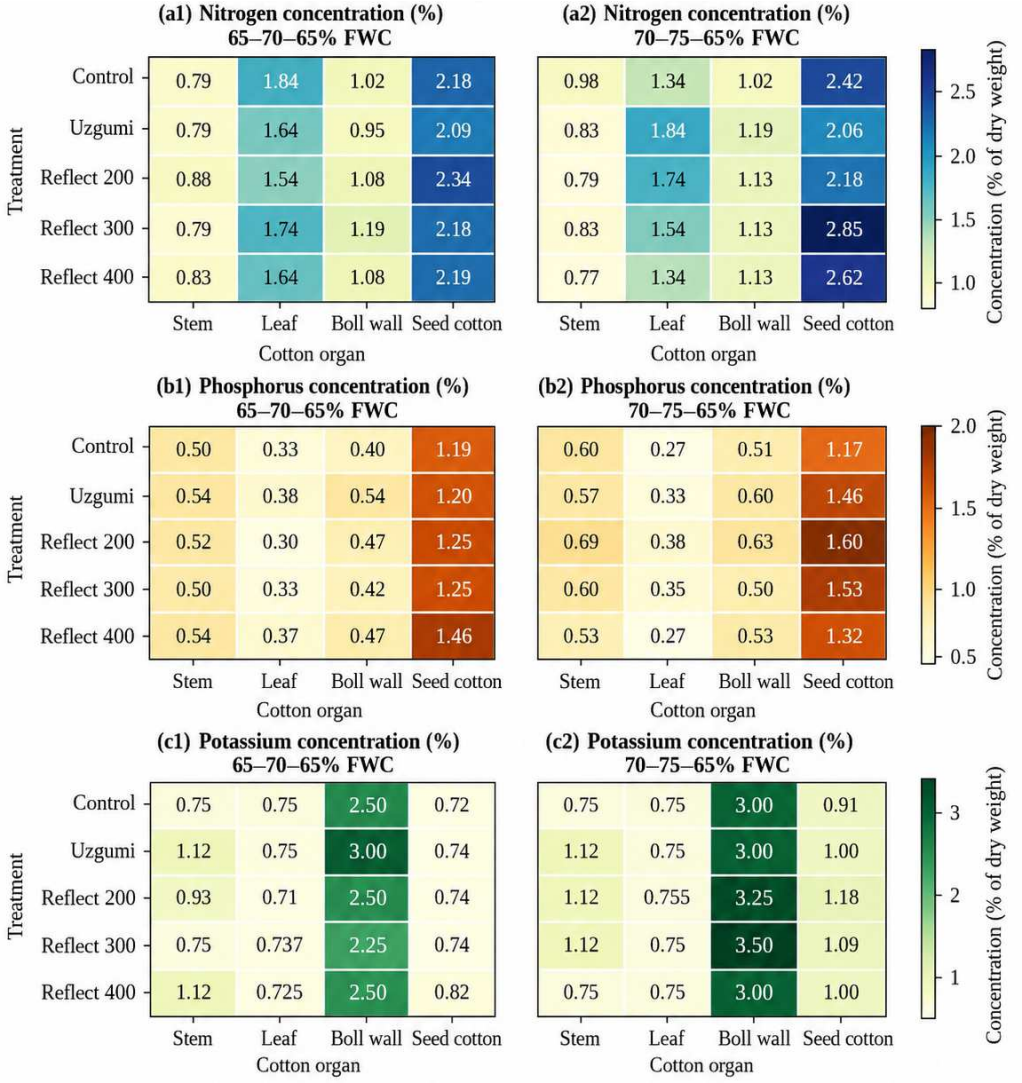



**Fig. 6.** Nitrogen, phosphorus, and potassium concentration (% of dry mass) in cotton stem, leaf, boll-wall, and seed-cotton tissue at the boll-opening stage, under three Reflect rates and two irrigation regimes, UzPITI-103 cultivar, Tashkent province, 2021. Values in each cell represent treatment means rather than individual replicate observations.

Overall, certain organ–nutrient–rate combinations under Reflect showed higher NPK concentrations than the control at every stage, most consistently in square/square-flower potassium and phosphorus, boll-wall nitrogen, and seed-cotton phosphorus and potassium; a uniform response was not observed in all organs, and — because individual-replicate data were unavailable — these are descriptive rather than statistically confirmed differences.

## Residual Soil Agrochemical Status under Reflect

At the end of the 2020 growing season, humus, total N and P, and available $NO_3^-$–N, $P_2O_5$, and $K_2O$ in the 0–30 cm layer changed in different directions depending on Reflect rate and irrigation regime, with no uniform pattern (Fig. 7; treatment means, not statistically tested). Under the 65–70–65% FWC regime, the 200 mL/t rate showed humus of 0.771%, total N of 0.067%, and total P of 0.115% — above the control's 0.749%, 0.064%, and 0.076% — while the 300 and 400 mL/t rates fell below the control for humus (0.695–0.698%) and total N (0.056–0.058%) even as total P remained elevated (0.100% at both rates). A similar pattern occurred under the 70–75–65% regime, where the 200 mL/t rate again showed the most consistent numerical gain (humus 0.726%, total N 0.061%, total P 0.115%, versus a 0.713%, 0.056%, and 0.100% control). Exchangeable $K_2O$ across nearly all Reflect treatments and both regimes was at or below the corresponding control (200–220 mg/kg versus a 220–240 mg/kg control).

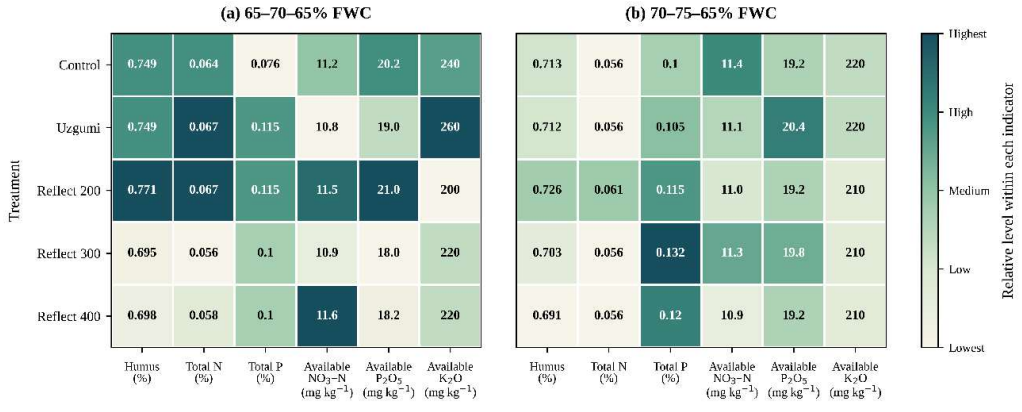



**Fig. 7.** Agrochemical indicators of the 0–30 cm soil layer at the end of the 2020 growing season, under three Reflect rates and two irrigation regimes: humus, total nitrogen, total phosphorus, $NO_3^-$–N, available $P_2O_5$, and exchangeable $K_2O$, UzPITI-103 experiment, Tashkent province. Values in each cell represent treatment means; because colors are normalized separately (min–max) for each indicator, color intensity is not comparable across different indicators.

These results do not support a general conclusion that Reflect slowed the seasonal decline

of soil fertility. Because pre-sowing soil indicators were determined for the trial field as a whole rather than by treatment, the season-long change cannot be precisely attributed to Reflect for each treatment; the results indicate only descriptive end-of-season differences among treatments.

## Tissue N, P, and K under Geohumate and Bactofert against a Reduced Fertilizer Background

Tissue N, P, and K under the reduced N120P84K60 background (Geohumate, Bactofert, and their combination) were compared descriptively with the N200P140K100-background control at budding, flowering, and boll opening (2020–2021 treatment means; Fig. 8).

At budding, root nitrogen (1.55–1.58%) and phosphorus (0.59–0.65%) in all three preparation treatments were at or above the 1.55% and 0.53% control; root potassium exceeded the control in the Geohumate (1.755%) and Bactofert (1.500%) treatments but fell below it in the combined treatment (1.010% versus 1.235%). Stem potassium across all three reduced-fertilizer preparation treatments exceeded the fully-fertilized control (2.010–2.520% versus 1.500%), and square N, P, and K were numerically higher in all three treatments (square nitrogen 3.11–3.55% versus 3.00%; phosphorus 1.11–1.24% versus 0.76%; potassium 1.125–1.500% versus 1.100%). Leaf indicators showed a less consistent direction.

At flowering, stem N, P, and K and square/flower N and P exceeded the control in all three reduced-fertilizer treatments, most markedly in the Geohumate+Bactofert package (stem N, P, K of 2.04%, 1.26%, and 2.00% versus a 1.61%, 0.81%, and 1.10% control). Square/flower nitrogen (1.74–1.88% versus 1.60%) and phosphorus (1.02–1.36% versus 0.93%) were likewise elevated across all three preparations. Leaf indicators varied in direction: leaf nitrogen rose in the Geohumate and combined treatments (2.33–2.41% versus 2.25%) but fell in the Bactofert treatment (2.17%), while leaf potassium was elevated in all three (1.24–1.74% versus 0.99%).

At boll opening, the most consistent differences occurred in seed-cotton tissue: nitrogen, phosphorus, and potassium in the Geohumate, Bactofert, and Geohumate+Bactofert treatments were at or above the fully-fertilized control (nitrogen 2.26–2.77% versus 2.00%; phosphorus 1.32–1.46% versus 1.25%; potassium 1.25–2.75% versus 1.19%). Boll-wall nitrogen (0.83–

0.88% versus 0.82%) and phosphorus (0.50–0.53% versus 0.43%) were also numerically higher across the three treatments. Stem and leaf results were more variable, with some values below the fully-fertilized control.

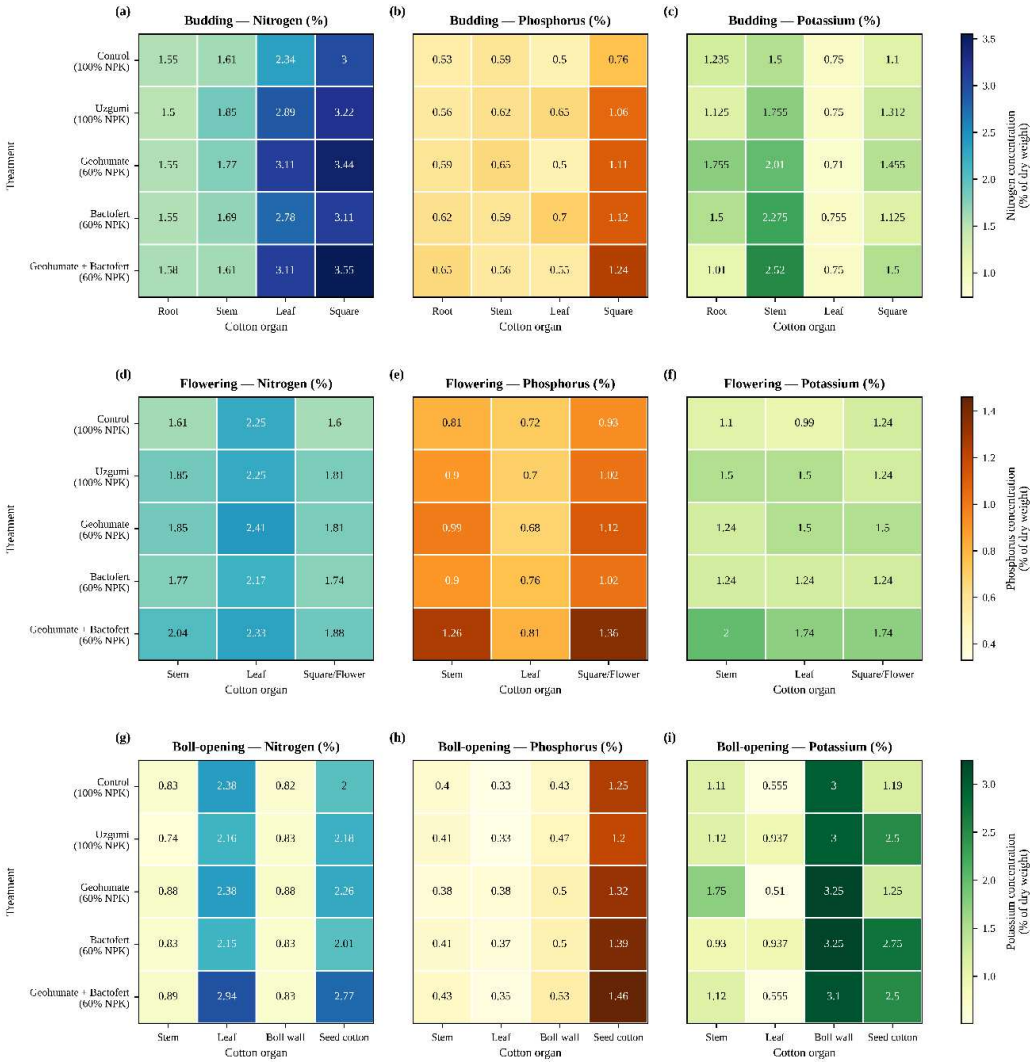



**Fig. 8.** Nitrogen, phosphorus, and potassium concentration (% of dry mass) in cotton organs at the budding, flowering, and boll-opening stages, under the N200P140K100-background control and Uzgumi treatments and the N120P84K60-background Geohumate, Bactofert, and Geohumate + Bactofert treatments, Andijon-37 cultivar, Tashkent province, 2020–2021. Values in each cell represent treatment means rather than individual replicate observations.

Because no biostimulant-free control was included at the N120P84K60 background, these differences cannot be attributed separately to the fertilizer reduction, Geohumate, Bactofert, or their interaction; they represent the descriptive performance of the tested reduced-fertilizer-plus-preparation packages relative to the fully-fertilized control.

## Residual Soil Fertility under Geohumate

At season's end, humus in the 0–30 cm layer under the Geohumate and Geohumate+Bactofert treatments (0.839–0.870%) approached or exceeded the fully-fertilized control (0.880%), and available N and P were comparable to the control (Fig. 9; treatment means, not statistically tested). Despite the 40% fertilizer reduction, these treatments did not show a greater decline in soil fertility than standard practice; exchangeable $K_2O$ was in fact higher under Geohumate and Geohumate+Bactofert (240–280 mg/kg) than under the fully-fertilized control (260 mg/kg).

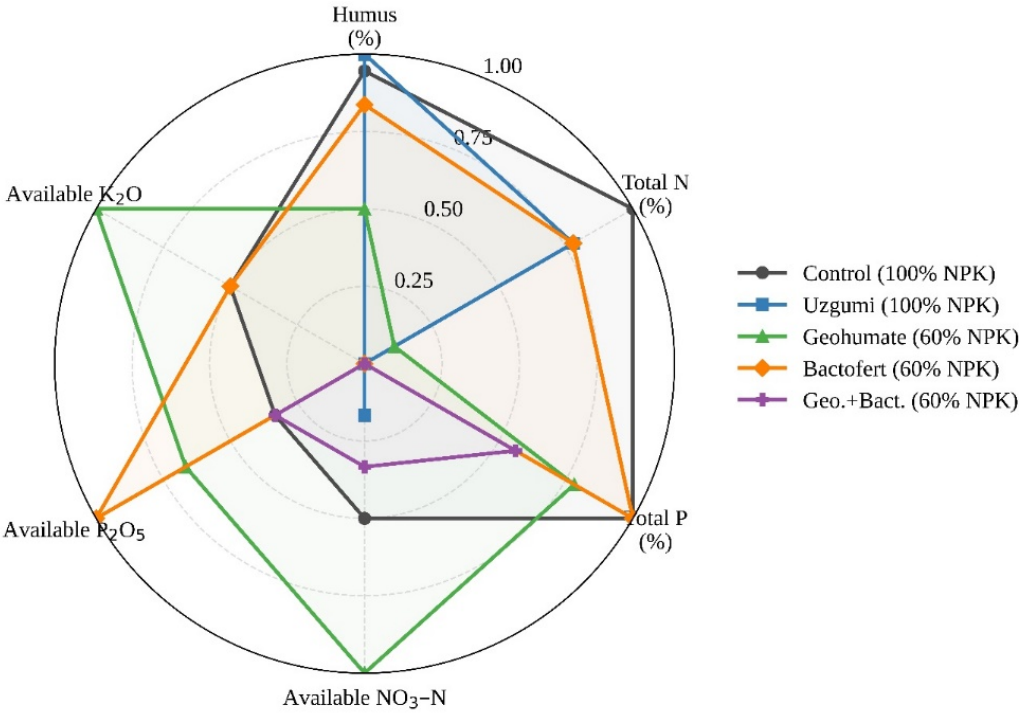



**Fig. 9.** Agrochemical status of soil at the end of the growing season under Geohumate/Bactofert against a 40%-reduced mineral-fertilizer background (Tashkent, 0–30 cm layer, 2021; treatment means, n = 5 treatments; values are min–max normalized separately for each indicator, so radial position reflects relative rather than absolute level).

## Economic Performance

Economic performance was calculated by applying 2024 prices to field-trial yields; conditional net profit is gross revenue minus total production cost, and profitability is their ratio expressed as a percentage. These are retrospective calculations based on treatment field yields rather than individual economic replications.

In the Reflect experiment, yield, net profit, and profitability rose with Reflect rate under

both irrigation regimes (Fig. 10). Under the 65–70–65% FWC regime, the highest values occurred at 400 mL/t (35.3 c/ha, 10,006.3 thousand UZS/ha, 59.5%), exceeding the control (30.1 c/ha, 6097.3 thousand UZS/ha, 36.3%) by 5.2 c/ha, 3909.0 thousand UZS/ha, and 23.2 percentage points. Under the 70–75–65% regime, the highest outcome occurred at 300 mL/t (37.1 c/ha, 11,378.6 thousand UZS/ha, 67.6%) rather than at 400 mL/t (36.8 c/ha, 11,146.3 thousand UZS/ha, 66.2%), exceeding the control (31.8 c/ha, 7389.3 thousand UZS/ha, 44.0%) by up to 5.3 c/ha, 3989.3 thousand UZS/ha, and 23.6 percentage points. Raising the rate to 400 mL/t thus did not provide an additional economic benefit under every irrigation regime, though net profit and profitability for all Reflect rates exceeded the control under both regimes.

In the Geohumate/Bactofert experiment, the N200P140K100 control yielded 35.1 c/ha (net profit 7977.3 thousand UZS/ha, profitability 47.5%); Uzgumi raised this to 38.9 c/ha (10,598.7; 62.9%). Against the reduced N120P84K60 background (Fig. 11), Geohumate gave the highest outcome in the experiment — 40.1 c/ha, 14,932.0 thousand UZS/ha, and 111.8% profitability — exceeding the fully-fertilized control by 5.0 c/ha, 6954.7 thousand UZS/ha, and 64.3 percentage points.

Bactofert (39.6 c/ha; 13,753.4; 97.0%) and Geohumate+Bactofert (39.7 c/ha; 13,649.9; 95.1%) likewise exceeded the control, though combining the two products did not improve on Geohumate applied alone. Total production cost in the reduced-fertilizer treatments (13,350.5–14,350.5 thousand UZS/ha) was below the fully-fertilized control (16,778.7 thousand UZS/ha), so the higher profitability reflects both higher yield and lower fertilizer cost.

Because no biostimulant-free control was included at the N120P84K60 background, and a detailed cost breakdown was unavailable for the second experiment, these elevated economic indicators cannot be attributed solely to Geohumate or Bactofert, nor can the separate contributions of fertilizer, product, labor, and irrigation costs be assessed. Overall, conditional net profit and profitability in the Reflect, Geohumate, and Bactofert treatments exceeded their respective controls at 2024 prices; because these values were not statistically tested with individual economic replications and are sensitive to price changes, they should be interpreted as the calculated economic outcomes of the tested technological variants rather than firm recommendations.

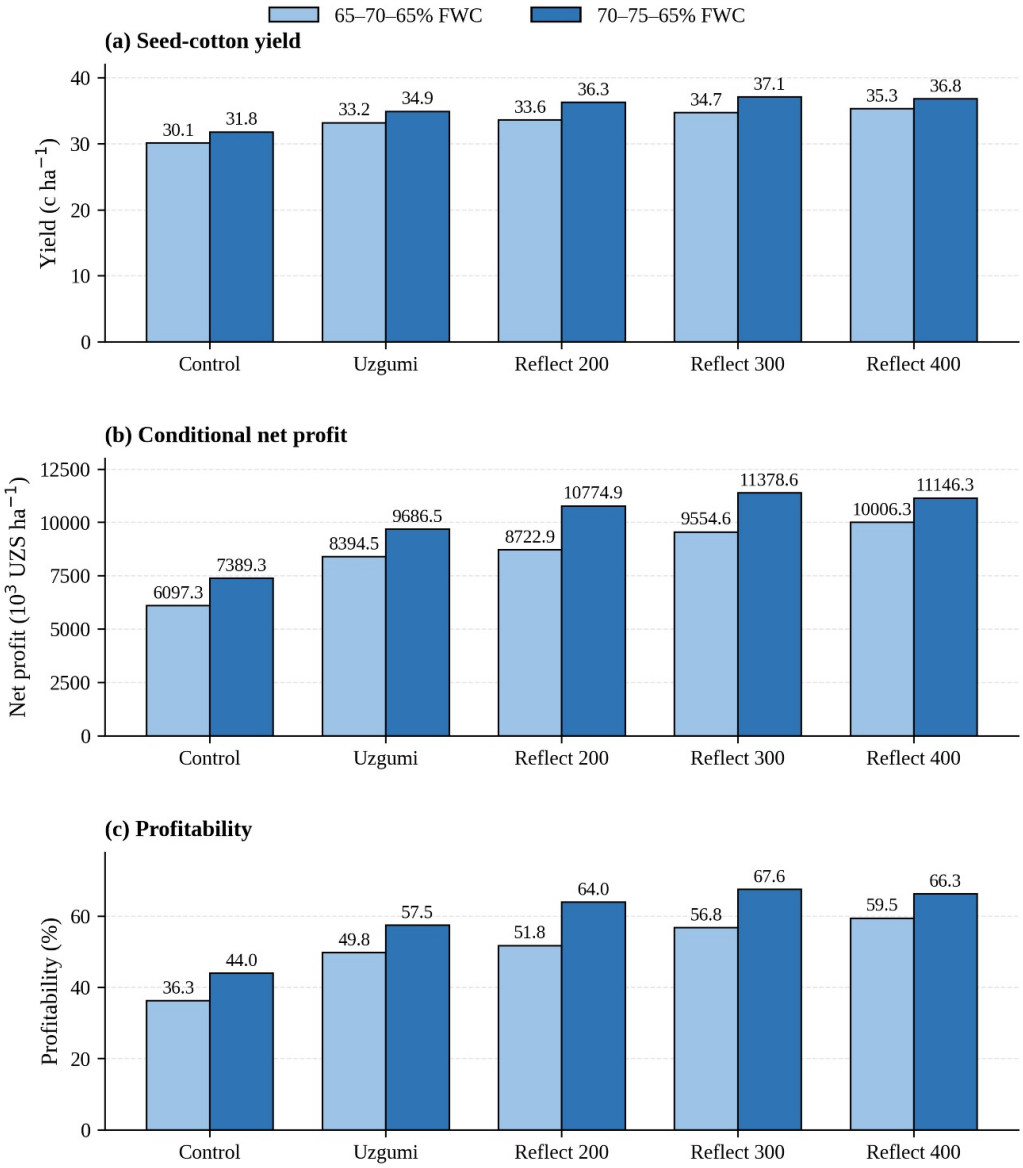



**Fig. 10.** Economic performance of Reflect biostimulant treatment (seed-cotton yield, conditional net profit, and profitability), UzPITI-103 cotton, two irrigation regimes (Tashkent province, 2024 prices; treatment means, n = 4 treatments $\times$ 2 irrigation regimes). The dark orange line marks the treatment with the highest value in each panel.

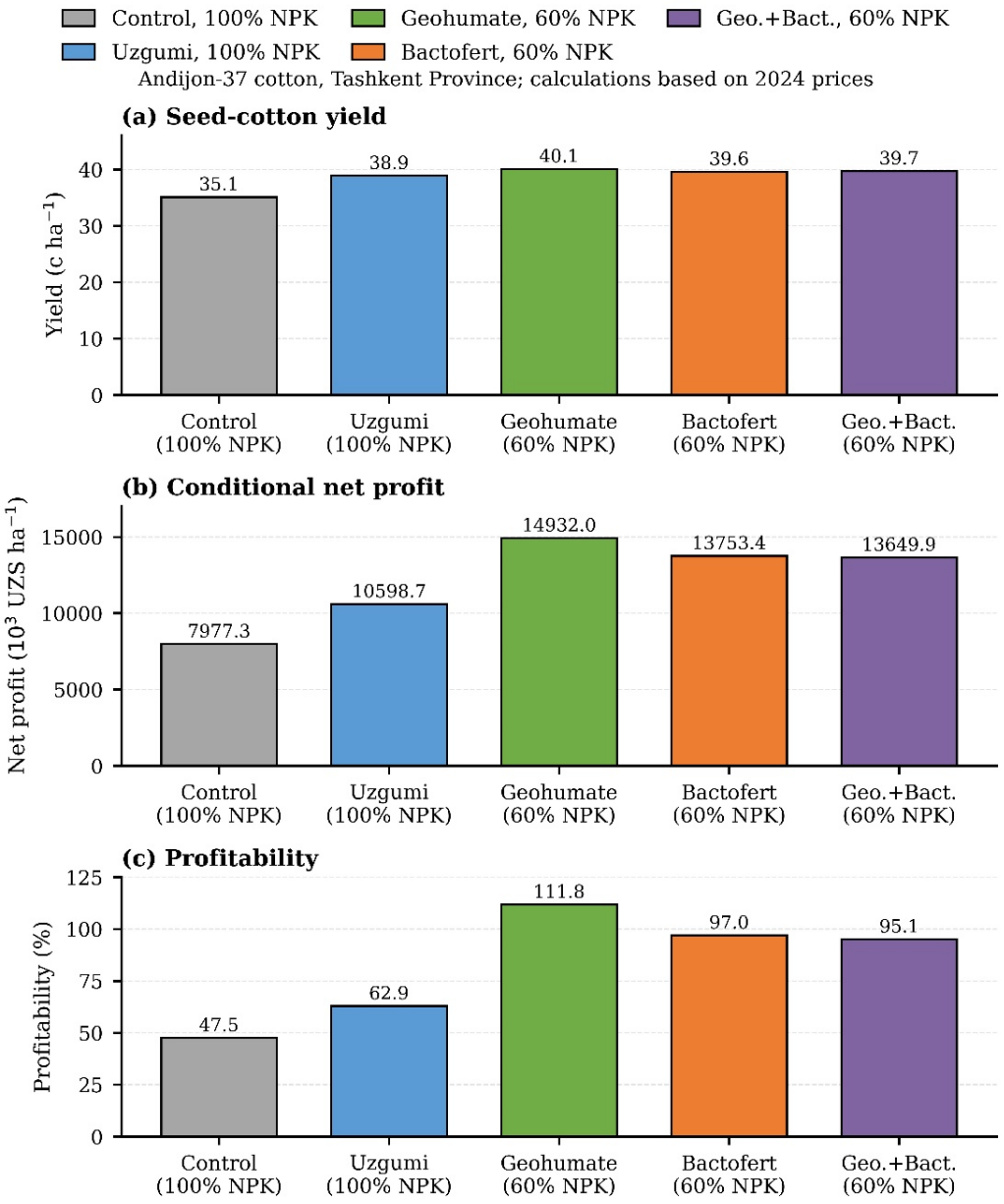



**Fig. 11.** Economic performance of Geohumate/Bactofert biostimulant treatment (seed-cotton yield, conditional net profit, and profitability), Andijon-37 cotton, full and 40%-reduced mineral-fertilizer backgrounds (Tashkent province, 2024 prices; treatment means, n = 4 treatments). The dark orange line marks the treatment with the highest value in each panel.

# Discussion

## Effect of Reflect on Cotton Mineral Nutrition under Different Irrigation Regimes

Reflect's effect on tissue N, P, and K varied with irrigation regime, rate, stage, and organ, with no uniform increase across all elements — indicating that the product changes how nutrients are taken up, diluted, and redistributed among organs rather than raising concentration everywhere. Effects were most consistent for square/square-flower phosphorus and potassium at budding and flowering and for boll-wall nitrogen and seed-cotton phosphorus and potassium at boll opening, suggesting Reflect may particularly influence phosphorus allocation to reproductive organs (important for energy metabolism and boll formation) and potassium-related assimilate transport. These patterns are broadly consistent with Zhang et al. (2025), who found that fulvic acid raised boll number and phosphorus-use efficiency without a uniform rise in tissue phosphorus, and with Li et al. (2025a), whose finding that a moderate phosphorus rate combined with fulvic acid outperformed a higher rate helps explain why the 400 mL/t Reflect rate did not outperform 300 mL/t on every indicator here. Where NPK concentrations fell below the control under certain Reflect treatments, this need not indicate reduced overall nutrient uptake, given the well-documented "dilution effect" whereby fast biomass gain can lower percentage tissue concentration even as total uptake rises (Pettigrew and Meredith 1997).

## Interaction between Irrigation Regime and Biostimulant Effect

Some of the higher NPK values occurred under the 65–70–65% FWC regime and others under 70–75–65%, so Reflect's effectiveness cannot be assessed as uniform across water-supply conditions. This is consistent with reports that irrigation and nitrogen fertilization interact to affect cotton nutrition (Ibrahim et al. 1997) and that tissue nutrient concentration and whole-plant accumulation do not always move together as irrigation volume changes (Li et al. 2025b); it also parallels findings that fulvic acid's benefits are bounded by water supply, improving indicators under moderate-to-good irrigation but not under severe deficit (He et al. 2026), and that biostimulant/water-technology combinations can raise water- and fertilizer-use efficiency

under managed irrigation (Sun et al. 2024). Notably, the 65–70–65% regime in this trial should not be described as "unirrigated" or "severe drought" — it represents a controlled, relatively lower soil-moisture threshold whose seasonal water volume was not lower than that of the 70–75–65% regime. The results therefore show Reflect's effectiveness across different managed soil-moisture thresholds, not evidence of irrigation-water savings.

## Soil Agrochemical Status Following Reflect Treatment

No uniform pattern was found in end-of-season soil humus, total N and P, or available nutrients under Reflect. A decline in available soil nutrients should not automatically be read as a negative effect on soil fertility — it could equally reflect greater plant uptake, leaching, conversion to less-available forms, or sampling variability, which cannot be distinguished without plant dry-mass and hectare-based nutrient-removal data. Humic acid combined with mineral fertilizer has improved soil properties on saline-alkaline soil (Hu et al. 2021), though that trial's soil differed considerably from the typical serozem used here; biostimulant efficacy may depend on initial humus, pH, salinity, texture, and moisture, none of which (beyond humus) were measured in this trial. Reduced mineral fertilizer combined with organic fertilizer has raised available N, P, and beneficial-microorganism abundance elsewhere (Shi et al. 2023); because soil microbial composition was not assessed here, a similar mechanism can be discussed as plausible but not confirmed.

## Effect of Geohumate and Bactofert against a Reduced Fertilizer Background

Geohumate, Bactofert, and their combination against the N120P84K60 background maintained N, P, and K in several organs at or above the fully-fertilized control, most consistently in the Geohumate+Bactofert package — elevated square nutrients at budding, stem and square/flower nutrients at flowering, and seed-cotton nutrients at boll opening — indicating improved nutrient supply from early reproductive development through to yield-forming organs. Hao et al. (2022) similarly found that an organic component combined with 80% of the mineral rate raised cotton N and P uptake, reproductive-organ partitioning, yield, and fertilizer-use efficiency, though

their design better separates the organic and fertilizer-reduction contributions than the present one allows. Shi et al. (2023) found reduced chemical fertilizer combined with biological-organic fertilizer outperformed plain organic fertilizer for soil N, P, and yield, supporting the plausibility of a Bactofert-related mechanism, though rhizosphere microbial or enzyme activity was not measured here. The elevated yield and profitability under Geohumate are broadly consistent with the average gains reported in the humic-acid meta-analysis of Ma et al. (2024), though that synthesis spans diverse crops and conditions and cannot specify Geohumate's precise effect here; as in Zhang et al. (2025), yield gains need not require a uniform rise in tissue NPK, since biostimulants can also act through root activity, photosynthesis, boll retention, and assimilate partitioning. Critically, because no biostimulant-free control was included at the N120P84K60 background, these results demonstrate the performance of reduced-NPK-plus-biostimulant packages, not the separate contributions of fertilizer reduction and biostimulant; the defensible conclusion is that these packages matched or exceeded the fully-fertilized control, not that Geohumate "allowed" the 40% reduction.

## Interpretation of the Economic Results

Reflect raised net profit and profitability relative to the control under both irrigation regimes, with the highest outcome at 400 mL/t under 65–70–65% FWC and at 300 mL/t under 70–75–65% — showing that the economically optimal rate does not always coincide with the biologically maximal one, since additional product cost at 400 mL/t was not always matched by a sufficient yield gain. In the second experiment, N120P84K60+Geohumate gave the best outcome overall, and Bactofert and Geohumate+Bactofert also exceeded the fully-fertilized control, though the combined treatment was not superior to Geohumate alone. These are retrospective, 2024-price-based calculations without a detailed cost breakdown for the second experiment, so they represent a relative economic comparison among treatments rather than firm production forecasts; a sensitivity analysis across product, fertilizer, water, fuel, and cotton prices would be needed to confirm their robustness.

## Practical Significance and Limitations

Reflect's effect on nutrition and economic performance depended on soil-moisture level, with 300–400 mL/t giving the most promising reproductive-organ and economic results at particular stages and regimes; the optimal rate should be verified across other soil-climatic conditions before a single recommendation is made. Reduced-fertilizer Geohumate/Bactofert packages showed higher agronomic and economic outcomes than full-NPK, relevant to fertilizer-saving technology, but the 40% reduction level cannot be independently validated without a biostimulant-free reduced-fertilizer control. Tissue NPK values are treatment means without retained replicate variance, so statistical reliability cannot be assessed, and percentage concentration alone cannot establish total nutrient uptake or removal — future trials should measure organ dry mass, NPK accumulation, and nutrient-use efficiency. Because seed treatment was combined with foliar feeding in the Reflect, Geohumate, and Uzgumi treatments, their separate contributions cannot be determined; a factorial design, together with confirmed randomization and soil pH/EC/bulk-density measurements, would strengthen future work. Overall, biostimulant effects on cotton nutrition and yield were neither simple nor unidirectional, depending on product, rate, irrigation regime, fertilizer background, stage, and organ; Reflect performed promisingly across managed soil-moisture conditions, and Geohumate/Bactofert within a reduced-fertilizer package, but these findings should be treated as a basis for further factorial, multi-site trials rather than final confirmation of water savings or a validated 40% fertilizer reduction.

## Conclusion

Overall, this study indicates that humic-based biostimulants are associated with a descriptive, resource-saving pattern of maintaining cotton yield and profitability under reduced mineral fertilization in Tashkent province; because the underlying tissue and soil data are treatment means without individual replicate observations, these results are hypothesis-generating rather than confirmatory.

Reflect was associated with numerically higher nitrogen, phosphorus, and potassium content in cotton tissue relative to an untreated control at budding, flowering, and boll-opening stages

under both irrigation regimes, with a larger relative difference under the better-irrigated (70–75–65% FWC) regime; this pattern was not independently statistically tested and should be regarded as descriptive.

In Experiment 2, the Geohumate and Geohumate+Bactofert treatments (40%-reduced fertilizer, N120P84K60) showed tissue N-P-K content numerically at or above the fully-fertilized control at all three sampling stages, together with a smaller seasonal decline in soil humus and available nutrients than the control; because no biostimulant-free N120P84K60 treatment was included, this descriptive observation concerns the reduced-fertilizer-plus-biostimulant package as a whole and is not evidence that the biostimulants compensated for the fertilizer reduction.

In these same trials, seed-cotton yield — independently measured and analyzed using two-factor ANOVA in the Reflect experiment and one-factor ANOVA in the Geohumate/Bactofert experiment, both with $LSD_{0.05}$ — was statistically significantly higher under Reflect (3.1–5.3 c/ha) and Geohumate/Bactofert (3.4–6.6 c/ha) than under the respective controls, and at 2024 prices both biostimulants raised calculated net profit and profitability relative to their controls; because yield and tissue nutrient content were not analyzed jointly, this finding should be regarded as independent, supporting context rather than as statistical confirmation of the nutrient-content results.

These descriptive findings support further, deeper investigation of Reflect and Geohumate for cotton nutrition and profitability on the phosphorus-poor irrigated serozem soils of Tashkent province, but a firm recommendation — particularly for the 40% fertilizer-reduction level drawn from the manufacturer's source — requires replicated tissue and soil sampling within a fully factorial design (including a biostimulant-free reduced-fertilizer control), biomass measurements suitable for calculating true mass-based nutrient uptake, and confirmation across additional sites and seasons.

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