

Induced Resistance Against *Tuta absoluta* in Tomato Cultivars Using Eco-friendly Plant Growth Regulators in Egypt

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Received: May 15, 2026 | Review began: June 03, 2026 | Review ended: September 08, 2026 | Published: September 09, 2026

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Abstract

Background

The South American tomato pinworm, *Tuta absoluta*, represents a severe threat to tomato production in Egypt, capable of causing up to 100% yield loss and devastating economic damage, a challenge intensified by climate change and escalating insecticide resistance. This study aimed to evaluate the efficacy of eco-friendly plant growth regulators (PGRs) - Salicylic acid, Benzyladenine, and Kinetin - as elicitors of induced resistance against *T. absoluta* across three distinct tomato cultivars: Hybrid Super Strain B, Baladi, and Castle Rock.

Methods

A split-split-plot randomized complete block design was employed to assess infestation levels over a 3-week period. We first characterized the constitutive (pre-treatment) biochemical baseline of the three cultivars by measuring primary nutrients, proteins, and defensive enzymes (Alpha- and Beta-esterase). Subsequently, the efficacy of PGR applications was evaluated through phenotypic assessment of larval mine density. Data were subjected to analysis of variance appropriate for a split-split-plot design to quantify the interactions between biochemical profiles and resistance outcomes.

Results

All PGR treatments significantly reduced larval mine density compared to the control. Cytokinins (benzyladenine and kinetin) demonstrated superior efficacy, particularly by the third week. Among the cultivars, Castle Rock exhibited the highest constitutive resistance, supported by significantly higher initial levels of total nitrogen, proteins, and defensive enzymes. Principal Component Analysis effectively separated Castle Rock from susceptible cultivars, confirming that constitutive esterase activity and nutrient status are the primary discriminants of resistance.

Conclusions

These 2016 screenhouse data establish a physiological proof-of-concept, demonstrating that constitutive biochemical machinery modulates plant resistance via synergistic "bottom-up" effects. While these findings highlight a sustainable pathway for non-chemical pest management, they underscore the need for future multi-season field trials to validate these biochemical insights against current 2026 climate dynamics and evolving pest biotypes, aligning with modern Integrated Pest Management standards.

Categories: Organic agriculture, Disease & pests of crops, Use of biostimulants, biofertilizers, bioinoculants in sustainable agriculture

Keywords: *tuta absoluta*, tomato cultivars, salicylic acid, cytokinins, elicitors, antioxidant enzymes, resistance

How to cite this article:

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Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most strategically important vegetable crops globally and specifically in Egypt, where it serves as a primary driver of horticultural export growth. It contributes significantly to the national agricultural trade balance, particularly toward high-value European markets [1]. However, tomato production in Egypt currently faces severe economic and environmental challenges. Recent climate changes, characterized by extreme temperature fluctuations and shifting weather patterns, have the potential to drastically affect agricultural productivity. These environmental shifts often lead to declining crop yields, posing a substantial threat to the sustainability of vital vegetable sectors [2].

The most significant biotic threat contributing to these losses is the South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Since its invasion, this pest has caused severe economic damage, with larvae capable of destroying up to 100% of the yield by mining leaves and boring into fruits [1,3,4]. Historically, control strategies relied heavily on the intensive application of synthetic insecticides. However, this over-reliance has led to the rapid development of widespread resistance in *T. absoluta* populations, environmental degradation, and the suppression of beneficial non-target arthropods, which undermines natural biological control services [3,4].

Furthermore, the presence of toxic pesticide residues in fruits poses significant health risks to consumers and acts as a major barrier to international trade. The Egyptian horticulture sector faces increasing pressure to comply with stringent international food safety standards and the "EU Green Deal" regulations, which strictly limit Maximum Residue Limits (MRLs) and restrict various synthetic insecticides. Compliance with these standards is now critical to avoid border rejections and maintain competitive market access [1]. Consequently, there is an urgent need for capacity building and the adoption of sustainable, eco-friendly pest control practices that minimize chemical residues while ensuring high productivity [1,5].

Recent advancements in biotechnological and agronomic solutions emphasize a transition toward integrated systems that decrease dependency on synthetic pesticides. Agronomic strategies, including the evaluation of resistant cultivars, have emerged as key components of Integrated Pest Management (IPM) [4]. However, as domesticated tomato cultivars often exhibit diminished natural resistance compared to their wild relatives, the use of external elicitors has become essential to boost plant defense mechanisms [6].

Plants deploy complex molecular signaling networks involving phytohormones to trigger biochemical deterrents against herbivores. The application of chemical elicitors, such as Plant Growth Regulators (PGRs), can be sprayed on crops to build up the plant's natural defense system by inducing "Induced Resistance" [7]. These PGRs trigger significant biochemical changes, including the accumulation of secondary metabolites such as phenols, flavonoids, and terpenoids, which make plant tissues less palatable or even toxic to larvae [7,6]. For instance, salicylic acid has been shown to induce systemic resistance by strengthening cell walls and inhibiting the digestive enzymes of leaf miners [7]. Moreover, the inherent resistance of tomato cultivars is fundamentally linked to their internal biochemical machinery [8]. The baseline levels of primary nutrients and the activity of defensive enzymes, such as esterases and peroxidases, act as the first line of defense that can be further amplified by PGR applications. The plant's nutrient status plays a decisive role in modulating these defense responses through "bottom-up" effects, shifting the biological focus from a growth-defense trade-off to a synergistic defense state [9]. Understanding these constitutive chemical profiles is crucial for explaining why certain cultivars respond more effectively to elicitors than others, as the success of induced resistance is significantly dictated by the host's underlying genetic and biochemical background [10]. Despite the known potential of PGRs in eliciting defense responses, there remains a critical gap in our understanding of how the constitutive biochemical landscape of diverse tomato cultivars interacts with these treatments to suppress *T. absoluta*. Specifically, it is unclear whether the varying resistance levels observed in field conditions are merely phenotypic or if they are dictated by underlying stoichiometric constraints such as nitrogen/protein-mediated enzymatic activity. We hypothesize that the constitutive biochemical profile, particularly the baseline levels of nitrogen, total protein, and esterase activity serve as the primary metabolic

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determinant for the efficacy of PGR-induced resistance. By characterizing these constitutive traits in Hybrid Super Strain B, Baladi, and Castle Rock cultivars, this study aims to resolve this limitation and provide a mechanistic framework for optimizing PGR-based integrated pest management strategies.

Materials And Methods

Experimental design and conditions

The study was conducted during the 2016 growing season at the experimental farm of the Faculty of Agriculture, Ain Shams University, Qaliubya, Egypt. The experiment was arranged in a split-split-plot design with three replicates, resulting in 36 experimental units. Main plots were assigned to three tomato cultivars (Hybrid Super Strain B, Baladi, and Castle Rock), sub-plots to four treatments (three PGRs and a control), and sub-sub-plots to three inspection dates. Plants were grown in large-capacity pots (15-20 L) containing homogenized clay soil. Standard agricultural practices, including irrigation, fertilization, and manual weed control, were uniformly applied to all units to isolate the effects of the experimental variables.

Pest infestation and management

Experiments were performed under semi-field conditions in a screenhouse to minimize environmental fluctuations. While the screenhouse protected the plants from external factors, infestation by *T. absoluta* was artificially initiated to ensure experimental precision. A standardized population of *T. absoluta* adults was collected from heavily infested open-field tomato crops and released manually and uniformly throughout the screenhouse at the beginning of the experiment. This systematic release guaranteed that all 36 experimental units were exposed to an equal pest density, ensuring that variations in larval mine density were driven by the inherent resistance of the cultivars rather than stochastic colonization. No synthetic pesticides were applied throughout the study.

Plant materials

Three tomato cultivars (Hybrid Super Strain B, Baladi, and Castle Rock) were used. Seedlings were obtained from a certified commercial nursery in Dokki, Giza, and transplanted on April 16, 2016. The biochemical and enzymatic baselines (as described in subsequent sections) were determined from these plants to quantify constitutive resistance mechanisms.

Chemical treatments

Three PGRs were utilized: salicylic acid (2 g L⁻¹), benzyladenine (1 g L⁻¹), and kinetin (1 g L⁻¹). Treatments, alongside a distilled water control, were applied as a single foliar spray 2 weeks post-transplanting using a standard hand-sprayer, ensuring uniform canopy coverage until runoff. PGRs were obtained from El Gomhouria Co. for Drugs, Chemicals, and Medical Supplies (Cairo, Egypt).

Sampling and assessment

Sampling commenced 2 weeks post-transplanting and continued weekly for 3 consecutive weeks. A total of 360 leaflets were randomly collected (10 leaflets × 4 treatments × 3 cultivars × 3 replicates). The entomological evaluation was strictly phenotypic, where the number of *T. absoluta* larval mines was recorded using a stereomicroscope. The mean number of mines per 10 leaflets was calculated to quantify the induced resistance.

Baseline phytochemical analysis

To establish the initial constitutive defense machinery of the tested tomato cultivars prior to any PGR application, dry leaf samples were collected during the vegetative stage exclusively from the untreated control plants. These samples were used to determine the baseline biochemical profile of the cultivars. The following components were determined:

Nutrients (N, P, K): Total nitrogen was determined by the micro-Kjeldahl method [11]. Inorganic phosphorus was measured photometrically at 650 nm [12], and Potassium (K⁺) was determined using a flame photometer [13].

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Primary Metabolites: Total free amino acids were assayed using the ninhydrin reagent [14]. Total soluble proteins were estimated using the Bradford method, and total carbohydrates were determined using the phenol-sulfuric acid method [15].

Secondary Metabolites and Enzymes: Total phenols were determined by the Folin-Ciocalteu method [16]. Alpha-esterases (α -esterases) and beta-esterases (β -esterases) activity was determined using the Van Asperen method [17]. Peroxidase activity was determined using the Vetter method [18].

All biochemical analyses were conducted at the Central Laboratory, Faculty of Agriculture, Ain Shams University, Egypt.

Statistical analysis

Data were subjected to a three-way analysis of variance (ANOVA) to evaluate the effects of cultivars, chemical treatments, and inspection dates, as well as their interactions. Mean comparisons were performed using the Least Significant Difference (LSD) test at $P < 0.05$. All statistical computations were performed using CoStat software (Version 6.4).

Results And Discussion

The infestation level of *T. absoluta*, expressed as the mean number of mines per 10 leaflets, was significantly influenced by PGRs, tomato cultivars, and inspection dates, as well as their interactions (Table 7). The three-way ANOVA revealed highly significant main effects for PGRs ($F = 128.6$, $P < 0.001$), cultivars ($F = 28.2$, $P < 0.001$), and time ($F = 553.5$, $P < 0.001$). Furthermore, all two-way interactions were highly significant, including PGR $\times$ Cultivar ($F = 11.0$, $P < 0.001$), PGR $\times$ Time ($F = 18.0$, $P < 0.001$), and Cultivar $\times$ Time ($F = 10.5$, $P < 0.001$). Crucially, the three-way interaction among plant growth regulators, cultivars, and time (R $\times$ C $\times$ T) also exerted a highly significant combined effect on the infestation rate ($F = 15.7$, $P < 0.001$; Table 7), demonstrating that the efficacy of the inducing agents varies depending on both the genetic background of the host and the duration of exposure.

Effect of inspection dates

Inspection dates had a highly significant effect on infestation levels ($F = 553.5$, $P < 0.001$). The mean number of mines decreased significantly over time, recording 8.8 mines/10 leaflets during the first week, 3.4 mines/10 leaflets in the second week, and reaching the lowest level (1.5 mines/10 leaflets) in the third week (Table 2). It is important to note that a natural decline in larval mine density was observed across all groups, including the control, likely due to the completion of the pest's larval developmental stages and subsequent pupation under screenhouse conditions. However, a comparative analysis reveals that PGR-treated plants exhibited a significantly steeper decline in infestation levels compared to the untreated control. The significant divergence in infestation levels observed during the initial 2 weeks post-application - prior to the natural population collapse - strongly supports the efficacy of the PGRs in suppressing larval activity. This indicates that while the pest population naturally fluctuated, the PGR treatments provided an additional, robust suppressive effect that curtailed larval development more efficiently than the plant's inherent resistance alone. Thus, the observed reduction represents an interaction between the pest's natural life-cycle constraints and the heightened phenotypic protection induced by the exogenous application of cytokinin-based regulators. Consequently, the enhanced suppression observed by the third week reflects a sustained metabolic reinforcement, where the plant's biochemical arsenal remains primed and potent following the initial application. Regarding the application frequency, it is critical to note that the PGRs in this study were applied as a single treatment. While this single application successfully demonstrated an immediate, potent 'priming effect' that significantly suppressed larval activity during the critical initial weeks, we acknowledge that the defense-inducing effects of elicitors typically diminish over time and require multiple applications for sustained, season-long resistance. Therefore, for practical agricultural applications, we recommend that farmers implement sequential, multi-application schedules (e.g., bi-weekly intervals) during peak pest pressure periods rather than relying on a single spray. This would ensure sustained metabolic activation and continuous crop protection against *T. absoluta*.

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Effect of plant growth regulators

Plant growth regulators significantly affected *T. absoluta* infestation ($F = 128.6$, $P < 0.001$). The highest infestation was observed in the untreated control (7.5 mines/10 leaflets), followed by salicylic acid (4.5 mines/10 leaflets). Kinetin (3.3 mines/10 leaflets) and benzyladenine (2.9 mines/10 leaflets) showed the lowest infestation levels, with no significant difference between them (Table 2). These results confirm the role of PGRs as potent "Plant Activators" capable of priming the plant's innate immune system [19]. This approach characterizes a shift toward next-generation plant defense stimulators that meet modern societal demands for environmentally friendly alternatives to conventional biocides [20].

The superior efficacy of cytokinins (benzyladenine and kinetin) can be attributed to their dual role in plant physiology. Cytokinins not only delay leaf senescence but also actively modulate plant immunity through hormonal crosstalk [21]. This effect is further explained by their role in nutrient allocation and photosynthetic performance; as signaling agents, cytokinins modify plant metabolism to adapt to biotic stresses, thereby strengthening the plant's physiological resilience and making the tissues less susceptible to larval establishment [22]. By enhancing the plant's metabolic status through molecular signaling, these PGRs likely rendered the leaflets less suitable for *T. absoluta* oviposition and larval development [6].

Similarly, the significant reduction in infestation caused by salicylic acid is consistent with its established role as a "central integrative hub" for systemic acquired resistance [21]. Salicylic acid triggers the accumulation of secondary metabolites, such as phenols and flavonoids, which increase toxicity or decrease palatability to larvae [7]. The involvement of the salicylic acid signaling pathway in tomato defense is further supported by the observation that tomato leaves rapidly accumulate salicylic acid and up-regulate marker genes such as PR1 and BGL2 in response to herbivore attack [23]. Moreover, exogenous salicylic acid dramatically increases endogenous H_2O_2 levels, acting as a secondary messenger for activating defense-related genes [23]. This proactive defense activation, or "priming" effect, activates internal stress-response mechanisms before peak larval activity, providing a robust chemical and physical barrier that hindered the mining activity of *T. absoluta* [19,24].

Effect of tomato cultivars

A significant effect of tomato cultivars on infestation was observed ($F = 28.2$, $P < 0.001$). Hybrid Super Strain B (5.1 mines/10 leaflets) and Baladi (4.9 mines/10 leaflets) recorded higher infestation levels, whereas Castle Rock showed significantly lower infestation (3.6 mines/10 leaflets) (Table 2). These differences underscore the importance of genetic background in pest resistance. Castle Rock's performance suggests inherent morphological or biochemical traits that act synergistically with PGR treatments, supporting the integration of resistant cultivars as a primary component of sustainable IPM programs [4]. Conversely, the higher susceptibility of Hybrid Super Strain B may reflect the domestication process, which often prioritizes yield over natural defense traits [6]. Consequently, the enhanced suppression observed by the third week reflects the prolonged physiological window of the initial application, though future studies must evaluate whether multiple applications are required to sustain this primed metabolic state over extended crop cycles.

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Inspection dates	Tomato cultivars	Plant growth regulators (PGRs)				Dates mean
		Control $\pm$ SE	Salicylic acid $\pm$ SE	Kinetin $\pm$ SE	Benzyladenine $\pm$ SE	
1 st week	Castle rock	9.0 $\pm$ 0.5	7.0 $\pm$ 0.5	8.0 $\pm$ 0.5	4.3 $\pm$ 0.6	8.8 a
	Baladi	12.6 $\pm$ 0.8	13.0 $\pm$ 0.5	5.0 $\pm$ 0.5	5.6 $\pm$ 0.3	
	Hybrid Super strain B	19.3 $\pm$ 1.3	7.0 $\pm$ 0.5	9.0 $\pm$ 0.5	6.0 $\pm$ 0.5	
2 nd week	Castle rock	5.0 $\pm$ 0.5	2.0 $\pm$ 0.5	1.6 $\pm$ 0.6	2.0 $\pm$ 0.5	3.4 b
	Baladi	5.3 $\pm$ 0.6	2.3 $\pm$ 0.3	3.0 $\pm$ 0.5	3.0 $\pm$ 0.5	
	Hybrid Super strain B	7.6 $\pm$ 0.3	5.3 $\pm$ 0.3	0.6 $\pm$ 0.3	3.0 $\pm$ 0.5	
3 rd week	Castle rock	2.6 $\pm$ 0.3	1.3 $\pm$ 0.3	0.0 $\pm$ 0.0	0.6 $\pm$ 0.3	1.5 c
	Baladi	4.3 $\pm$ 0.6	1.3 $\pm$ 0.3	2.3 $\pm$ 0.6	1.6 $\pm$ 0.6	
	Hybrid Super strain B	2.6 $\pm$ 0.3	1.3 $\pm$ 0.3	0.3 $\pm$ 0.3	0.0 $\pm$ 0.0	
PGRs mean		7.5 A	4.5 B	3.3 C	2.9 C	
Cultivars mean	Hybrid Super strain B = 5.1 a		Baladi = 4.9 a		Castle Rock = 3.6 b	

TABLE 1: Mean number of *T. absoluta* mines per 10 leaflets ($\pm$ SE) as influenced by plant growth regulators, tomato cultivars, and inspection dates during the growing season.

Means within a row or column followed by the same letter are not significantly different at $P < 0.05$ (LSD test). Capital letters (A, B, C) refer to PGRs means, while small letters (a, b, c) refer to dates and cultivars means. Values represent mean $\pm$ Standard error. LSD, Least Significant Difference

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LSD	Fvalue	MS	SS	df	Source of variance
0.52	128.6***	120.3	360.9	3	Plant growth regulator (R)
0.45	28.2***	26.4	52.9	2	Tomato cultivar (C)
0.45	553.5***	517.6	1035.3	2	Time (T)
0.97	11***	10.3	62.2	6	R * C
0.97	18***	16.8	101.3	6	R * T
0.90	10.5***	9.8	39.3	4	C * T
1.32	15.7***	14.7	176.6	12	R * C * T
-	-	0.93	67.3	72	Error
-	-	-	1896	107	Total

TABLE 2: Summary of analysis of variance for the effects of plant growth regulators, cultivars, and time on *T. absoluta* infestation.

***Significant at $P < 0.001$.

df, degrees of freedom; LSD, Least Significant Difference; MS, mean squares; SS, Sum of Squares

Biochemical basis of resistance (the chemical profile)

To understand the underlying mechanisms of resistance, the baseline phytochemical profile of the three cultivars was analyzed (Table 3). The results revealed significant variations in primary nutrients and metabolites. Castle Rock, which exhibited the lowest infestation levels, was characterized by the highest baseline of Total Nitrogen (11.5 mg/g DW), Total Protein (69.8 mg/g DW), and Phosphorus (1,317.0 mg/g DW). Similarly, Table 4 shows that Castle Rock maintained high levels of esterase activities, specifically Alpha-esterase (423.7 $\mu\text{g } \alpha\text{-naphthol/min/g DW}$) and Beta-esterase (323.3 $\mu\text{g } \beta\text{-naphthol/min/g DW}$). Although "Castle Rock" displayed lower baseline levels of total phenols (4,833.7 $\mu\text{g GAE/g DW}$) compared to "Hybrid Super Strain B" (5,650.0 $\mu\text{g GAE/g DW}$), its superior resistance strongly indicates that resistance is not defined by static phenolic quantity alone. Instead, this observed biological alignment suggests that resistance in these tomato lines is driven primarily by the rapid, efficient mobilization of the metabolic baseline—specifically the constitutive esterase activity—rather than static pre-existing phenolic pools. These specific enzyme profiles serve as crucial biochemical barriers, where enhanced esterase activity facilitates the detoxification of herbivore-induced metabolites and strengthens plant tissue resilience. We hypothesize that the efficacy of the chemical defense in "Castle Rock" is attributed to its specific qualitative phenolic composition or a more dynamic induced-defense response triggered post-infestation, which works synergistically with the observed esterase activity to mitigate *T. absoluta* damage.

This dynamic response can be explained by downstream phytohormone signaling pathways. Cytokinins, such as kinetin and benzyladenine, play a dual role; they not only delay leaf senescence and improve chloroplast differentiation but also actively induce resistance by modulating plant immunity and interacting with other defense-related phytohormones [21]. This complex hormonal crosstalk significantly influences the oviposition preference and larval

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development of *T. absoluta*, offering a sustainable and innovative tool for enhancing crop protection in modern agriculture [21]. The inherent resistance of tomato cultivars is fundamentally linked to this biochemical machinery [8]. High nutrient levels, particularly nitrogen and protein, play a decisive role in modulating defense responses through "bottom-up" effects [9]. This metabolic baseline in Castle Rock likely provided the necessary fuel for the rapid synthesis of defensive compounds, shifting the plant toward a synergistic defense state.

Interaction effects and agricultural implications

All interaction effects were highly significant, including the three-way interaction ($R \times C \times T$) ($F = 15.7$, $P < 0.001$). This indicates that the combined effect of PGRs, cultivars, and time played a crucial role in determining infestation levels. In general, all PGR treatments reduced infestation compared with the control across all cultivars and dates, with kinetin and benzyladenine showing the highest reduction, particularly during the third week where infestation approached zero in some treatments. In the context of Egypt's current challenges, including climate-induced productivity losses [2] and stringent export regulations [1], using PGRs as eco-friendly alternatives offers a double advantage: enhancing plant resilience and ensuring the production of pesticide-free tomatoes [1]. Recent studies have demonstrated the potential of elicitors to trigger biochemical defense responses in tomato plants [25]. Our results confirm that PGRs like salicylic acid and cytokinins enhance the enzymatic antioxidant system (e.g., peroxidase and esterases), thereby providing a sustainable alternative to synthetic pesticides against the invasive *T. absoluta*. While the current study demonstrates a clear phenotypic reduction in *T. absoluta* infestation following PGR applications, it is important to acknowledge a methodological limitation: post-treatment biochemical profiling was not sequentially conducted after the elicitor applications. Consequently, while we cannot directly quantify the immediate dynamic shifts in phenolic pools or enzymatic activities post-application within these specific trials, extensive literature confirms that exogenous applications of salicylic acid and cytokinins act as potent elicitors that upregulate defense-related pathways. The observed resistance, particularly in "Castle Rock," strongly implies an effective utilization of its robust baseline metabolic resources. Future research should focus on time-course biochemical profiling post-PGR application to explicitly map the chronological induction of these defense mechanisms.

Practical application recommendations for farmers

Based on the screened phenotypic and biochemical responses, integrating PGRs offers a pragmatic strategy for tomato growers facing heavy *T. absoluta* pressure. To maximize the efficacy of this eco-friendly approach, farmers should prioritize selecting cultivars with robust baseline metabolic profiles, such as "Castle Rock," which demonstrate a superior inherent capacity to utilize exogenous elicitors. Regarding the application regime, while a single application activates immediate defensive pathways, sustainable field control necessitates a multi-application schedule. It is recommended to implement sequential foliar sprays of cytokinins (kinetin or benzyladenine) at bi-weekly intervals, starting immediately at the vegetative nursery stage or upon the first localized detection of adult moths. This proactive "priming" ensures that the plant's enzymatic and secondary metabolic defenses remain continuously activated throughout the critical growth windows when larvae are most destructive.

Economic cost-benefit analysis: PGRs vs. conventional pesticides

A crucial factor dictating the adoption of sustainable pest management is its economic viability compared to conventional chemical control. Conventional management of *T. absoluta* in Egypt typically demands intensive, high-frequency applications of synthetic insecticides (often 10-12 sprays per season), resulting in escalating input costs, rapid pest resistance, and severe international market border rejections due to pesticide residues. In contrast, utilizing PGRs as elicitors drastically reduces the required frequency of chemical interventions. Biochemically, the cost of raw PGR compounds (such as kinetin or salicylic acid) per feddan is highly competitive or lower than premium synthetic biocides. Operationally, reducing the chemical spray calendar by 50-60% directly translates into significant savings in labor, water, and fuel costs for farmers. Most importantly, the economic "benefit" is amplified at the market level; producing pesticide-free tomatoes ensures compliance with stringent international food safety standards and the "EU Green Deal" MRLs. This unlocks premium pricing in export markets and eliminates the risk of catastrophic financial losses from border rejections, making PGR-induced resistance a highly profitable investment for sustainable modern agriculture.

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Tomato cultivars	Mean no. of <i>T. absoluta</i> mines	Nitrogen (mg/g DW)	Phosphorus (mg/g DW)	Potassium (μ Eq/g DW)	Total protein (mg/g DW)	Total carbohydrate (mg/g DW)	Free amino acid (μ g alanine/g DW)
Hybrid super strain B	19.3 $\pm$ 1.3 ^a	7.0 $\pm$ 0.5 ^b	781.0 $\pm$ 28.8 ^b	433.0 $\pm$ 13.2 ^a	45.4 $\pm$ 3.1 ^b	107.0 $\pm$ 4.0 ^a	4.6 $\pm$ 0.4 ^b
Baladi	12.6 $\pm$ 0.8 ^b	10.1 $\pm$ 0.2 ^a	1144.3 $\pm$ 65.8 ^a	389.3 $\pm$ 11.5 ^a	61.8 $\pm$ 2.8 ^a	113.0 $\pm$ 6.3 ^a	13.4 $\pm$ 0.8 ^a
Castle Rock	9.0 $\pm$ 0.5 ^b	11.5 $\pm$ 0.8 ^a	1317.0 $\pm$ 57.7 ^a	436 $\pm$ 20.7 ^a	69.8 $\pm$ 3.3 ^a	124.0 $\pm$ 4.0 ^a	5.8 $\pm$ 0.2 ^b
F value	28.5***	13.9**	26.4**	2.7	15.8**	3.1	76.2***
P value	0.0009	0.005	0.001	0.14ns	0.004	0.12ns	0.0001
LSD	3.3	2.1	184.2	54.4	10.8	17.1	1.9

TABLE 3: Baseline nutritional status and primary metabolites of the three tested tomato cultivars.

Values represent mean $\pm$ SE. Means within a column followed by the same letter are not significantly different ($P < 0.05$) according to LSD test.

Significance levels: ** Significant at $P < 0.01$; *** Significant at $P < 0.001$.

ns, Non-Significant; DW, Dry Weight; LSD, Least Significant Difference

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Tomato cultivars	Mean no. of <i>T. absoluta</i> mines	Total phenol (μg GAE/g DW)	Alpha esterase (μg α -naphthol/min/g DW)	Beta esterase (μg β -naphthol/min/g DW)	Peroxidase ($\Delta\text{O.D.405/min/g}$ DW)
Hybrid super strain B	19.3 $\pm$ 1.3 ^a	5613.0 $\pm$ 115.4 ^a	227.0 $\pm$ 15.5 ^b	192.0 $\pm$ 6.9 ^b	18.3 $\pm$ 1.3 ^c
Baladi	12.6 $\pm$ 0.8 ^b	5650.0 $\pm$ 86.6 ^a	405.7 $\pm$ 8.5 ^a	283.0 $\pm$ 13.3 ^a	54.5 $\pm$ 2.6 ^a
Castle Rock	9.0 $\pm$ 0.5 ^b	4833.7 $\pm$ 173.3 ^b	423.7 $\pm$ 13.2 ^a	323.3 $\pm$ 7.6 ^a	32.4 $\pm$ 1.4 ^b
F. value	28.5***	12.5**	72.2***	47.9***	95.7***
P value	0.0009	0.007	0.0001	0.0002	0.0000
LSD	3.3	450.4	44.3	33.6	6.4

TABLE 4: Defensive enzymes, phenolic content, and their correlation with *T. absoluta* infestation levels.

Values represent mean $\pm$ SE. Means within a column followed by the same letter are not significantly different ($P < 0.05$) according to LSD test.

Significance levels: ** Significant at $P < 0.01$; *** Significant at $P < 0.001$.

ns, Non-Significant; DW, Dry Weight; LSD, Least Significant Difference

Conclusions

This study demonstrates that eco-friendly PGRs, particularly cytokinins (benzyladenine and kinetin), are highly effective in suppressing *T. absoluta* infestation by significantly reducing larval mine density. Crucially, the host plant's initial status - characterized by high baseline levels of nitrogen, proteins, and esterase activity - serves as a vital constitutive indicator of inherent cultivar resistance. PGR treatments provided robust phenotypic protection that worked in tandem with these natural defense baselines.

While these findings establish a strong physiological foundation for induced resistance, we acknowledge that this study, conducted under controlled greenhouse conditions using 2016 experimental data, represents a proof-of-concept rather than a complete field-scale recommendation. To transition these findings into viable IPM strategies for 2026 agricultural realities, future research must focus on multi-season open-field trials that validate these biochemical responses under varying climate dynamics and contemporary pest biotypes. Integrating such cultivars with targeted PGR applications offers a sustainable, non-pesticidal pathway that aligns with environmental standards, providing a significant step toward pesticide-free agriculture and long-term environmental resilience.

How to cite this article:

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Appendices

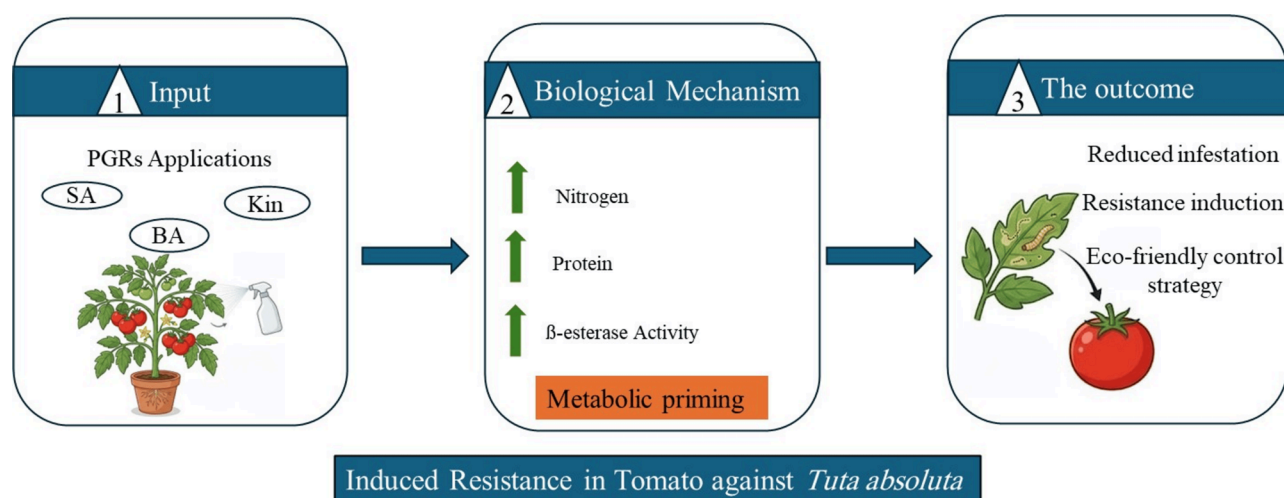


FIGURE 1: Graphical abstract.

PGR, Plant Growth Regulator; SA, Salicylic Acid; BA, Benzyladenine; Kin, Kinetin

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Eman M. Abdelmaksoud, Shoukry A. Elrefai, Ashraf Helmi

Acquisition, analysis, or interpretation of data: Eman M. Abdelmaksoud

Drafting of the manuscript: Eman M. Abdelmaksoud

Critical review of the manuscript for important intellectual content: Eman M. Abdelmaksoud, Shoukry A. Elrefai, Ashraf Helmi

Supervision: Eman M. Abdelmaksoud, Shoukry A. Elrefai, Ashraf Helmi

Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen.

Human subjects: All authors have confirmed that this study did not involve human participants or tissue.

Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue.

Conflicts of interest: In compliance with [the ICMJE uniform disclosure form](#), all authors declare the following:

- **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work.
- **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.

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- **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request. All data generated or analyzed during this study are included in this published article and/or its appendices.

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