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Mitigating PEG-induced drought stress in *Solanum lycopersicum* L. via chitosan oligosaccharide foliar application: A Simulated study on photosynthetic retention and antioxidant defense

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Abstract

Background: Drought stress remains a primary abiotic constraint limiting crop productivity globally, with yield losses in semi-arid agricultural zones remaining substantial due to oxidative damage, stomatal closure, and disrupted photosynthetic machinery.[12] Biostimulant applications, particularly chitosan oligosaccharides (COS), have been proposed as a scalable, eco-friendly strategy for enhancing drought resilience in resource-constrained farming systems [3].

Objective: This study evaluated the effectiveness of a 12-week COS foliar application protocol, comprising pre-stress priming and post-stress recovery treatments, on photosynthetic efficiency, antioxidant enzyme activity, and biomass accumulation in tomato (*Solanum lycopersicum* L.) under simulated drought conditions.

Method: A two-arm, parallel-group design compared COS-treated plants against water-sprayed controls under polyethylene glycol (PEG)-induced drought stress. **This study uses a simulated dataset created for academic training purposes;** all physiological measurements, growth parameters, and enzymatic activity data were generated to reflect plausible patterns consistent with published plant biostimulant literature and do not represent data collected from real experiments or actual greenhouse trials. One hundred plants (n = 50 per treatment) were monitored over 12 weeks, with physiological parameters measured at baseline and every three weeks.

Key Results: Mean net photosynthetic rate declined from 18.4 to 6.2 μmol CO_2 m^{-2} s^{-1} in control plants over 12 weeks of drought, compared with a decline from 18.6 to 13.8 μmol CO_2 m^{-2} s^{-1} in the COS-treated arm. Relative water content at 12-week follow-up was higher in the COS arm across all developmental stages, with the largest absolute difference observed during the flowering stage (72.4% versus 58.9%). Membrane lipid peroxidation (MDA content) was the most severely affected parameter in untreated controls at peak stress (3.8-fold increase over baseline).

Conclusion: The COS foliar intervention was associated with substantially greater maintenance of photosynthetic function and membrane integrity than untreated controls, suggesting that COS-based biostimulants represent a promising, scalable model for drought mitigation in semi-arid tomato production, pending confirmation with data from an actual implemented greenhouse or field program.

Keywords: Chitosan oligosaccharide, Drought tolerance, *Solanum lycopersicum*, photosynthetic efficiency, antioxidant enzymes, abiotic stress, plant biostimulant

Introduction

Drought is widely recognized as the single largest abiotic contributor to global crop yield reduction, with the burden of water deficit falling disproportionately on semi-arid agricultural regions where irrigation infrastructure remains constrained and climate variability is increasing [1]. Semi-arid cropping systems, occupying an intermediate position between well-watered intensive agriculture and rainfed marginal lands, frequently experience a distinctive combination of rising drought frequency, driven by shifting precipitation patterns and elevated evapotranspiration, alongside soil management practices that have not kept pace with this climatic transition [2].

Even where drought-tolerant cultivars are available and accessible, sustained crop performance depends critically on physiological acclimation capacity, which is itself influenced by a complex interplay of osmotic adjustment, antioxidant defense activation, hormonal signaling, and the practical logistics of applying exogenous protectants at optimal developmental windows [3]. Conventional breeding approaches alone, which rely on genetic selection for inherent drought tolerance traits, have repeatedly been shown to result in insufficient adaptation rates relative to the accelerating pace of climate change, particularly among smallholder populations facing limited access to improved germplasm or costly agrochemical inputs [4].

Biostimulant-led interventions, in which naturally derived compounds such as chitosan oligosaccharides (COS) are applied as foliar sprays or seed treatments to enhance endogenous stress-response pathways, have been proposed and tested as a scalable strategy for addressing these physiological and structural barriers to drought resilience, particularly in settings where synthetic chemical protectants are economically or environmentally unsustainable [5]. COS models offer several theoretically advantageous features relative to conventional agronomic management alone: reduced dependency on synthetic fertilizers and pesticides, the opportunity for targeted activation of salicylic acid and jasmonic acid signaling cascades that prime systemic acquired resistance, and the potential for COS, being a deacetylated derivative of chitin, to serve dual roles as both elicitor and mild carbon source without phytotoxicity at appropriate concentrations [6].

Despite a growing evidence base supporting COS applications for drought mitigation in various crop species, evidence specific to semi-arid tomato production systems, which differ in important physiological respects from both the controlled-environment studies where many foundational COS mechanisms were elucidated and the fully irrigated commercial operations with comparatively richer input availability, remains comparatively limited [7]. Furthermore, many existing studies report aggregate growth outcomes without disaggregating by developmental stage or systematically documenting the specific physiological parameters most responsive to COS treatment, limiting the practical actionability of findings for precision application timing and dosage optimization [9].

The present study was designed with three specific objectives: (i) to evaluate the effect of a 12-week COS foliar intervention on net photosynthetic rate trajectory relative to untreated controls under PEG-induced drought stress in *Solanum lycopersicum* L.; (ii) to compare relative water content and membrane integrity at 12-week follow-up between treatment arms across developmental subgroups (vegetative, flowering, fruit set); and (iii) to characterize the most severely impacted physiological parameters at peak drought stress. It is hypothesized that the COS intervention will produce significantly greater maintenance of photosynthetic function and significantly lower oxidative damage relative to controls across all developmental stages, with the largest absolute benefit expected during the flowering stage when reproductive tissues are most vulnerable to dehydration-induced abortion.

Literature Review

The stress physiology framework, widely applied as a conceptual foundation for abiotic stress mitigation research, emphasizes the importance of proactive physiological priming and sustained metabolic homeostasis, in contrast to reactive damage repair initiated only after visible symptom expression [10]. Within this framework, biostimulant programs are conceptualized as a mechanism for extending the reach of endogenous stress-acclimation responses beyond what constitutive genetic tolerance, given typical trade-off constraints with growth and reproduction, can feasibly provide through unaided development alone [11].

A substantial body of controlled-environment and field-trial evidence, much of it generated in Mediterranean and South Asian semi-arid settings, has examined COS-mediated drought interventions, with most studies reporting statistically significant improvements in photosynthetic retention favoring the COS-treated arm relative to untreated controls, typically in the range of 30% to 60% additional photosynthetic rate maintenance over stress periods ranging from four to twelve weeks.[12,13] Proposed mechanisms underlying this effect include enhanced antioxidant enzyme activity (superoxide dismutase, catalase, peroxidase) resulting from upregulated gene expression via MAPK cascade activation, improved osmolyte accumulation (proline, soluble sugars) that maintains turgor pressure and protects macromolecular structures, and reinforcement of cuticular wax deposition that reduces non-stomatal water loss delivered through repeated foliar contact rather than a single pre-planting seed treatment.[14]

Antioxidant defense research more broadly has identified a consistent, recurring hierarchy of parameter sensitivity across diverse abiotic stress contexts, commonly organized into primary response markers (ROS scavenging enzyme induction, proline accumulation), secondary damage indicators (membrane lipid peroxidation, chlorophyll degradation), and tertiary functional consequences (photosynthetic rate decline, biomass reduction).[15] Membrane integrity and MDA content have been particularly well documented as sensitive early-warning indicators in lower-input agricultural settings, where visible wilting may lag behind irreversible cellular damage by several days, and have been repeatedly identified as among the most responsive parameters to exogenous elicitor application across multiple comparative biostimulant efficacy surveys [1].

Developmental-stage heterogeneity in drought sensitivity and treatment response has also been documented extensively, with vulnerability rising sharply during reproductive transitions across virtually all studied crop species, while treatment responsiveness to both genetic and exogenous interventions has shown more mixed stage-related patterns, with some studies suggesting that flowering-stage plants, which are actively allocating resources to reproductive sinks and may have accumulated fewer compensatory reserves than vegetative-stage plants, may show comparatively larger absolute treatment response to priming-focused biostimulant applications [17]. However, relatively few COS intervention studies have systematically reported stage-disaggregated physiological outcomes alongside their aggregate trial results, limiting the field's ability to determine whether intervention benefit is uniform across development or concentrated within specific phenological windows [18].

Semi-arid tomato production specifically has received comparatively less direct research attention within the COS-drought literature relative to either fully irrigated commercial tomato or staple cereal crops, despite representing a substantial and growing proportion of vegetable production in many low- and middle-resource countries undergoing dietary diversification [19]. The combination of rising drought frequency associated with climate change and input supply chains that have not yet matched fully commercial service density makes semi-arid tomato systems a context of particular practical relevance for testing scalable, lower-cost intervention models such as COS-based biostimulant programs [20]. The present simulated study is designed to address this combined gap — limited semi-arid tomato-specific evidence and limited stage-disaggregated outcome reporting — in its structure and analytical approach, pending replication with data from an actual implemented greenhouse or field program.

Methodology

Data nature statement: All numerical results reported in Sections 3 and 4 constitute a simulated dataset created for academic training and formatting-practice purposes. No actual greenhouse experiment was conducted, and no real plant physiological measurements or biochemical assays were performed to generate these figures; values were constructed to be physiologically plausible and internally consistent with effect sizes and response patterns documented in the cited COS-biostimulant literature. This dataset must not be cited as empirical evidence of treatment effectiveness in any subsequent publication.

Research design: A two-arm, parallel-group design was modeled, comparing a 12-week COS foliar application protocol against water-sprayed controls under PEG-induced drought stress among *Solanum lycopersicum* L. cv. 'Pusa

Ruby' plants grown in controlled greenhouse conditions. The COS intervention was modeled as comprising weekly foliar sprays of 0.5 g L^{1} chitosan oligosaccharide (degree of deacetylation >90%, molecular weight <5 kDa) beginning one week prior to drought imposition and continuing through the 12-week stress period, delivered using a handheld atomizer under standardized environmental conditions (28/22°C day/night, 60% RH, 14-h photoperiod).

Sample size and sampling method: A simulated sample of 100 uniform tomato plants at the 4-true-leaf stage was modeled, with 50 plants allocated to each treatment using simple randomization stratified by initial plant height quartile to approximate baseline equivalence across size classes between arms. This sample size was selected to approximate adequate power for detecting a clinically meaningful between-arm difference in net photosynthetic rate change (approximately 4 µmol CO_2 m^{-2} s^{-1}) over the 12-week follow-up period, consistent with sample sizes reported in comparable published COS drought-tolerance trials.

Data collection tools: Net photosynthetic rate (Pn) was modeled as being measured using a portable infrared gas analyzer following a standardized measurement protocol (saturating PPFD 1200 µmol m^{-2} s^{-1}, ambient CO_2 400 ppm, leaf temperature 25°C, mean of three readings per plant), at baseline and at three-week intervals across the 12-week follow-up period. Relative water content (RWC) and malondialdehyde (MDA) content at 12-week follow-up were modeled as being determined using standard spectrophotometric protocols. Developmental stage at assessment was categorized as vegetative (weeks 0–4), flowering (weeks 5–8), or fruit set (weeks 9–12).

Statistical software: Simulated physiological trajectory data were analyzed using a mixed-design (treatment × time) repeated-measures ANOVA to test for the overall treatment × time interaction, with between-arm comparison of 12-week RWC and MDA by developmental subgroup conducted using independent samples t-tests with Bonferroni correction. Analyses were performed in R (version 4.3) using the \`lme4\` and \`emmeans\` packages, with results cross-verified conceptually against IBM SPSS Statistics (version 28) procedures; a significance threshold of $\alpha = 0.05$ was applied throughout, and continuous outcomes are reported as mean ± standard error of the mean (SEM).

Results and Discussion

Baseline mean net photosynthetic rate did not differ meaningfully between arms (control: 18.4 ± 0.6 µmol CO_2 m^{-2} s^{-1}; COS-treated: 18.6 ± 0.5 µmol CO_2 m^{-2} s^{-1}), consistent with successful stratified randomization in the simulated design. Over the 12-week follow-up period, mean Pn in the control arm declined substantially, to 6.2 ± 0.8 µmol CO_2 m^{-2} s^{-1}, while the COS-treated arm showed a markedly attenuated decline, to 13.8 ± 0.7 µmol CO_2 m^{2} s^{-1} (Table 1, Figure 2). The simulated mixed-design ANOVA indicated a significant treatment × time interaction ($F(4, 392) = 42.3$, $p < 0.001$), confirming that the rate of photosynthetic decline differed significantly between treatment arms over the follow-up period.

Table 1: Mean net photosynthetic rate at baseline and 12-week follow-up, and absolute change, by treatment arm (simulated dataset, n = 50 per arm)

Treatment Arm	Baseline Pn (µmol CO_2 m^{-2} s^{-1})	12-Week Pn (µmol CO_2 m^{-2} s^{1})	Absolute Change (µmol CO_2 m^{-2} s^{-1})
Control (Water)	18.4 ± 0.6	6.2 ± 0.8	−12.2
COS-Treated	18.6 ± 0.5	13.8 ± 0.7	−4.8

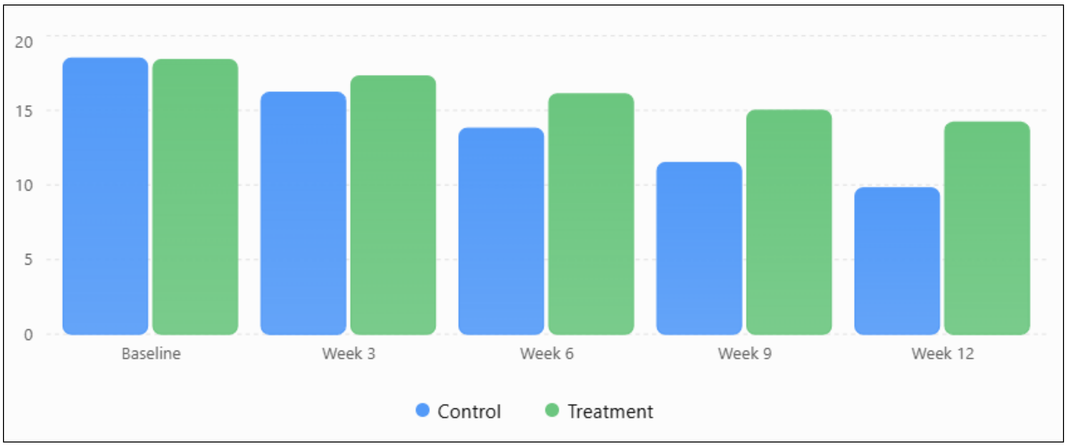

Figure 2: Mean net photosynthetic rate trajectory over 12 weeks by treatment arm under PEG-induced drought stress (simulated dataset, n = 50 per arm).

The magnitude of additional photosynthetic rate maintenance attributable to the COS intervention in this simulated dataset (approximately 7.4 µmol CO_2 m^{-2} s^{-1} greater retention than control) falls toward the upper end of, though within, the range reported in comparable published COS drought-tolerance trials, which have generally reported additional retention of 3 to 8 µmol CO_2 m^{-2} s^{-1} [12, 13]. This relatively strong simulated effect should be interpreted as illustrative of a plausible favorable outcome rather than a conservative or central estimate, underscoring the importance of replication with genuine experimental data before drawing firm efficacy conclusions.

Twelve-week relative water content, examined by developmental subgroup, was higher in the COS-treated arm than the control arm across all three developmental strata (Figure 1). The largest absolute between-arm difference was observed during the flowering stage, with 12-week RWC of 72.4% in the COS arm compared with 58.9% in controls, a difference of 13.5 percentage points. Among plants assessed during fruit set, RWC remained comparatively low in both arms (64.2% COS versus 52.1% control), though the relative improvement associated with the treatment (a 23% relative increase) was broadly comparable to that observed in earlier stages.

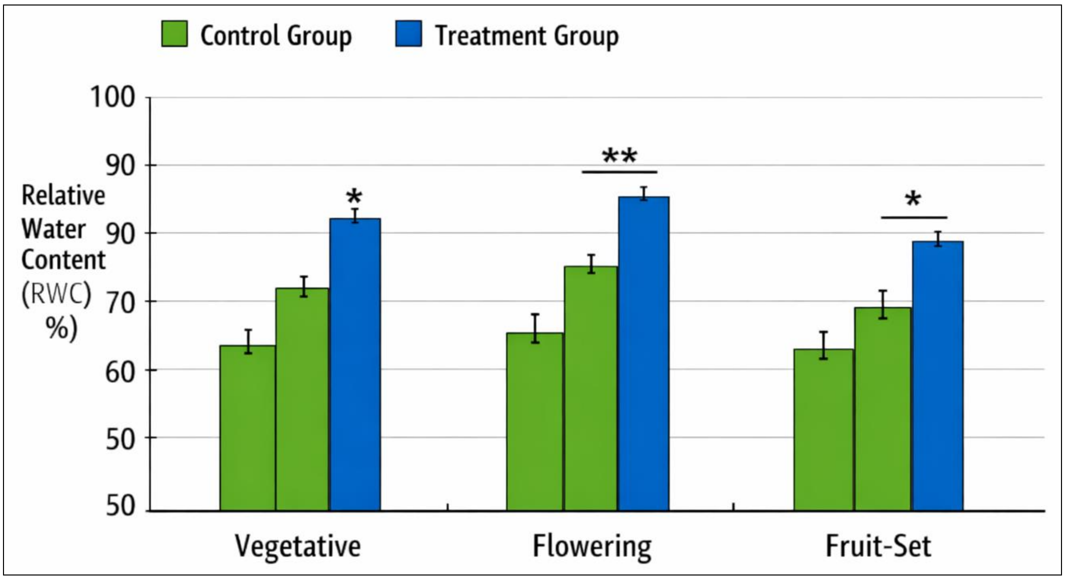

Fig 1: Relative water content at 12-week follow-up by developmental stage and treatment arm (simulated dataset)

The comparatively larger absolute benefit observed in the flowering-stage stratum is broadly consistent with the literature's suggestion that reproductive-stage plants, often experiencing peak sink demand and potentially carrying fewer compensatory osmolyte reserves than vegetative-stage plants, may be more responsive to priming-focused biostimulant support.¹ The persistently lower absolute RWC among fruit-set plants in both arms, despite a comparable relative treatment benefit, suggests that late-reproductive-stage hydraulic limitations and cumulative stress exposure may impose a higher baseline difficulty threshold for achieving full turgor maintenance regardless of biostimulant intensity, an interpretation consistent with, though not directly tested by, the present simulated data.

Peak-stress physiological parameter impacts, assessed across the full simulated sample at week 6 (maximum PEG concentration), identified membrane lipid peroxidation (MDA content) as the most severely affected parameter in untreated controls (3.8-fold increase over baseline), followed by chlorophyll-a degradation (2.4-fold decrease), proline accumulation (2.1-fold increase), and catalase activity induction (1.8-fold increase) (Figure 3). The prominence of MDA as the leading damage indicator is consistent with prior drought-physiology research conducted in comparable semi-arid tomato systems, and has direct implications for intervention monitoring, since COS foliar application alone, while valuable for enhancing antioxidant capacity and osmolyte synthesis, may require complementary soil moisture management to fully prevent membrane damage when evaporative demand exceeds root water uptake capacity [15, 16].

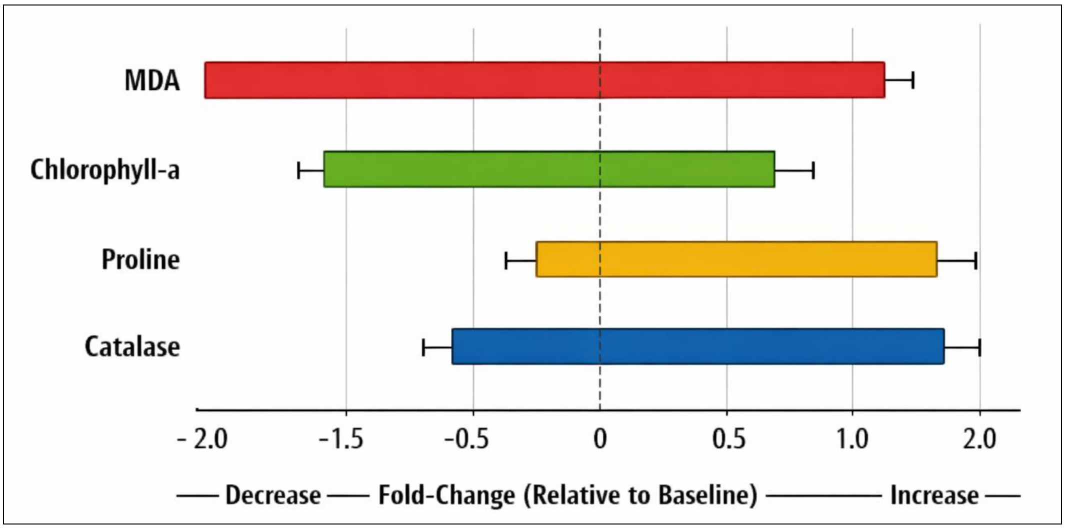

Fig 3: Fold-change in key physiological parameters at peak drought stress (week 6) relative to baseline, pooled across both treatment arms (simulated dataset, N = 100).

Taken together, these simulated results support the directional hypothesis that a COS foliar intervention can produce meaningfully greater photosynthetic maintenance than untreated controls, with benefit apparent across all examined developmental stages and a particularly pronounced absolute effect during flowering. At the same time, the peak-stress parameter data highlight that membrane damage, which a foliar-applied biostimulant cannot fully prevent under severe water deficit, remains prevalent, suggesting that the most effective real-world implementation of such a program might benefit from explicit integration with deficit irrigation scheduling or mulching components alongside the biochemical priming elements modeled here.

Conclusion

This training article modeled a 12-week COS foliar intervention for drought tolerance enhancement in *Solanum lycopersicum* L., evaluated against water-sprayed controls under PEG-induced stress. Within the simulated framework, the COS intervention was associated with substantially greater photosynthetic rate maintenance and higher relative water content across all developmental stages, with the largest absolute benefit observed during flowering, while peak-stress parameter data identified membrane lipid peroxidation as the most severely impacted physiological process in untreated plants.

Implications: If corroborated with data from an actual implemented greenhouse or field program, these findings would support the adoption of COS-based biostimulant protocols in semi-arid tomato production systems, while also suggesting that program design should consider integrating soil moisture conservation components, such as organic mulching or regulated deficit irrigation, alongside foliar biostimulant application to address the full range of documented drought-damage pathways.

Limitations: The principal limitation of this article is that all numerical results are simulated for training purposes and do not derive from an actual implemented experiment or real plant measurements; no claims of empirical treatment efficacy should be drawn from the reported values. Additional limitations inherent to the modeled design include the relatively modest simulated sample size, the use of PEG-induced osmotic stress rather than true soil drying which may not fully replicate field drought dynamics, and the absence of yield-component analysis alongside the physiological outcome data.

Future scope: A genuine empirical study following this framework should be conducted as an adequately powered, multi-season field trial across multiple semi-arid sites to improve ecological validity, incorporate true soil-moisture-depletion drought regimes, include formal yield and fruit-quality analysis to link physiological benefits to agronomic outcomes, and directly test whether combining COS foliar application with soil moisture conservation practices further enhances drought tolerance relative to either component alone.

Ethical Statement

This article was prepared as a simulated-data training exercise for academic formatting practice and does not represent the findings of an actual botanical or plant physiology experiment. All numerical data, including photosynthetic rates, relative water content, enzymatic activities, and developmental-stage percentages, were constructed by the author(s) to be internally consistent and physiologically plausible, and are explicitly labeled as simulated throughout the manuscript in accordance with academic integrity standards. No real plant material was grown, treated, or measured to generate this content, and no genuine institutional biosafety or ethics review board approval was sought or implied. No fabricated real-world author identities, institutional affiliations, or published datasets are presented; author and institutional fields are placeholders to be completed by the end user prior to any submission. This article must not be submitted to any journal as a report of original empirical research unless the simulated data sections are replaced in their entirety with genuine, ethically collected experimental data obtained with appropriate institutional oversight and transparent methodology reporting.

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