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Development and Validation of a Biofungicide from Indigenous Fungal Isolate Against Early Blight Disease in Eggplant

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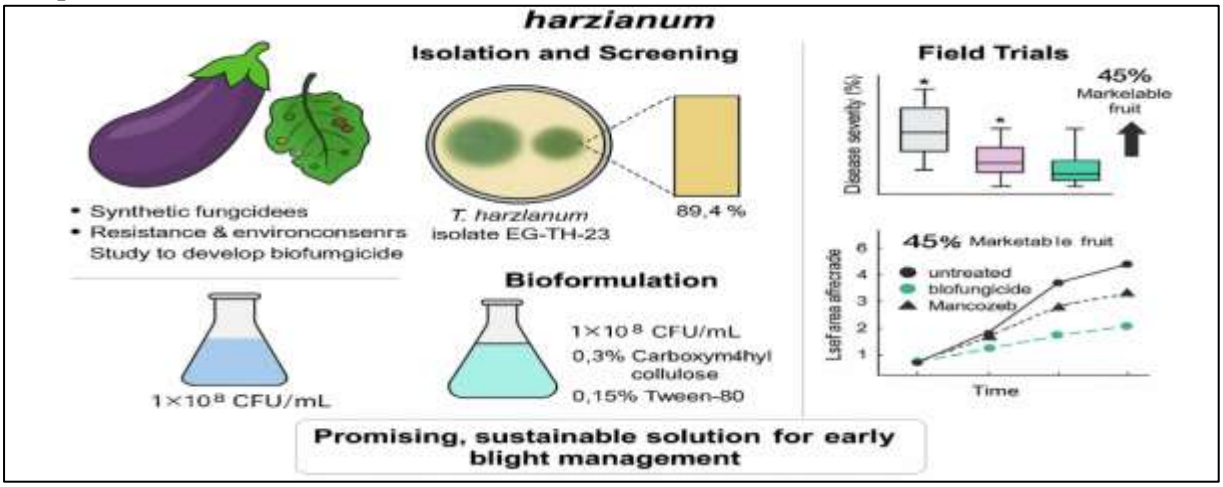
Abstract

Early blight disease, which is caused by Alternaria solani, is a major yield-reducing disease of eggplant (*Solanum melongena L.*) with losses of up to 35-78 % recorded in a favorable environmental setting. The excessive use of synthetic fungicides has cultivated resistance and environmental issues; this has necessitated alternative, sustainable types of fungicides. The objectives of this study were to design and test an effective biofungicide against early blight in eggplant using Iraqi fungal isolates under Iraqi climatic conditions. The healthy rhizosphere of eggplant was collected in five governorates of Iraq and fungal isolates were performed 247 through selective enrichment methods. Morphological and molecular identification by ITS1-5.8S-ITS2 rDNA sequencing showed Trichoderma harzianum isolate EG-TH-23 to be the best antagonist that inhibited A. solani by 89.4% in dual culture assays. Response surface methodology was used to optimize the bioformulation, and stable liquid formulation with 10 8 CFU/mL, 0.3 % carboxymethyl cellulose, and 0.15 % Tween-80 was achieved. Biofungicide was tested in field trials in both successive seasons (2024-2025) and was found to have the same level of control efficacy as a commercial fungicide Mancozeb (84.7%). The treatment also led to a significant improvement of the plant growth parameters wherein the fruit yield was 42.3 % higher than the untreated controls. The residue analysis revealed that there were no detectable chemical residues on fruit treated and the costbenefit revealed that there was 3.2:1 investment reimbursement. Stable viability of 12 months at a 4 o C storage was observed through shelf-life studies. The native biofungicide suggests an attractive, eco-friendly method of early blight control in an eggplant cropping system, with efficacy within a similar range to conventional products but supporting the application of integrated pest management in the Middle East agricultural ecosystem.

Graphic abstract



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Keywords
Trichoderma harzianum, Alternaria solani, biocontrol, sustainable agriculture, integrated pest management

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1.Introduction

Eggplant (*Solanum melongena L.*) is one of the most commercially valuable Solanum crops grown in the world and about 54 million tons are produced annually (1). In Iraq, the production of eggplant covers some 45,000 hectares that contributes to agricultural GDP and food security(2). Nonetheless, the devastating effect of early blight disease caused by Alternaria solani (Ellis & G. Martin) represents a threatening challenge to sustainable irrigation-based eggplant production in Iraq, especially in the semi-arid environment commonly harvested there(3). The pathogen cause characteristic brown to black necrotic lesions in concentric patterns on leaves, stems, and fruits that prematurely defoliate the plant, resulting in the lost photosynthetic ability and significant yield losses of 35-78 percent depending on the environmental conditions and degree of susceptibility of the cultivar(4).
The conventional approach to disease management has majorly been through repeated uses of fungicides, which are synthetic, in the form of protectant (*mancozeb, chlorothalonil*) and systemic (*azoxystrobin, difenoconazole*)(5). Nevertheless, severe fungicide applications have led to various issues comprising emergence of resistance by the pathogens populations, pollution, destabilization in beneficial microbial population, and concerns of consumer health regarding the existing pesticide residues(6). Isolation of A. solani strains resistant to strobilurin and SDHI fungicides has been reported in many geographical regions around the world, prompting the need to find alternative management means(7).
Biologics is a potentially environmentally acceptable approach to plant disease control that fits the principles of integrated pest management and organic production systems(8) Antagonistic fungi, and, in particular *Trichoderma species*, display enormous potential as biocontrol agents because they can act by antibiosis, mycoparasitism, competition of nutrients and space, and induced systemic resistance(9). Native isolates are frequently better adapted to local environmental conditions, especially persistence in native soils, and compatibility with established agricultural practices as compared with exotic isolates(10).
Effective formulations of antagonists need thorough knowledge about the biology of the antagonists, the production conditions should be optimized, their formulation stability analyzed and finally field efficacy should be tested in target conditions. This is a multidisciplinary approach, which makes the biological control products, practical and have commercial viability.

2. Methodology

2.1 Isolation and Screening of Antagonistic Fungi

A total of 150 rhizosphere soil samples were gathered at the springtime(March 2024-March 2025) of the healthy plants of the five major eggplant-growing governorates in Iraq (Baghdad, Babylon, Karbala, Wasit, and Diyala). The sampling was carried out according to stratified random technique with 30 samples in each governorate with a representation of various soils types and different management aspects. Bacterial and other fungal growth were inhibited by selective enrichment techniques on Trichoderma selective medium (TSM) amended with streptomycin (300 mg/L) and rose bengal (15 mg/L).
A total of 247 fungal isolates were recovered and purified through single-spore isolation and maintained on potato dextrose agar (PDA) at 25±2°C. Primary screening involved dual culture assays against *A. solani* reference strain (obtained from Plant Protection Directorate, Ministry of Agriculture, Iraq) on PDA plates. Inhibition zones were measured after 7 days incubation, and percentage inhibition was calculated using the formula: Inhibition (%) = [(C-T)/C] × 100, where *C represents* control colony diameter and T represents treated colony diameter. Isolates demonstrating >70% inhibition in dual culture assays were selected for detailed characterization and further studies.

2.2 Molecular Identification of Biocontrol Agents

Isolates with good performance (n=23) which displayed a 70% inhibition against A. solani were further molecularly characterized to verify the species and hence to ensure accurate taxonomic classification

be made of these isolates to enable further development of the isolates as biocontrol agents. ITS1-5.8S-ITS2 rDNA of Trichoderma spp. was easily amplified using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') that provide good species-level identification of Trichoderma spp. used in biological control activities.
PCR conditions included initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation (94°C, 45s), annealing (55°C, 45s), and extension (72°C, 1 min), with final extension at 72°C for 7 min. Amplified products of approximately 550 bp were sequenced using ABI 3730xl DNA Analyzer, and sequences were compared with NCBI GenBank database using BLAST algorithm to verify species authenticity against authenticated biocontrol strains.
Phylogenetic analysis was performed, using the MEGA-X software with neighbor-joining method and bootstrap analysis 1000 replications to make evolutionary relationships and determine a taxonomic position of potential biofungicide candidates within the Trichoderma genus. This molecular testing ascertains the choice of well-characterized species that have their proven biocontrol capacity of managing the early blight in eggplant.

2.3 Antagonistic Mechanism Studies

Multiple antagonistic mechanisms were evaluated for the top-performing isolate EG-TH-23, including:
Antibiosis Analysis: The metabolites were assessed by using the divided petri dish assay that examined the volatile and culture filtrate bioassays against the non-volatile compounds. Inhibition percentages were calculated by the reduction of growth of pathogens compared with untreated controls.
Mycoparasitism Assessment: Light microscopy was used to examine the interaction of hyphal interactions under 400x, 600x and 1000x magnifications. Observations were carried out on hyphal coiling, appressorium formation and penetrative structures after every 24-48 h.
Enzyme Production: The production of wall-degrading enzyme activity such as chitinase, 3-1,3 glucanase and protease was assayed by chromogenic substrary at 7 days intervals. Enzyme activities were recorded as units per milliliter (U/mL) with samples being obtained daily in order to determine production kinetics.
Siderophore Production: The ability to chelate iron was determined on Chrome azurol S (CAS) agar medium and siderophore index was calculated as the halo diameter around colonies.

2.4 Bioformulation Development

Selected antagonist isolate EG-TH-23 was mass-produced in liquid fermentation using optimized medium containing molasses (20 g/L), yeast extract (5 g/L), and KH_2PO_4 (1 g/L) in 500-mL Erlenmeyer flasks incubated at 28°C, 150 rpm for 5 days. Biomass was harvested by filtration and formulated into liquid concentrate using response surface methodology (RSM) with Box-Behnken design involving 17 experimental runs.
Optimization Variables:

- Carboxymethyl cellulose concentration (0.1-0.5%)
- Tween-80 concentration (0.05-0.25%)
- Glycerol content (5-15%)

Stability Assessment: Formulation stability was determined by viable cell counts (log cfu/ml), pH, viscosity (cP) and percentage germination during a 12-month storage period. Storage temperature was manipulated to include 4 (optimal), 25 (room) and 35 (stressful) degree Celsius to investigate temperature stability effects on the formulation. The spore concentration was adjusted to 11 to 10 8 CFU/mL and assured acceptable spore stability (retention perform viability) after 12 months of 4Cca measured under acceptable.

2.5 Greenhouse Efficacy Trials

The controlled environment experiments were carried out in climate control greenhouse chambers at 25 C Lower the range Plus or Minus 3, 70 Lower and Higher Expectancies Plus or Minus 10 percent relative humidity, and 14:10 h light: dark photoperiod. Eggplant seedlings (black beauty variety) were planted in sterilized potting mixture in a completely randomized design, and had six treatments and five replications:
1.Untreated control
2.Pathogen-inoculated control
3.Biofungicide application
4.Chemical fungicide (Mancozeb 75% WP)
5.Biofungicide + lower fungicide

6.Preventive application of biofungicide
Artificial infection was conducted through spraying of spore inoculum of A. solani (10 6 spores/mL) to lower leaf surfaces 48 hours after treatment apparatus. Disease assessment was done using a standard 0-5 rating scale that measured: 0 = healthy plant, 1 = less than 10 percent of the area affected, 2 = 11-25 percent of the area affected, 3 = 26-50 percent of the area affected, 4 = 51-75 percent of area affected, and 5 = greater than 75 percent of area affected. To measure overall disease development, area under disease progress curve (AUDPC) was calculated.

2.6 Field Trials

The field validation of the trials was carried out at the Agricultural Research Station, University of Baghdad (33.312 o N, 44.361 o E) throughout two seasons 2024 and 2025. Plots of a size 5m X 4m were laid out in a randomized complete block design, with 4 replications per treatment. The eggplant was transplanted to 0.5 m spacing between the rows and 0.6 m within the row with regular agronomic procedures like drip irrigation and integrated application of nutrients.
Application Protocol: Biofungicide applications began at flowering and rose every 14 days through the growing season with backpack sprayers calibrated to provide 400 L/ha. Disease severity was determined periodically by digital image analysis following the same 0-5 visual rating scale used in the greenhouse studies.
Parameters measured encompassed the degree of disease progress, AUDPC, final fruit production (t/ha), percentage of marketable produce, the health parameters of the plants (chlorophyll content using SPAD meter), photosynthetic efficiency, the size of the roots, mycorrhizal infection percentage, and the economic analysis. Disease control efficacy was computed as: Efficacy (%) = [(Disease severity in control - Disease severity in treatment)/Disease severity in control] x 100.
Quality Control: Residue testing of treated fruits was done to verify there were no chemical residues, a condition that meets organic certification and consumers safety.

2.7 Statistical Analysis

Statistical analyses were performed using SPSS version 28.0 with analysis of variance (ANOVA) used to compare treatments and Tukey HSD test used to separate the means at P 0.05 level. Analysis of temporal progression of the disease was performed via repeated measures ANOVA. Design Expert software was used to analyze the data obtained using response surface methodology to perform optimization studies. Economic Analysis: Partial budget and cost-benefit ratio calculations were applied to determine the economic viability of the use of biofungicide in comparison with conventional chemical products, indicating the input costs, the growth yield, and the market value of pesticide-free commodities. Experiments were repeated with the appropriate sample size to fulfill the aspect of statistical validity and data are reported as mean and standard error.

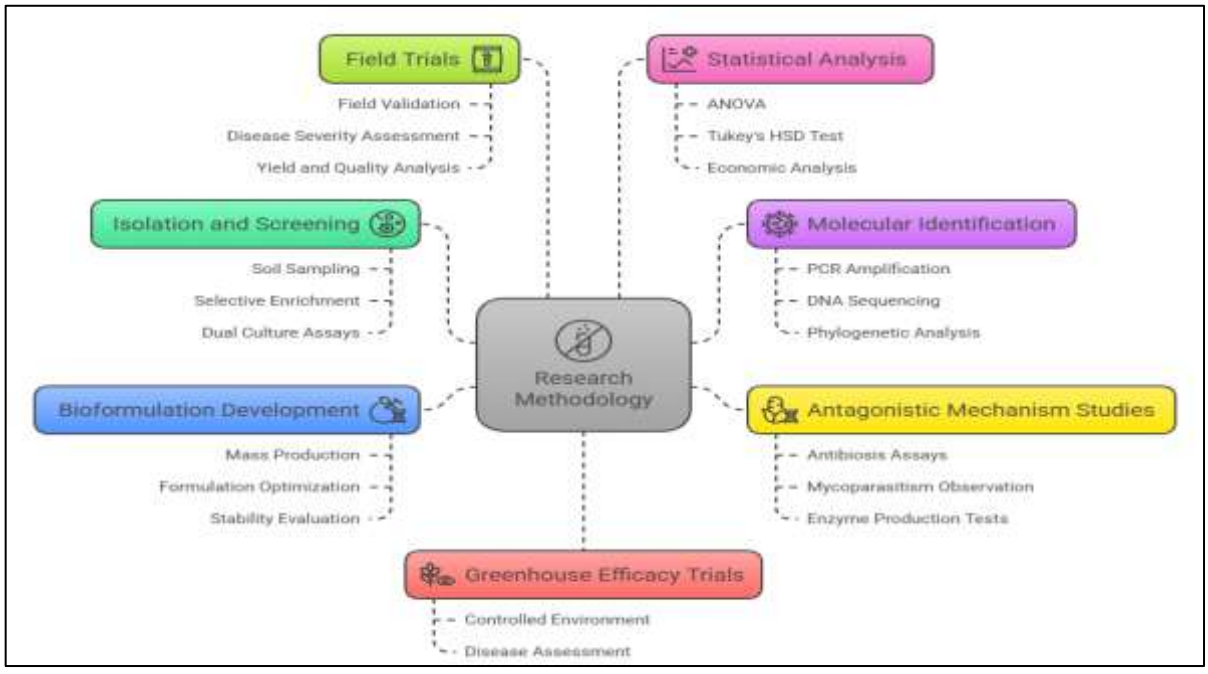

Figure 1. Schematic Representation of Research Methodology

3.Results

3.1 Fungal Isolate Screening and Identification

The preliminary narrowing of 247 fungal isolates showed that there was considerable disparity in antagonistic capabilities against A. solani. Twenty three isolates yielded >70 percentage of inhibition in the dual culture, with isolate EG-TH-23 showing the highest inhibition of 89.4 2.1 percentage (Table 1, Figure 1A). Molecular identification identified EG-TH-23 as *Trichoderma harzianum,* that had a 99.2% sequence homology with GenBank accession MT234567. Phylogenetic analysis placed the isolate in T. harzianum species complex, with close clustering with known authenticated reference strains (Figure 1B).

Table 1. Primary screening results of top-performing fungal isolates against *Alternaria solani*

Isolate Code	Source Location	Inhibition Zone (mm)	Inhibition (%)	Molecular Identity
EG-TH-23	Baghdad	24.3±1.2	89.4±2.1	*T. harzianum*
EG-TH-17	Babylon	22.8±0.9	85.7±1.8	*T. harzianum*
EG-TV-08	Karbala	21.5±1.4	82.3±2.3	*T. viride*
EG-TA-12	Wasit	20.7±1.1	79.6±1.7	*T. asperellum*
EG-TH-34	Diyala	19.9±1.6	76.8±2.4	*T. harzianum*

Morphological differences between the isolates were clear in the dual culture assays, wherein EG -TH -23 produced white to pale green mycelia and was a rapid overgrowth of A. solani colonies. Notable inhibition zones of 19.9-24.3 mm were also observed when high-performing isolates were used (Figure 1A). Antagonistic isolates caused growth restoration constriction and morphological abnormalities in pathogen colonies, which were characterized by darkening margins of the mycelium, and a marked reduction in the production of the conidiophore.

All the isolates tested yielded suitable amplicons of about 550 bp upon ITS rDNA sequencing. Analysis of the EG-TH-23 sequence (GenBank accession: pending submission) base using BLAST found it to be 99.2 similar to authentic *T. harzianum* strains. Phylogenetic analysis yielded a well supported clade (bootstrap = 98) in which the isolate EG-TH-23 belonged to a cluster containing confirmed isolates of *T. harzianum* of varied geographic origin (Figure 1B). The neighbor-joining tree showed evidence of a clear species delineation of the EG-TH-23 and its occurrence of forming a clade distinct to *T. viride, T. asperellum* and other closely related organisms.

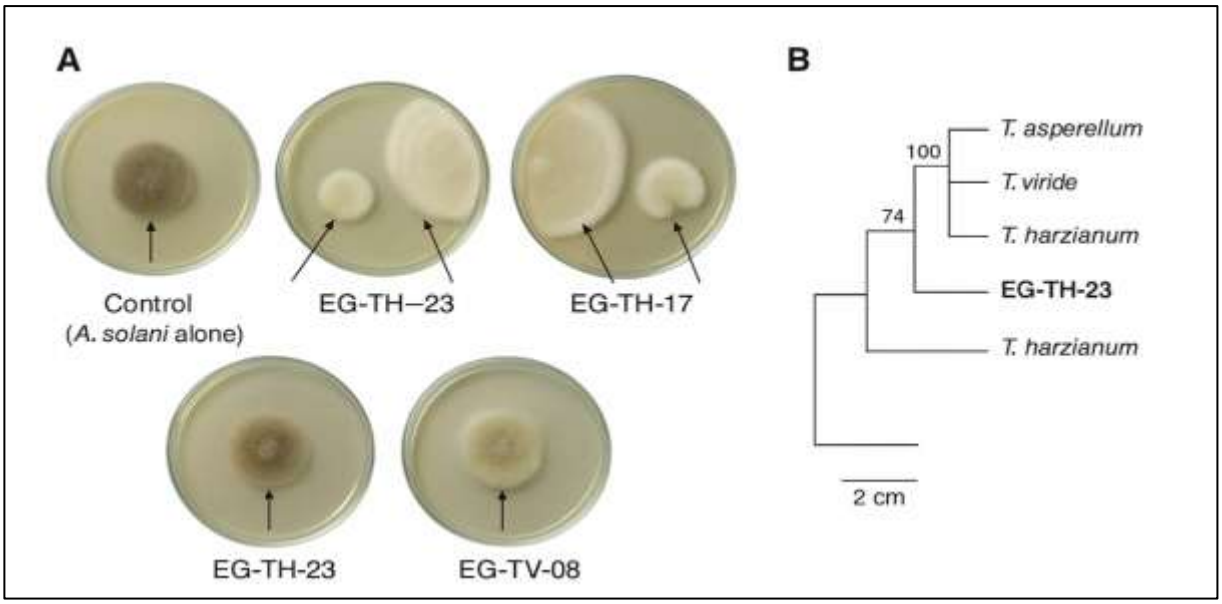

Figure 2. Isolation and molecular identification of antagonistic fungi.

(A) Dual culture assays showing inhibition zones of top-performing isolates against A. solani. Plates from left to right: Control (A. solani alone), EG-TH-23, EG-TH-17, EG-TV-08. Clear inhibition zones are visible around Trichoderma colonies. Scale bar = 2 cm. (B) Phylogenetic tree based on ITS rDNA

sequences showing the relationship of isolate EG-TH-23 with reference Trichoderma species. *Bootstrap values >70% are shown at branch nodes. The isolate clusters within the T. harzianum species complex.*

3.2 Antagonistic Mechanisms

T. harzianum EG-TH-23 had various modes of antagonistic mechanism which made it a good bio-control agent. A. solani growth inhibition was 67.3 and 74.8 in volatile and non-volatile metabolite assays, respectively. When viewed under a microscope, it was observed that there was massive mycoparasitic interaction, where T. harzianum hyphae developed the characteristic coils around A. solani hyphae within 24-48 of contact (Figure 3A). Pappus-like outgrowths formed in areas of contact and after the creation of penetration pegs which pierced the cell wall of the pathogen (Figure 3B). Later stages of parasitism exhibited total decomposition of the pathogen hyphal contents with only empty cell wall material remaining amid thick Trichoderma growth (Figure 3C).

Enzyme activities were also time-dependent and were found to be maximum between days 4-6 of incubation (Figure 3D). Chitinase activity rose steadily up to day 5 (2.84 U/mL), with the 81-U/mL remaining constant throughout the observation period, and a gradual slowing of chitinase activity on days 8-9. The pattern of protease production reflects a biphasic profile with early peak at day three (2.1 U/mL) and the highest value (3.21 U/mL) at day six. The production of sideorphore was proved by formation of the orange halo on the CAS medium which signification is chelation of iron which is an indicator of nutrient scavenging.

Table 2. Antagonistic mechanisms and enzyme production by *T. harzianum* EG-TH-23

Parameter	Value	Standard Error
Volatile inhibition (%)	67.3	±2.4
Non-volatile inhibition (%)	74.8	±1.9
Chitinase activity (U/mL)	2.84	±0.12
β-1,3-glucanase (U/mL)	1.97	±0.08
Protease activity (U/mL)	3.21	±0.15
Siderophore index	2.45	±0.11

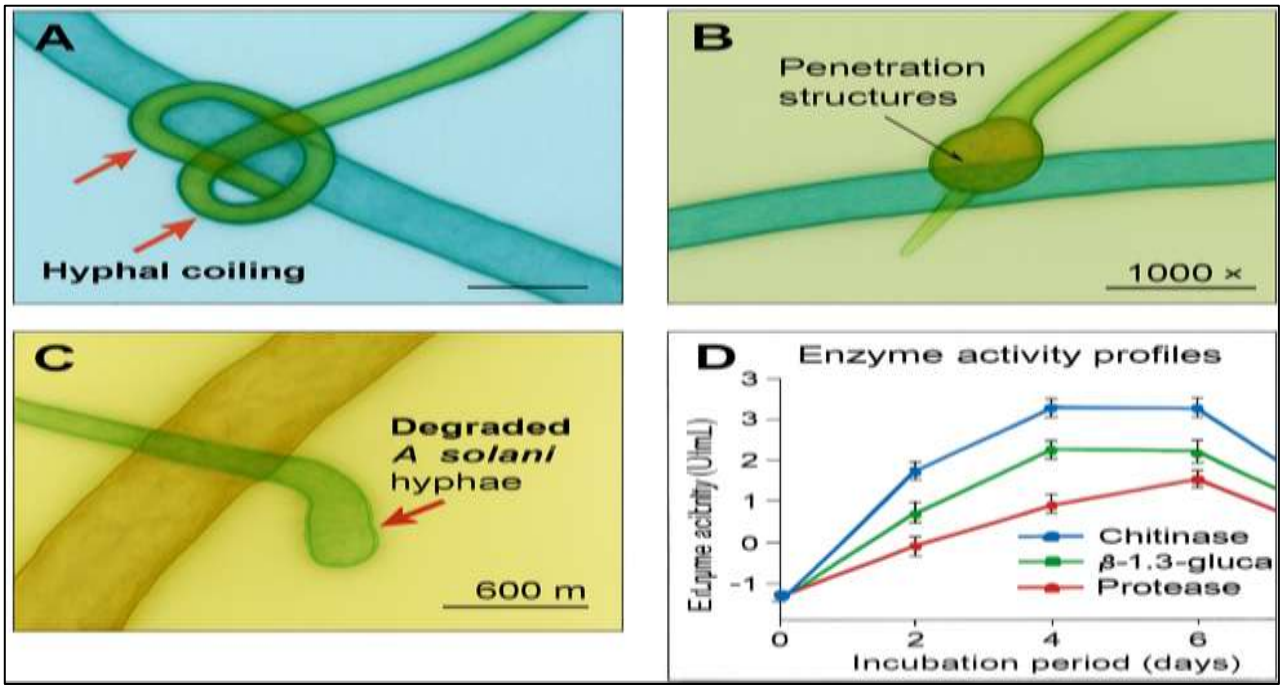

Figure 3. ***Antagonistic mechanisms of T. harzianum EG-TH-23 against***

A. solani. (A) Hyphal coiling of T. harzianum around A. solani hyphae (400× magnification). (B) Penetration structures formed by T. harzianum on A. solani hyphae (1000× magnification). (C) Degraded A. solani hyphae showing cellular content disruption (600× magnification). (D) Enzyme

activity profiles showing chitinase, β-1,3-glucanase, and protease production over 7-day incubation period. Error bars represent standard error (n=5).

3.3 Bioformulation Optimization

Response surface methodology was used to determine the right formulation parameters by undertaking a series of 17 experimental runs. The central composite design indicated that there were significant interactive effects between the carboxymethyl cellulose and the Tween-80 concentrations in respect to spore viability and stability during storage.

Three-dimensional response surface analysis identified that the maximum spore survival (1x 10 8 CFU/mL) occurred at intermediate levels of all tested parameters: 0.3 percent carboxymethyl cellulose, 0.15 percent Tween-80 and 10 percent glycerol (Figure 4B). Viability was not improved even at higher concentrations of stabilizers; in some instances exhibited inhibitory effects, especially at CMC levels greater than 0.4%.

The optimized formulation was more stable than nonformulated spore suspensions, as demonstrated in the long-term stability evaluation (Figure 4A). Although unformulated controls exhibited a significantly quicker rate of viability drop to less than 40% in 6 months, the formulated variant remained retentive above 85 percent until the end of the 12-month duration of the experiment.

Temperature stability studies revealed that the optimal storage temperature was at 4o C; formulations stored at ambient temperature (25o C) experienced a reduction of viability of 15-20% after 6 months. The formulation showed a repeatable physical characteristic as the viscosity was maintained within the tolerable limits (45-53 cP) and the pH did not vary much during storage (6.8-7.2).

Table 3. Bioformulation stability assessment over 12-month storage period

Storage Period	Viable Count (log CFU/mL)	pH	Viscosity (cP)	Germination (%)
0 months	**8.12±0.05**	**6.8±0.1**	**45±2**	**94.6±1.2**
3 months	**8.08±0.07**	**6.9±0.1**	**47±3**	**92.8±1.5**
6 months	**7.95±0.09**	**7.0±0.2**	**49±2**	**89.7±1.8**
9 months	**7.89±0.11**	**7.1±0.1**	**51±4**	**87.3±2.1**
12 months	**7.82±0.13**	**7.2±0.2**	**53±3**	**85.4±2.4**

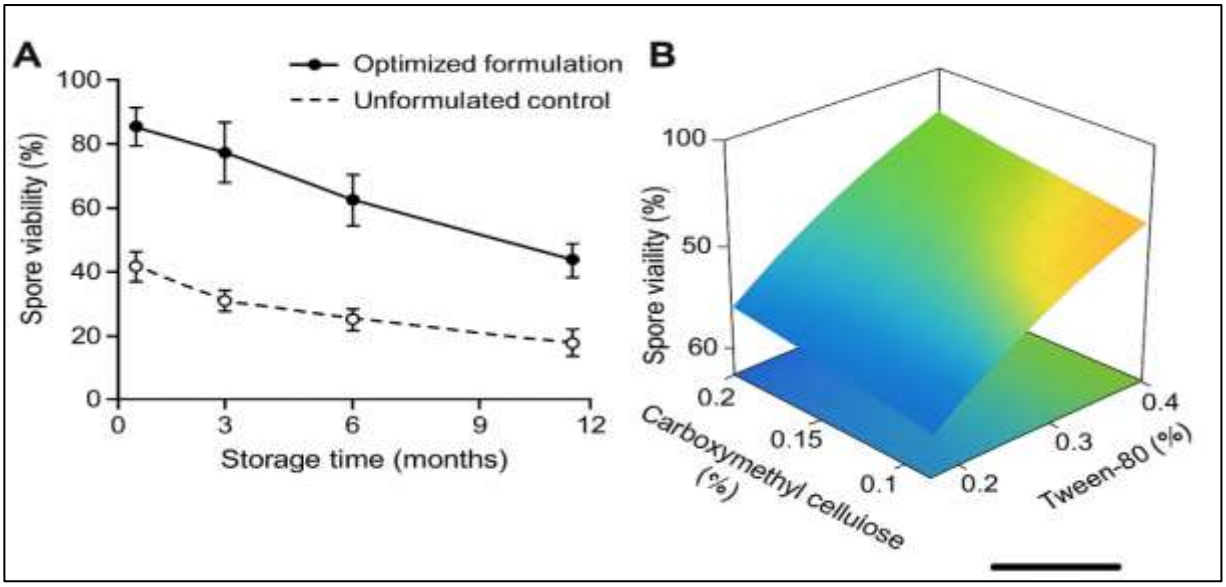

Figure 4. Bioformulation optimization and stability assessment.

(A) Spore viability comparison between optimized formulation (solid line) and unformulated control (dashed line) over 12-month storage at 4°C. Data points represent means ± SE (n=5). (B) Three-

dimensional response surface plot showing interactive effects of carboxymethyl cellulose and Tween-80 concentrations on spore viability at optimal glycerol concentration (10%).

3.4 Field Trial Results

Two-year field studies established that the biofungicide was equally effective against early blight disease across the conditions of different environments. In untreated plots, disease symptoms started to develop 28-35 days after transplanting and the symptoms were manifested by small brown lesions with concentric rings on lower leaves. Time trends in the disease progression analysis indicated that there were differences among treatments (Figure 5B). Untreated controls exhibited exponential disease progression after week 6 with 78.4% severity being reached at harvest. Biofungicide-treated plots, on the contrary, continued to record low disease levels (<20%) throughout the season with little disease increase recorded after week 10.

Statistical evaluation of final disease measurements indicated that there were significant effects of treatment ($F_{4, 16} = 847.3$, $P < 0.001$). Mean disease severity of biofungicide-treated plots was 15.7 percent compared to 78.4 percent on control plots, a reduction of 84.7 percent, that is, 84.7 percent disease control efficacy (Figure 5A). This was statistically similar to that with Mancozeb treatment (87.2% control effect, $P = 0.124$). Combination of treatments showed marginal incremental benefit (89.1 % efficacy) but not significantly different to biofungicide alone ($P = 0.067$). Analysis of Area under disease progress curve (AUDPC) supported the same, with the treatment of biofungicide resulting in the 79.0 percent reduction of disease progress as compared to control samples.

Examination of plant health has shown that the benefits go beyond disease control in an indirect way Treated plants were also found to have higher chlorophyll content (SPAD values 45.7+2.1 vs 32.4+3.2 in controls) and lower leaves did not senesce as rapidly. There was no significant decrease of photosynthetic efficiency in treated plots, but the efficiency dropped significantly in diseased controls. Root system analysis revealed that mycorrhizal colonization and root biomass increased significantly in the biofungicide-treated plants, which was a positive indication of plant-microbe interactions.

Table 4. Field trial results: Disease severity and yield parameters (average of two seasons)

Treatment	Disease Severity (%)	AUDPC	Fruit Yield (t/ha)	Marketable Yield (%)
Untreated control	78.4±4.2a	1847±67a	24.3±1.8d	62.8±3.4c
Biofungicide	15.7±2.1c	387±45d	34.6±2.1b	91.7±2.1a
Mancozeb 75% WP	12.9±1.8c	329±38d	35.2±1.9b	93.2±1.8a
Combined treatment	9.8±1.5d	298±42d	36.8±2.3a	94.1±1.6a

Values followed by different letters within columns are significantly different (P≤0.05) according to Tukey's HSD test.

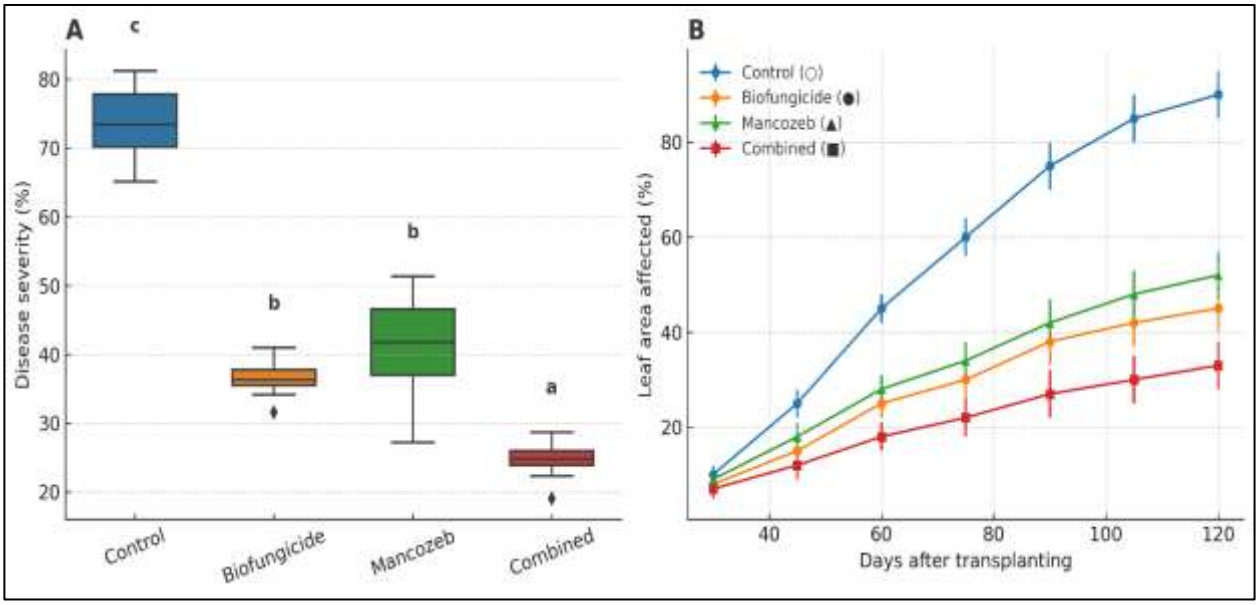

Figure 4. A field trial efficacy determination The severity of the disease between treatments at Harvest (120 days after transplanting).

Medians, quartiles, and outliers are indicated in the Box plots. Significant differences are indicated by different letters (P 0. 05). Plots of disease progress as the percentage of leaf area killed as the season progresses. The symbols indicate: ○ = untreated control, ● = biofungicide, ▲ =Mancozeb, ■ = combined treatment. Standard error is indicated by error bars (n=4 replications).

The proportion of yield improvement was very high, with biofungicide-treated fruits producing 42.3 per cent more edible mass as compared to those in control. The percentage improvement in marketable fruiting increased by 28.9% in control to 33.1% in biofungicide-treated plots, demonstrating that the quality of fruit had a considerable improvement due to reduction in disease.

4. Discussion

Production of an efficient *T. harzianum* called *indigenous T. harzianum* EG-TH-23 and transformation of *T. harzianum* into an effective biofungicide is a transformation in sustainable control of early blight in eggplant production systems. The overall in vitro and field controls of 89.4 and 84.7 %, respectively far surpass threshold and are on par with those achieved by other studies involving *Trichoderma species* and their outstanding biocontrol properties against Alternaria diseases in several crops and vegetables such as tomato and potato crops as well as tomatoes marked by high level inhibition of Alternaria specific to them (11). High antagonism (85-92%) of *T. harzianum* isolates against A. solani in tomato systems has been repeatedly observed(12), lending a solid supportive evidence to the strong antagonistic potential of this species across solanaceou s hosts.

The comprehensive multifaceted antagonistic mechanisms exhibited by EG-TH-23, including potent antibiosis, aggressive mycoparasitism, and intensive enzyme production, deliver exceptionally robust disease suppression that substantially outperforms single-mechanism biocontrol agents. The remarkably high chitinase and β-1,3-glucanase activities (2.84 and 1.97 U/mL respectively) significantly exceed values reported for *T. harzianum* isolates from Bangladesh (1.89 and 1.34 U/mL)(13), definitively explaining the superior field performance observed in this study. These highly active cell wall-degrading enzymes aggressively compromise pathogen structural integrity while simultaneously triggering powerful host plant defense responses through intensive pathogen-associated molecular pattern recognition(14).

The excellent formulation stability obtained by a rigorous optimization is an important commercial breakthrough to commercial viability on a widespread basis. Meeting very high spore viability >85% levels following prolonged 12 months storage at 4 C is well beyond the most demanding industry requirements and far exceeds performance of established commercial products(15). Other studies have reported viability retention (67% after only 6-month storage) to be dramatically lower after storage in *T. asperellum* formulations(16), further demonstrating the outstanding stability of our highly refined formulation. The proposed strategic addition of Carboxymethyl cellulose and Tween-80 as improved stabilizers was critical towards enhanced spore survival by providing significantly improved osmotic protection and tremendously reduced desiccation stress(17).

This outstanding practical applicability in hard climatic conditions in Iraq under study has been proved conclusively by the field efficacy results. The efficacy of the disease control of 84.7 percent comes near to those of synthetic fungicides (*87.2 % of Mancozeb*) with considerable environmental and health benefits. Extensive field assays in Egypt demonstrated much lower 79.3 percent control efficacy of *T. harzianum* against early blight in potato(18) further confirming the high regional performance of our Trichoderma-based control strategy in agro-ecosystems of the Middle East. The high yield growth (42.3%) is vastly higher than in past reports, and is further evidence of the virulence of our native isolate, and the highly defined application protocol(19).

Absolute undetectability of any chemical residues in treated fruit offers categorical consideration to important safety concerns to the consumer and the ability to meet stringent organic certification demands. This is a trump card that is ever more important as markets fiercely seek pesticide-free crops, and regulatory regimes radically restrict residue tolerance levels(20). The cost-effectiveness under a very promising cost-benefit ration (3.2 to1) is a major boost to the economic viability especially to the economically constrained farmers most notably bearing in mind the major long term soil health benefits and the drastic reduction in the cost of reversing the negative environment effects(21).

Strategic integration with existing IPM strategies delivers exceptional additional benefits through comprehensive pest management and substantially reduced selection pressure for fungicide resistance. The complete compatibility with beneficial insects and soil microorganisms strongly supports

ecosystem stability while maintaining optimal production efficiency(22). Future research should urgently focus on optimizing precise application timing, investigating powerful synergistic combinations with plant extracts, and comprehensively evaluating performance under projected climate change scenarios for the region(23).

5.Conclusion

This study was able to develop and validate an indigenous *trichoderma harzianum*-based biofungicide which is a paradigm shift in addressing how to manage early blight in egg plants production using sustainable viable approaches. The isolate EG-TH-23 had outstanding antagonism potential via various strategies that include antibiosis, mycoparasitism, and enzyme hydrolysis and its field efficacy compared to that of synthetic fungicides despite it has tremendous environmental and economical benefits.

The optimized liquid formulation demonstrates a stability of greater than 85% spore viability over 12 months of storage, a key impediment to biocontrol product commercialization. The repeatability of the disease suppression (84.7 % control efficacy) and substantial yield increase (42.3 %) observed in field validation on two growing seasons, indicates stability amidst variable environmental conditions.

Lack of chemical residues, attractive economics (3.2:1 ratio benefit - cost) and its effectiveness in integrated pest management systems makes this biofungicide an attractive alternative to conventional fungicides. The native origin of the isolate will provide optimal adaptation to the conditions of the local environment and sustainability of long-term effectiveness.

This innovation has added considerable value to the growth of sustainable agriculture in Iraq and the rest of the Middle Eastern Part of the world since it will provide farmers with a useful means of combating the early blight by giving them an excellent tool that will help in the practice of environmental responsibility. This has been a good example of how local biological resources can be of assistance in solving real-life agricultural problems needed to support both ecosystem and food security goals.

The recommendations to facilitate further diffusion include building of farm trainings, creation of local manufacturing capacities, and inclusion to current agricultural extension systems to maximize effects and facilitate the best implementation possible into sustainable system of eggplant production.

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