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HPLC-BASED IDENTIFICATION OF GOSSYPOL AS A PHYTOALEXIN IN SELECTED COTTON (*GOSSYPIUM HIRSUTUM* L.) VARIETIES UNDER BIOTIC STRESS CONDITIONS

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Abstract

Gossypol, a sesquiterpenoid phytoalexin, plays a significant role in the defense response of cotton (*Gossypium hirsutum* L.) against biotic stress factors. In this study, six cotton varieties (*Gulbahor-2, Bukhara-102, Afsona, Sadaf, UzRFA-710, and UzRFA-709*) were evaluated under four treatment conditions: control (untreated), inoculation with *Fusarium oxysporum* f.sp. *vasinfectum*, *Azotobacter chroococcum* K2020, and a combined inoculation of both microbes.

High-Performance Liquid Chromatography (HPLC) was employed to identify and quantify gossypol content as a biochemical marker of induced resistance. The chromatographic analysis was carried out using an Agilent-1200 HPLC system equipped with a diode-array detector (DAD) at 254 nm. Preliminary results indicated that *Fusarium oxysporum* f.sp. *vasinfectum* inoculation significantly increased gossypol synthesis compared to the control. In contrast, *Azotobacter chroococcum* K2020 treatment showed a suppressive effect, reducing gossypol accumulation in most varieties. Interestingly, combined inoculation resulted in an intermediate gossypol level, suggesting microbial interaction effects on phytoalexin pathways.

Among the six cultivars of *Gossypium hirsutum* L. evaluated, the *Afsona* variety was selected for in-depth HPLC-based analysis of gossypol synthesis under various biotic treatments. Chromatographic and quantitative data revealed notable differences in gossypol accumulation depending on microbial inoculation.

In the control group, gossypol concentration was **1.17 mg/g**, representing the basal level of phytoalexin production under non-stress conditions. This served as a reference point for assessing the relative effects of microbial treatments.

In plants inoculated with *Azotobacter chroococcum* K2020, gossypol levels significantly decreased to **0.86 mg/g,** indicating the immunomodulatory influence of this beneficial microbe. *Azotobacter* appears to downregulate the stress-induced biosynthesis of secondary metabolites, including phytoalexins such as gossypol. This response may reflect a strategy to maintain physiological balance and avoid unnecessary metabolic expenditure in the absence of severe pathogenic threat. Thus, *Azotobacter* modulates the plant's defense metabolism and prevents overactivation of stress responses.

In contrast, inoculation with *Fusarium oxysporum* f.sp. *vasinfectum* resulted in the highest gossypol content (**1.84 mg/g**), reflecting a strong activation of the plant's innate immune system. This elevated synthesis of gossypol is indicative of a typical resistance response against fungal pathogen invasion.

The most notable finding emerged in the combined treatment (*Azotobacter* + *Fusarium)*, where gossypol content reached **1.25 mg/g**, representing an intermediate level. This suggests a complex biological interaction between the two microorganisms. While *Fusarium* acted as a pathogenic elicitor triggering the defense response, *Azotobacter* appears to have partially attenuated this reaction through its immunomodulatory effect. As a result, gossypol synthesis was enhanced relative to the control but remained lower than in the *Fusarium*-only treatment.

Overall, the study demonstrates that gossypol biosynthesis is not solely driven by pathogen exposure but is also significantly shaped by the presence of beneficial microbes. This has important implications for developing biotechnological strategies aimed at optimizing plant defense responses through microbial inoculants.

Gossypol content and HPLC peak characteristics in *Afsona* cotton variety under different biotic treatments

Treatment Variant	Retention Time (min)	Peak Area (mAU·s)	Gossypol Concentration (mg/g)
Control	12.728	4310.17	1.1747
Azotobacter chroococcum K2020	12.720	3149.73	0.8584
Fusarium oxysporum f.sp. vasinfectum	12.733	6759.81	1.8423
Combined treatment	12.684	4585.50	1.2497

The results of this study demonstrate that gossypol, a key phytoalexin in *Gossypium hirsutum* L., is differentially synthesized in response to various microbial treatments. Among the tested conditions, *Fusarium oxysporum* f.sp. *vasinfectum* elicited the strongest defense response, resulting in the highest gossypol levels. In contrast, *Azotobacter chroococcum* K2020 suppressed gossypol synthesis, likely through immunomodulatory activity that attenuates unnecessary stress responses. Interestingly, the combined treatment produced intermediate results, suggesting a balance between elicitation and suppression mechanisms.

These findings underscore the complex nature of plant–microbe interactions and the importance of understanding how beneficial microorganisms can modulate defense pathways. The use of HPLC as a sensitive tool for phytoalexin quantification proved effective in assessing the biochemical basis of induced resistance. This approach could be valuable for screening cotton genotypes with improved resilience and for developing sustainable biotechnological strategies aimed at enhancing crop protection.