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Susceptibility assessment and induced systemic resistance in cucumber genotypes to *Fusarium Wilt*

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Abstract

This study aimed to investigate the efficacy of the bio-fungus *Trichoderma viride* and the fungicide Hymexazol in inducing systemic resistance (ISR) against Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cucumerinum* (F.o.c) in cucumber (*Cucumis sativus* L.). Two genotypes were evaluated: a susceptible local cultivar and a partially resistant genotype imported from Monsanto Holland B.V. (Seminis). Plants were treated with the inducers, and the expression levels of key defense-related genes were assessed at 24, 48, and 96 hours post-inoculation (hpi) using quantitative real-time PCR (qRT-PCR). The results revealed that both *T. viride* and Hymexazol significantly enhanced the expression of *Chi2* and *PR-1* genes in both genotypes compared to untreated controls. The highest gene expression levels were generally observed at early time points, indicating rapid activation of plant defense responses. In contrast, the untreated resistant genotype showed only moderate induction of defense-related genes, while the untreated susceptible genotype exhibited minimal or no significant response, highlighting inherent genetic differences in disease resistance. These findings demonstrate the effectiveness of biological and chemical inducers in activating plant defense mechanisms and enhancing ISR. The study provides important insights into the molecular basis of induced resistance and suggests that integrating bio-fungicides with conventional fungicides can be an effective and sustainable strategy for managing Fusarium wilt in cucumber cultivation.

Keywords: *Fusarium oxysporum*, hymexazol, induced systemic resistance, qRT-PCR, *Trichoderma viride*

Introduction

The cucumber (*Cucumis sativus* L.) is one of the most important vegetable crops worldwide, valued for its nutritional content and wide use in fresh consumption and processing. It is rich in water, fiber, vitamins, and minerals, and holds considerable economic importance in many countries (Lida et al., 2025). However, cucumber production is highly affected by plant diseases, particularly fungal pathogens, which significantly reduce growth, yield, and quality. Among these, Fusarium wilt is a major constraint, causing substantial economic losses globally (Kaur et al., 2025).

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cucumerinum* (F.o.c), is one of the most destructive diseases of cucumber. It affects plants at all growth stages by invading vascular tissues, disrupting water and nutrient transport, and leading to symptoms such as yellowing, wilting, and eventual plant death. This disease results in severe yield and quality losses, particularly under intensive cultivation systems (Din et al., 2020). Such diseases negatively impact crop productivity and farmers' income, emphasizing the need for effective disease management strategies (Kaur et al., 2025).

Traditionally, chemical control methods have been widely used to manage plant diseases. Certain chemical compounds can act as resistance inducers, enhancing plant defense mechanisms through signaling pathways such as those involving salicylic acid (Sachin et al., 2024). However, increasing concerns about environmental impact have encouraged the search for sustainable alternatives.

Biological control agents, particularly fungi of the genus *Trichoderma*, have gained attention due to their dual role in suppressing soil-borne pathogens and inducing systemic resistance (ISR) in plants. These fungi activate defense-related signaling pathways, including

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jasmonic acid and ethylene pathways, leading to modulation of gene expression and reduced disease severity, while also promoting plant growth (Alkooranee et al., 2017 and Yuan et al., 2019). Plant resistance genes play a crucial role in defense responses by regulating the production of proteins such as pathogenesis-related (PR) proteins and other defense enzymes. Their activation is triggered by chemical and biological signals, making gene expression analysis a key tool for understanding plant defense mechanisms (Elsharkawy et al., 2013 and Alkooranee et al., 2017).

Therefore, this study aimed to evaluate the efficiency of *T. viride* and the fungicide Hymexazol in inducing systemic resistance against Fusarium wilt in two cucumber genotypes differing in susceptibility, by analyzing the expression of *Chi2* and *PR-1* genes at different time points after treatment.

Materials and Methods

Sample Collection

Fusarium oxysporum f. sp. *cucumerinum* (F.o.c) was isolated from roots and stems of the diseased cucumber plants exhibiting Fusarium wilt symptoms (32.377124,46.23839) in 2025 as described in Ma et al (2024). F.o.c. is grown on potato dextrose agar (200 g sliced potatoes, 20 g dextrose and 20 g agar powder in 1000 mL water).

The *Trichoderma viride* fungus was obtained from Microbiology Laboratory, Plant Protection Department, College of Agriculture, Wasit University, Wasit, Iraq. Resistant cucumber seeds were purchased from Monsanto Holland B.V. (Seminis) and cucumber seeds of the sensitive genotype (locale) and the chemical pesticide Hymexazol liquid concentrate (SL) with a concentration of 30% of the active ingredient hydroxy-5-methylisoxazole-3 from the commercial markets.

Morphological and Microscopic Diagnosis

The fungal isolate f was identified morphologically based on the shape and color of the surface and basal colonies and the growth rate. Microscopic diagnosis was also performed using a compound light microscope to determine the genus and species based on the shape of the sporophyte, the number of septa in the spores, and the type and shape of the spores (Macroconidia, Microconidia, Chlamydospores, etc.) and other methods mentioned in the taxonomy (Booth, 1977; Leslie and Summere, 2006).

Pathogenicity Tests

The pathogenicity test of the fungal isolate F.o.c was performed to confirm its ability to cause Fusarium wilt in cucumber plants, as described by Leslie and Summere (2006). The fungal isolate was cultured on potato and sucrose agar (PDA) for 5–7 days at 25 ± 2°C. A spore suspension was then prepared by adding sterile distilled water to the fungal colonies and gently scraping them off. The spore concentration was adjusted to approximately 1×10^6 spores/ml using a hemocytometer. Young test plants from the susceptible host, aged 2–3 weeks, were used. The soil was carefully removed from the roots, and the roots were then immersed in the spore suspension for 10 minutes. Control plants were immersed in sterile distilled water only. The plants were then returned to pots containing sterile soil and maintained under suitable greenhouse conditions. The appearance of disease symptoms, such as yellowing, wilting, or plant death, was recorded during the observation period, and the severity of the infection was assessed. To prove the relationship between the fungal isolate and the disease symptoms, it was re-isolated from infected plant tissues and re-cultured on PDA medium, and its characteristics were compared with the original isolate to fulfill Koch's postulates. The severity of Fusarium wilt disease in cucumber plants was assessed using a scale based on the foliage symptoms, as follows: 0 = Healthy plant with no symptoms, 1 = Mild yellowing of lower leaves without wilting, 2 = Yellowing and partial wilting of leaves with stunted growth, 3 = Significant wilting affecting most leaves with general weakness of the plant, 4 = Severe wilting or death of the entire plant. Based on this scale, the Disease Severity Index was calculated to evaluate the pathogenicity of the fungal isolate.

Plant Treatment with Inducers and F.o.c

Two genotypes of cucumbers were used for the molecular analysis. The local genotype is sensitive, whereas the imported genotype is resistant to Fusarium wilt. Seeds were planted in seedling trays contain peat mixture and grown in a growth chamber under continuous white fluorescent light at 25 °C and a light intensity of 150 µE/m2/s1 for 12-h light/dark cycles. After 25 days the seedlings were removed, and soil and debris were carefully cleaned from the roots, followed by washing under running water. The seedlings were then mechanically inoculated with *T. viride* suspension and treated with Hymexazol fungicide at a concentration of 5%. This was done by placing the seedlings in containers containing the *T. viride* suspension, Hymexazol fungicide, and sterile distilled water for comparison, for 30 minutes. In another treatment, some seedlings were first placed in the *T. viride* suspension for 15 minutes and then again

in the fungicide for another 15 minutes. All seedlings were then planted in 1000 cm³ of a 1:1 sand-peat moss mixture, inoculated 48 hours prior with F.o.c. loaded onto millet seeds at a rate of 10 g/pot, according to Dewan's method (1989).

Roots of both genotypes were harvested from one plant per treatment at 24, 28, and 96 hours post-inoculation, with three seedlings per pot in three replicates (pots) at each time point. Liquid nitrogen was used to freeze the leaves. RNA extraction and subsequent analyses were performed on leaves stored at -80°C.

Gene Expression Assay

Isolation of total RNA, first-strand cDNA synthesis and quantitative real-time polymerase chain reaction (qRT-PCR) were used as described in detail previously (Alkooranee et al., 2015). Two primers for *Chi2* and *PR-1* genes (**Table 1**) were used for cucumber seedlings, in addition to the GAPDH gene, which was used as a housekeeping marker gene

Statistical Analysis

The experimental design was completely randomized, with three replicates for all treatments. Data of the results were subjected to analysis of variance using GenStat software, and the means ($P < 0.05$) were compared between treatments of cucumber genotypes using least significant difference tests.

fungus *F. oxysporum* (Leslie and Summerell 2006). The fungus completely covered the plate within 7 days.

Pathogenicity Tests

Pathogenicity tests for the fungal isolate F.o.c demonstrated its ability to induce Fusarium wilt symptoms in cucumber plants during the controlled growth chamber period. Symptoms began to appear several days after inoculation, with mild yellowing observed in the lower leaves of the treated plants, followed by gradual wilting and spread of symptoms to other parts of the plant. As the infection progressed, signs of growth retardation and general weakness appeared, ultimately leading to complete wilting and plant death in some plants. In contrast, the control plants (treated with sterile distilled water) showed no symptoms and remained healthy throughout the experiment.

The results also revealed that the imported (resistant) genotype exhibited significantly less severe symptoms compared to the local (susceptible) genotype when exposed to the same disease conditions. Symptoms in the local genotype included a faster and more pronounced onset of yellowing and wilting, while these symptoms in the imported genotype were limited or delayed in onset and less severe. This is attributed to the imported genotype's ability to activate plant defense mechanisms more efficiently.

When assessing disease severity on a scale of

Table 1. qRT-PCR Primers Used in Study

Gene name	Gene ID		Sequence (5'-3')
Chi2	Csa1G267230	F	AACACAGGCAACAGTAGTA
		R	GTAGACTTAATGTCATCGTATCC
PR-1	Csa003481	F	TAGCAGGGTGTGTGGTCATT
		R	AATTGCCTGGAGGATCGTAG
houskeeping gene	GAPDH	F	CGCTTCCTTCAACATCATTCCCA
		R	TCAGATTCCTCCTTGATAGCCTT

Results and Discussion

Morphological and Microscopic Diagnosis of F.o.c

Morphological analysis of *Fusarium oxysporum* f. sp. *cucumerinum* colonies (**Figure 1** and **Table 2**) on the PDA medium revealed a dense, grayish rose-like mycelium on the upper surface, while the lower surface showed a grayish white-color. Microconidia ranged in shape from oval to kidney-shaped. The number of septa ranged from one to five. Macroconidia were ovoid-elliptical, slightly curved, pointed, and divided into 3 cells, ranging in shape from oblong and straight to curved. Chlamydospores were spherical (rounded) formed singly and in chains. These characteristics are typical of the

0–4, varying degrees of infection were recorded, ranging from 1 to 4, with most plants falling within the 2–3 range, indicating moderate to severe infection. The disease severity index was relatively high (54%). The fungus was also re-isolated from infected plant tissues and grew on PDA medium, exhibiting the same morphological characteristics as the original isolate, thus confirming Koch's postulates and proving that the fungal isolate is the main cause of the disease symptoms **Figure 2**.

The present results indicate that the fungal isolate possesses clear pathogenicity, causing symptoms of yellowing and gradual wilting. These symptoms are consistent with recent studies, where the disease begins with yellowing of the lower leaves and progresses to

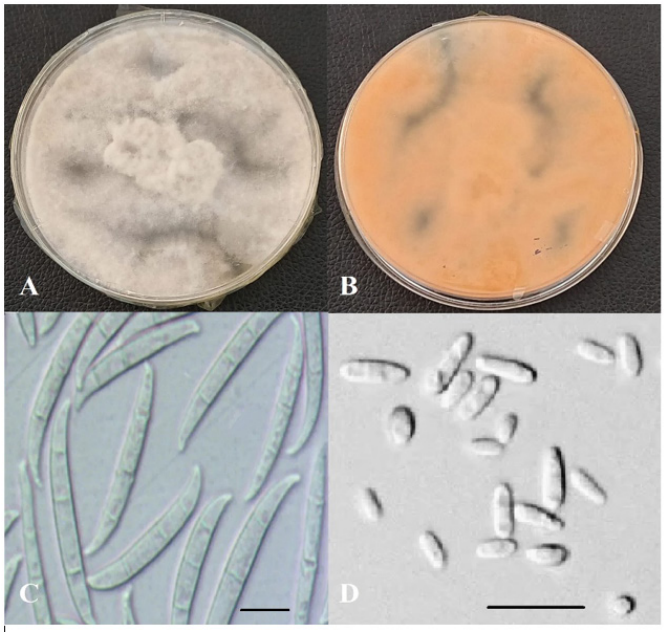

Figure 1. Morphological and Microscopic Characteristics of *F. oxysporum f. sp. cucumerinum* (F.o.c). A: Colony Aspect on PDA. B: Colony on the Lower Surface on PDA. C: Macroconidia. D: Microconidia

Table 2. Morphological and Microscopic Characteristics of F.o.c

Colony:	
growth rate on PDA, mm/day ± SE	7 days
Mycelium	grayish rose
Pigment	grayish white on day 7
type of conidiophores	short, slender, and unbranched (monophialidic)
Microconidia:	
size, µm	average 7.0 × 3.5 µm
Septation	unicellular
Shape	ovoid-elliptical
Macroconidia:	
size, µm	18.5 × 4.0 µm
Septation	3-septate
Shape	ovoid-elliptical, slightly curved
Chlamydospores:	
size, µm	average 8.7 µm
Shape	spherical (rounded)

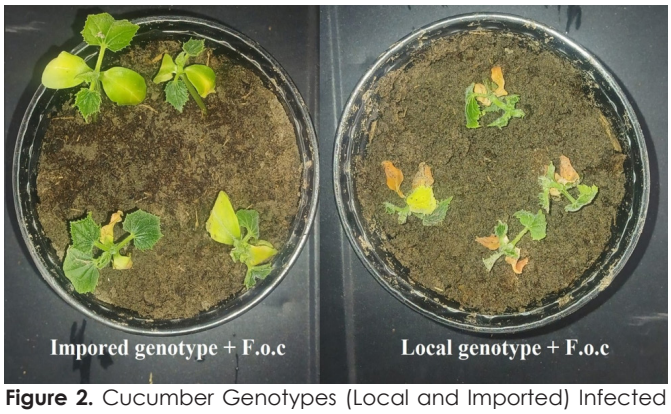

Figure 2. Cucumber Genotypes (Local and Imported) Infected with F.o.c.

wilting and plant death due to fungal invasion of the vascular system (Vasić et al., 2025). This is attributed to the fungus's ability to colonize the xylem vessels and impede water transport, leading to water imbalance within the plant—a well-known pathogenic mechanism in vascular wilt diseases (Yu et al., 2025).

Furthermore, the severity of infection recorded in this study aligns with recent research indicating that Fusarium wilt is one of the most destructive diseases affecting cucumber crops, causing significant losses that can reach high levels in agricultural fields (Wang et al., 2025).

Recent research trends point to a growing interest in studying this pathogen, particularly regarding understanding the plant-pathogen interaction and developing biological control and resistance strategies (Chaudhary et al., 2025).

Gene Expression Assay

Expression Levels of Resistance Genes in Imported and Locally Genotypes of Cucumber Infected by F.o.c.

The qRT-PCR results showed that the expression levels of *Chi2* and *PR-1* genes in imported genotype seedlings infected with F.o.c. were up-regulated by 1.71-fold (from 1.14- to 1.94-fold) and 1.35-fold (from 1.14- to 1.53-fold), respectively at 24 hpi, and up-regulated by 1.94-fold (from 1.14- to 2.21-fold) and 1.41-fold (from 1.14- to 3.87-fold), respectively at 48 hpi, and reached 2.20-fold (from 1.14- to 1.60-fold) and 1.62-fold (from 1.14- to 1.84-fold), respectively at 96 hpi. However, it did not rise in the locally genotype seedlings that is susceptible to infection at all tested time points (Figure 2). For the local genotype, the expression levels of the *Chi2* and *PR-1* genes did not increase in plants infected or uninfected with F.o.c. only the *Chi2* gene doubled after 96 hours of infection **Figure 3**.

The imported genotype is more efficient at activating defense genes upon exposure to the pathogenic fungus. Increased expression of the *Chi2* gene indicates the activation of a chitinase-dependent defense mechanism, which plays a crucial role in the degradation of fungal cell walls, primarily composed of chitin. The *Chi2* gene has been shown to be a potent activator of Fusarium wilt and contributes to enhanced plant resistance by inhibiting fungal growth or weakening its cellular structure (Xu et al., 2021). Recent studies have also demonstrated that high expression of chitinase genes is directly associated with increased cucumber resistance to Fusarium wilt, and that inhibition of these genes leads to increased susceptibility to infection (Xu et al., 2021).

The *PR-1* gene is one of the most important genes

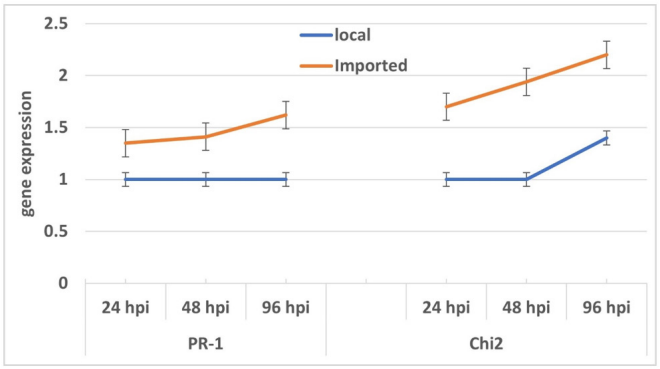

Figure 3. Relative Gene Expression for Local and Imported Genotypes of Cucumber Infected F.O.C. were Sampled 24, 48 and 96 hpi Analyzed with qRT-PCR and Compared with GAPDH Expression Levels

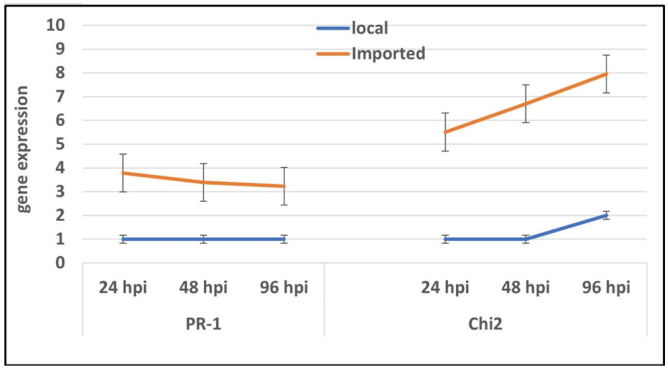

Figure 4. Relative Gene Expression for Local and Imported Genotypes of Cucumber Treated with *T. Viride* were Sampled 24, 48 And 96 Hpi Analyzed With qRT-PCR and Compared with GAPDH Expression Levels

associated with the acquired systemic resistance (SAR) pathway and is often used as an indicator of salicylic acid-dependent plant defense activation. Recent studies have shown that PR genes, including *PR-1*, are significantly activated upon pathogen infection and play a role in enhancing plant immunity by regulating local and systemic defense responses (Kim and Kang, 2023). Other studies have shown that *PR-1* expression may be an early, temporary response or a sustained response, depending on the severity of the infection and the host's interaction with the pathogen (Abdelkhalek et al., 2022).

The time pattern of increased gene expression in the imported strain (24, 48, and 96 hours) suggests a dynamic and escalating defense response, with the response starting early and continuing to build up. This reflects the plant's ability to recognize the pathogen and effectively activate defensive signaling pathways. In contrast, the absence of a response in the native strain indicates impaired pathogen recognition or dysfunction in immune-related signaling pathways, making it more susceptible to infection.

Expression Levels of Resistance Genes in Imported and Locally Genotypes of Cucumber Treated with Trichoderma

Gene expression analysis of the resistance genes *Chi2* and *PR-1* were performed to determine the ability of *T. viride* to induce systemic resistance in both imported and local genotypes seedlings at 24, 48, and 96 hpi. The results **Figure 4** showed that the bioagent induced varying increases in the expression levels of resistance genes, with a higher increase observed in the imported phenotype compared to the local genotype seedlings. The results also revealed that the bioagent stimulated the expression levels of the *Chi2* gene more than the *PR-1* gene. This demonstrates the ability of *T. viride* to activate the mechanisms of Induced Systemic Resistance (ISR).

The *Chi2* gene levels in imported genotype seedlings reached 13.42-fold and 17.04-fold, and 14.28-fold at 24, 48, and 96 hpi, respectively, while *PR-1* levels increased 6.30-fold, 7.38-fold and 7.26-fold at 24, 48, and 96 hpi, respectively (Figure 4).

In the local genotype, the gene expression in both *Chi2* and *PR-1* genes increased in both genotypes. *Chi2* gene expression increased 6.11-fold, 8.61-fold, and 8.18-fold at 24, 48, and 96 hpi, respectively. *PR-1* levels increased 5.10-fold, 5.98-fold, and 6.53-fold at 24, 48, and 96 hpi, respectively (Figure 4).

These results are consistent with Agrios' (2005) findings regarding the role of bio-stimulants in activating complex plant defense responses dependent on hormonal signaling and gene regulation.

The imported genotype exhibited a higher gene expression response compared to the native genotype across all time points (24, 48, and 96 hpi), suggesting a genetic difference in the efficiency of the response to bio-stimulants. This is attributed to the imported genotype's superior ability to recognize signals from Trichoderma and activate defense signaling pathways. Recent studies have shown that resistant varieties or genotypes exhibit a faster and more intense response to defense genes compared to susceptible varieties, resulting from higher efficiency in regulating gene expression associated with plant resistance (Hua et al., 2023).

The results also showed that the *Chi2* gene exhibited higher expression levels than the *PR-1* gene in both genotypes. This is because chitinase genes like *Chi2* play a direct role in the degradation of fungal cell walls composed of chitin, making them crucial primary lines of defense against fungal pathogens. In contrast, the *PR-1* gene is associated with the salicylic acid-dependent acquired systemic resistance pathway and acts as an indirect signaling pathway for plant immune

system activation. Studies have shown that Trichoderma strongly stimulates the production of lytic enzymes such as chitinase and β-1,3-glucanase, which explains the significantly higher expression of *Chi2* compared to *PR-1* (Yu et al., 2024).

The results also showed that the *Chi2* gene exhibited higher expression levels than the *PR-1* gene in both genotypes. This is because chitinase genes like *Chi2* play a direct role in the degradation of fungal cell walls composed of chitin, making them crucial primary lines of defense against fungal pathogens. In contrast, the *PR-1* gene is associated with the salicylic acid-dependent acquired systemic resistance pathway and acts as an indirect signaling pathway for plant immune system activation. Studies have shown that Trichoderma strongly stimulates the production of lytic enzymes such as chitinase and β-1,3-glucanase, which explains the significantly higher expression of *Chi2* compared to *PR-1* (Yu et al., 2024).

Expression levels of resistance genes inimported and locally genotypes of cucumber treated with fungicide hymexazol

Gene expression analysis of the resistance genes *Chi2* and *PR-1* were performed to determine the ability of fungicide Hymexazol to induce systemic resistance in both imported and local genotypes seedlings at 24, 48, and 96 hpi. The results **Figure 5** showed that the Hymexazol induced the expression levels of the gene *PR*-1 more than the *Chi2* gene.

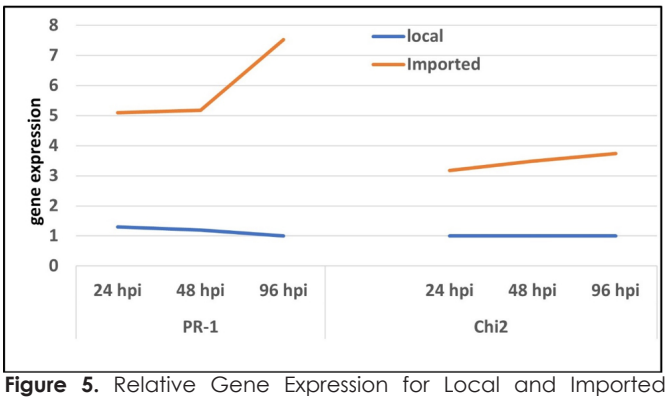

Figure 5. Relative Gene Expression for Local and Imported Genotypes of Cucumber Treated Fungicide Hymexazol with were Sampled 24, 48 and 96 Hpi Analyzed with qRT-PCR and Compared with GAPDH Expression Levels

the *Chi2* gene levels in imported genotype seedlings reached 3.18-fold and 3.49-fold, and 3.74-fold at 24, 48, and 96 hpi, respectively, while *PR-1* levels increased 5.10-fold, 5.18-fold and 7.52-fold at 24, 48, and 96 hpi, respectively (Figure 4).

In the local genotype, the gene expression in *Chi2* gene non-increased, but the *PR-1* gene expression increased 1.30-fold and 1.20-fold at 24 and 48 hpi, respectively (Figure 5).

Gene expression analysis indicates that treatment with the fungicide Hymexazol stimulated systemic resistance mechanisms in cucumber seedlings. However, the degree of response varied between the studied genes and genotypes. The results showed that the *PR-1* gene was the most responsive compared to the *Chi2* gene, indicating that Hymexazol's effect is more likely to activate the salicylic acid-associated defense pathway, of which *PR-1* is a key indicator.

The significant increase in *PR-1* gene expression levels over time (24, 48, and 96 hpi) reflects Hymexazol's ability to act as a chemical inducer that contributes to the activation of acquired systemic resistance (SAR) by enhancing defense signaling within the plant. Recent studies have shown that inducers can reprogram plant gene expression, enhancing its susceptibility to pathogens, particularly by activating PR genes associated with immune response (Yu et al., 2024). The *PR-1* gene is also considered a classic molecular marker for salicylic acid pathway activation and plant response to external stimuli (Wang et al., 2025).

In contrast, the *Chi2* gene showed a relatively limited increase in expression compared to *PR-1*, suggesting that the activation of genes involved in the enzymatic degradation of fungal cell walls may be less responsive to chemical stimuli than regulatory pathways associated with systemic immunity. *Chi2* is responsible for producing chitinase enzymes that target components of the fungal cell wall, but its activation is often more pronounced in cases of direct pathogen interaction or when using bio-stimuli (Xu et al., 2021).

The results also showed that the imported genotype outperformed the native genotype in gene expression levels for both *PR-1* and *Chi2*, indicating genetic differences in the ability to respond to chemical stimuli. These differences may be due to higher efficiency in signal perception or transduction within plant cells, as well as differences in the regulation of defensive hormonal pathways. Recent studies indicate that the plant's genetic background plays a crucial role in determining the intensity and speed of the immune response, with resistant phenotypes exhibiting a higher capacity to activate defensive genes compared to susceptible phenotypes (Hua et al., 2023). The chronological pattern of gene expression shows that the response to the *PR-1* gene was continuous and progressively increasing, particularly in the imported phenotype, indicating a sustained stimulation

of plant resistance rather than a momentary response. In contrast, the *Chi2* response was less stable, potentially reflecting differences in regulatory mechanisms among the various defensive genes. This suggests that hymexazol may primarily stimulate regulatory pathways associated with systemic immunity, with a secondary effect on genes involved in direct defense.

Conclusions

The imported resistant genotype exhibited a faster response in activating systemic induced resistance (ISR) compared to the native variety when exposed to the pathogen or treated with *Trichoderma viride* or with chemical fungicide. This is attributed to higher efficiency in the expression of resistance-related genes, resulting in a faster and more effective defensive response at both the systemic and local resistance gene levels.

Furthermore, the speed and intensity of the resistance gene response varied depending on the type of stimulant used. Bio-stimulants, such as beneficial microorganisms, stimulate a range of genes, including *Chi*, before stimulating the *PR-1* gene, whereas chemical stimulants may induce a response in *PR-1* before *Chi*,

Bio-stimulants is a promising and effective alternative to chemical stimulants, as it can sustainably activate the plant immune system, and maintain the efficiency of the defensive response against pathogens.

Author contribution

J.T.A. designed the research and supervised all the process, M.M.K. collected and analyzed the data and wrote the manuscript.

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