Cheatomum globousum Promotes Tomato Growth and Induces Systemic Resistance Against Tobacco mosaic virus

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Abstract

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Endophytic fungi are being explored as a promising approach for sustainable disease management. This study investigated the potential of *Cheatomum globosum*, an endophytic fungus, to enhance systemic disease resistance against tobacco mosaic virus (TMV) in tomato plants. Seedling root immersion with *C. globosum* culture filtrate under controlled greenhouse conditions resulted in several positive effects on plant growth. These included increased plant height (55.03 cm) and primary root length (14.56 cm), along with increased fresh (7.54 and 4.75 g) and dry (3.21 and 2.05 g) weight of both the aerial and root systems, respectively. Additionally, treated plants exhibited significantly higher total chlorophyll content (3.98 mg/1 g fresh weight), indicating improved photosynthetic potential. Furthermore, the activity of catalase (91.13 units g^{-1} ml^{-1} min^{-1}) and peroxidase (4.09 units g^{-1} ml^{-1} min^{-1}) enzymes, crucial for scavenging harmful reactive oxygen species, was significantly elevated compared to the untreated control group. Most importantly, *C. globosum* treatment led to a significant reduction in TMV accumulation within systemically infected leaves (disease severity index = 0.81%). These findings collectively suggest that *C. globosum* holds promise as a dual-purpose agent, promoting plant growth and at the same time acting as a potential biocontrol tool for combating plant viral diseases.

Keywords: Disease severity, endophytic fungi, growth parameters, sustainable agriculture, systemic disease resistance, viral disease.

Introduction

Tobacco mosaic virus (TMV) is a single-stranded positive-sense RNA belonging to the Tobacco virus genus that infects many plant species in several families, primarily tobacco and tomato plants as well as other *Solanaceae* family plants (Abdelkhalek *et al.,* 2022a). TMV infection results in mosaic signs on the leaves, yellowing plant tissue, and significant economic losses worldwide (Lyu *et al.,* 2023). Since TMV has an extensive host range, it has a devastating effect on the hosts' yields (Abdelkhalek *et al.,* 2022a). New approaches to controlling TMV are required because of the shortage of suitable and effective control (Grabka *et al.,* 2022).

Biological control agents have long been shown to improve plant defense systems and reduce disease severity and incidence (Morsy *et al.,* 2020; Rhouma *et al.,* 2023a; 2023b; 2023c). Plant growth-promoting-fungi are well known for their ability to minimize the disease incidence of many viral pathogens (including TMV) and trigger plant defense (Abdelkhalek *et al.,* 2022b; Adedayo & Babalola, 2023; Rhouma *et al.,* 2024).

The use of beneficial *Chaetomium* spp. to control TMV infection has recently attracted interest (Adedayo & Babalola, 2023). These endophytic fungi are free-living fungi known as bioagents because of their competence to suppress TMV infection (Haruma *et al.,* 2023). They can also elicit a host immune response against TMV with potential benefits for vegetative growth and enhance photosynthetic rates and respiration reactions by reconfiguring plant gene transcription (Kedves *et al.,* 2021).

Several workers reported that endophytic fungi (*Chaetomium* spp., *Ovatospora* spp., *Thielavia* spp., *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp., *Beauveria* spp. and *Metarhizium* spp.) had positive effects on the tomato defense system against mosaic virus infection (Morsy *et al.,* 2020; Grabka *et al.,* 2022; Shaalan *et al.,* 2022). Plants generally compensate for diseases through a variety of cellular processes (Sinno *et al.,* 2020). Pathogenesis-related proteins are primarily found in the systemic acquired resistance (SAR) pathway and are potent as antipathogenic agents (Abdelkhalek *et al.,* 2022b).

Treatment of tomato seeds with *Chaetomium globosum* relieved various biotic and abiotic stressors (Kedves *et al.,* 2021). Thus, after colonizing the roots, *Chaetomium* spp. can interact well with plants, and in some cases, chemically operate as endophytic symbiotic organisms (Nafaa *et al.,* 2023). As a result, they can modulate the activation of many plant genes and even plant physiology (Niu *et al.,* 2022). By penetrating the plant epidermal tissues, *C. globosum* can colonize plant roots, which usually results in triggering different metabolic pathways by modifying gene expression (Adedayo & Babalola, 2023; Haruma *et al.,* 2023). It has been observed that when a plant comes into contact with a pathogen, a SAR mechanism is triggered, but when it comes into contact with a nonpathogenic organism, an ISR (induced systemic resistance) mechanism is activated (Aggarwal, 2015). *C. globosum* was reported as a mediator of plant

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systemic immunity by significantly increasing the activity of glucanase, polyphenol oxidase, catalase, superoxide dismutase, peroxidase, and total phenolic content, all of which are essential SAR markers (Haruma *et al.,* 2023).

Due to the importance of the tobacco mosaic virus, and the need for more essential information to manage this viral disease and reduce economic and environmental damages arising from using chemical products, further research is necessary for the successful development of a reliable management program. This study aims to assess the morphometric, physiological, and biochemical effect of *C. globosum* against TMV infection of tomato plants.

Materials and Methods

Source of virus culture and endophyte

The tobacco mosaic virus and *C. globosum* were obtained from the Department of Plant Protection and Biological Sciences, College of Agriculture, University of Basrah, Iraq. All experiments were conducted within the Laboratory of Plant Protection of the same institution between August 2022 and June 2023.

***In vivo* evaluation of *C. globosum* on tomato plants inoculated with TMV virions**

Tomato seedlings (cv. "Super Marmond") were transplanted into pots filled with a 1:1 mixture of peat and vermiculite (three plants per pot). The experiment was set up as a randomized complete block design with three blocks, each containing 36 pots (AL-Taie *et al.,* 2024; Matrood & Rhouma, 2021a). Each treatment and block was replicated three times (9 plants per replicate). Tomato plants, 15 days after sowing, were inoculated. Seedlings (roots) were submerged for 30 min in a conidial suspension of *C. globosum*. The conidial suspension was filtered, diluted with sterile distilled water, and adjusted to a concentration of 10^7 spores/ml as determined by hemocytometer (Matrood *et al.,* 2021). One week later, the true leaves of each plant were mechanically inoculated with TMV virions (1 mL) as reported earlier (Abdelkhalek *et al.*, 2022a; 2022b). Four treatments were applied in each block: T1: plants inoculated only with TMV virions (positive control), T2: plants treated only with water (negative control), T3: plants treated only with *C. globosum*, and T4: plants treated with *C. globosum* and inoculated with TMV virions. Treated pots were incubated in a growth chamber under a 16-hour light/8-hour dark photoperiod, and at temperature of 20-22°C for 60 days.

Plant growth characteristics

Sixty days post-inoculation (dpi), tomato plants were carefully removed from their pots, and shoot and root fresh weight (g), dry weight (g), and shoot and root length (cm) were measured according to the methods described previously (Matrood *et al.,* 2022; Rhouma *et al.,* 2023a; 2023c).

Chlorophyll content was assessed by using the following formula (Hajji-Hedfi *et al.*, 2023a):

$$\text{Total chlorophyll (Chl T; mg/g fresh weight)} = (12.41 \times \text{absorbance}_{663} - 2.59 \times \text{absorbance}_{645}) + (22.90 \times \text{absorbance}_{645} - 4.68 \times \text{absorbance}_{663}).$$

Plant defense-related enzymes assay

The biochemical characteristics of catalase (CAT; units/g/ml/min) and peroxidase (POX; units/g/ ml/min) activity were evaluated in tomato leaves. Per treatment and block, three tomato leaves were assessed following the methods described by Hajji-Hedfi *et al.* (2023b) and Matrood & Rhouma (2021b) for CAT and POX activity, respectively. CAT activity determination involved a 3 mL reaction mixture comprised of 0.05 mL enzyme extract, 0.5 ml hydrogen peroxide (H_2O_2), 0.95 mL distilled water, and 1.5 mL phosphate buffer. The absorbance of this mixture was measured at 240 nm (Hajji-Hedfi *et al.,* 2023b). POX activity determination also utilized a 3 ml reaction mixture, but with differing components: 0.1 ml enzyme extract, 0.5 mL H_2O_2, 0.9 ml distilled water, 1 ml phosphate buffer, and 0.5 ml guaiacol. The absorbance of the POX reaction mixture was measured at 470 nm (Matrood & Rhouma, 2021b).

Disease assessment

A 0-4 disease severity scale was used to assess the severity of TMV infection on plants. This scale ranged from 0 (no symptoms) to 4 (complete death), with 1 indicating mild mosaic, 2 indicating mosaic and malformation, and 3 indicating severe mosaic and malformation (Zehnder *et al.,* 2000). The disease severity index (DSI) was then calculated using the following formula:

$$\text{DSI (\%)} = \frac{\Sigma\ \text{(number of diseased plants in a specific disease rating category} \times \text{disease rating)}}{\text{total number of plants investigated} \times \text{highest disease rating}} \times 100$$

This formula essentially calculates the average disease severity by weighting the disease rating of each plant according to the number of plants in that category and then standardizing by the maximum possible disease score (Aeini *et al.,* 2021; Matrood & Rhouma, 2022).

Statistical analysis

Statistical analysis was performed on the mean values of replicates to assess treatment effects. Analysis of variance (ANOVA) was conducted using SPSS version 20.0 (SPSS, SAS Institute, USA) to identify significant differences between treatments. Prior to ANOVA, homogeneity of variances and normality of the data were assessed. Duncan's Multiple Range Test was employed to determine significance between means at P=0.01.

Results

Plant growth characteristics

Table 1 summarizes the effects of a preventive treatment using *Chaetomium globosum* on tomato plants growth in the presence of TMV infection under different treatment conditions. TMV infection significantly reduced plant length, fresh weight, and dry weight compared to the healthy control. Plants treated with *C. globosum* showed significantly greater length (62.11 cm), fresh weight (11.33 g), and dry weight (4.08 g) compared to both the negative and positive control groups. This suggests that *C. globosum* treatment promotes overall plant growth (Table 1).

Table 1. Effect of preventive treatments using *Chaetomium globosum* on tomato plant length, fresh and dry weights of the aerial part in the presence of tobacco mosaic virus (TMV) infection.

	Length (cm)		Fresh weight (g)		Dry weight (g)	
Treatments	**Aerial part**	**Root system**	**Aerial part**	**Root system**	**Aerial part**	**Root system**
Negative control	45.14 c	11.41 c	7.59 b	4.11 c	3.23 b	2.03 b
Positive control	23.12 d	9.76 d	5.93 c	3.05 d	2.87 c	1.82 c
C. gloposum	62.11 a	19.04 a	11.33 a	5.94 a	4.08 a	2.64 a
C. gloposum + TMV	55.03 b	14.56 b	7.54 b	4.75 b	3.21 b	2.05 b

Values followed by the same letters in the same column are not significantly different at P= 0.05 according to Duncan's multiple range test.

Whereas, *C. globosum* treatment improved plant growth compared to TMV-infected plants (positive control), the values for the *C. globosum* + TMV group were still lower than the healthy control group (negative control) for all three growth parameters (length = 55.03 cm; fresh weight = 7.54 g; dry weight = 3.21 g). This indicates that *C. globosum* offers some level of protection against TMV but may not completely negate the negative effects of the virus infection (Table 1).

Plant growth was also measured by assessing primary root length, and fresh and dry weight of the root system. In all three parameters (19.04 cm, 5.94 g and 2.64 g, respectively), the *C. globosum* treatment (without TMV) resulted in significantly increased growth compared to the negative and positive control groups. When *C. globosum* was introduced alongside TMV, the root length (14.56 cm) and weight measures (4.75 and 2.05 g) were still significantly higher than the positive control (TMV only), but lower than the *C. globosum* treatment alone. This suggests that *C. globosum* can alleviate some of the negative effects of TMV on plant growth, but may not completely eliminate them (Table 1).

Plant defense-related enzymes assay

Table 2 investigates the effect of *C. globosum* on enzyme activity in plants infected with tobacco mosaic virus. Results obtained (Table 2) showed that activities of catalase (CAT) and peroxidase (POX), enzymes involved in plant defense mechanisms, were significantly affected by the treatments. Plants inoculated with only TMV had lower CAT (15.33 units/g/ml/min) and POX (1.10 units/g/ml/min) activity compared to the negative control (23.79 and 2.13 units/g/ml/min, respectively). This indicates that TMV infection suppresses these enzymes, potentially weakening the plant's defense system (Table 2). Interestingly, treatment with *C. globosum* alone (without TMV) significantly increased both CAT (118.15 units/g/ml/min) and POX (6.09 units/g/ml/min) activity compared to the negative control. This suggests that *C. globosum* might activate the plant's defense system. Even when *C. globosum* was applied along with TMV, CAT (91.13 units/g/ml/min) and POX (4.09 units/g/ml/min) activity remained significantly higher than that of the controls. However, these enzymes activity were lower compared to *C. globosum* treatment alone. This implies that *C. globosum* can still partially mitigate the negative effects of TMV infection on enzyme activity, potentially aiding plant defense against the virus (Table 2).

Table 2. Effect of preventive treatments using *Chaetomium globosum* on catalase and peroxidase activities (units/g/ml/min) in the presence of tobacco mosaic virus infection.

Treatments	Catalase	Peroxidase
Negative control	23.79 c	2.13 c
Positive control	15.33 d	1.10 d
C. gloposum	118.15 a	6.09 a
C. gloposum + TMV	91.13 b	4.09 b

Values followed by the same letters in the same column are not significantly different at P=0.05 according to Duncan's multiple range test.

Data presented in Table 3 show the effect of preventative treatments using *C. globosum* on Chlorophyll content in plants infected with TMV. Plants treated with *C. globosum* alone had significantly higher chlorophyll content compared to both the negative control (4.18 mg g-1) and the TMV-infected positive control (1.99 mg/g). However, when *C. globosum* was applied to plants already infected with TMV (*C. globosum* + TMV), chlorophyll content remained lower than the negative control, but still significantly higher than the TMV-infected positive control. This suggests that *C. globosum* may offer some protective effect against chlorophyll loss from TMV infection, but may not completely prevent it (Table3).

Disease assessment

Results obtained (Table 3) showed that the DSI of tomato plants in the negative control group was 0.0, indicating disease absence. Plants treated only with *C. globosum* showed a DSI of 0, similar to the healthy negative control group. This suggests that *C. globosum* itself is not harmful to the plants. On the other hand, plants in the positive control group, infected with TMV but received no treatment to combat the virus, had a significantly higher DSI of 4, signifying a severe virus infection. Tomato plants that were pre-treated with *C. globosum* before being infected with TMV had a DSI of 0.81, significantly higher than the negative control but significantly lower than the positive control. This finding suggests that pre-treating plants with *C. globosum* offers protection against TMV infection. Overall, results obtained provide evidence that *C. globosum* may act as a biocontrol agent to mitigate TMV infection of tomato plants (Table 3).

Table 3. Effect of preventive treatments using *Chaetomium globosum* on total chlorophyll content and disease severity index (DSI) in the presence of tobacco mosaic virus infection.

Treatments	Chlorophyll content (mg/g fresh weight)	DSI
Negative control	4.18 b	0.00 b
Positive control	1.99 c	4.00 a
C. gloposum	5.51 a	0.00 b
C. gloposum + TMV	3.98 b	0.81 b

Values followed by the same letters in the same column are not significantly different at P=0.05 according to Duncan's multiple range test.

Discussion

Crop losses due to plant viral infections are a major worldwide concern, significantly impacting global food security (Rhouma *et al.,* 2021; Shaalan *et al.,* 2022; Lyu *et al.,* 2023). The complex nature of plant viral infections and the ongoing challenge of managing them in a changing environment necessitates the discovery of novel biocontrol agents (Aeini *et al.,* 2021; Abdelkhalek *et al.,* 2022b). Plant growth-promoting microorganisms offer a safe and environmentally friendly alternative to conventional chemical controls for long-term disease management (Hajji-Hedfi *et al.,* 2023a; 2023b). While *C. globosum* proved to be effective in managing plant fungal and bacterial diseases, its potential role in inducing plant defenses against viral infections remains relatively unexplored (Ghazi Mohammed *et al.,* 2024). There is a significant gap in our understanding of this specific application of *C. globosum*, with previous research being focused on antifungal and antibacterial properties (Aggarwal, 2015; Elshahawy & Khattab, 2022; Grabka *et al.,* 2022; Tian *et al.,* 2022).

Under controlled conditions, this research investigated the effects of *C. globosum* on tomato plant growth and their defense against TMV infection. The findings revealed that *C. globosum* application, regardless of subsequent TMV infection, significantly enhanced various growth parameters in tomato plants. When measured at 60 dpi, the *C. globosum* treatment group exhibited the highest values for growth parameters, followed by the group receiving both *C. globosum* and TMV. Conversely, the group solely treated with TMV displayed a significant detrimental impact on all measured growth parameters. These results support what obtained by previous workers who reported that *C. globosum* application correlated with increased chlorophyll content, root length, shoot length, and dry weight in comparison to untreated control plants (Abou Alhamed & Shebany, 2012; Elshahawy & Khattab, 2022; Khan *et al.,* 2012; Kumar *et al.,* 2021; Tarroum *et al.,* 2021). Additionally, *Chaetomium* spp. has been documented to produce auxin-like molecules that directly stimulate plant cell division, elongation, and differentiation, all contributing to increased growth. Furthermore, their volatile organic compounds, hydrocyanic acid, indole-3-acetic acid, and extracellular enzymes might directly influence chlorophyll production and further improve plant growth parameters (Singh *et al.,* 2021; Spinelli *et al.,* 2022; Tarafdar & Gharu, 2006; Tian *et al.,* 2022).

This study investigated the mechanisms by which *C. globosum* protects tomato plants from TMV infection. The findings confirmed a dual protective effect: symptom modulation leading to reduced disease severity and direct viral load reduction within plant tissues. This was evidenced by both the observed decrease in symptom severity and the lower TMV accumulation levels in *C. globosum* treated plants compared to the control group (Abdelkhalek *et al.,* 2022a; 2022b). The data suggest *C. globosum* may trigger a plant defense response through the upregulation of peroxidase and catalase enzymes (Matrood & Rhouma, 2021b). Peroxidase is known to be involved in strengthening cell walls through the deposition of lignin and suberin, potentially creating a physical barrier against the virus (Elshahawy & Khattab, 2022). Catalase, with its dual enzymatic activity, can function as a specific peroxidase while also protecting cells from the damaging effects of hydrogen peroxide, a byproduct of plant defense reactions (Zehnder *et al.,* 2000). Alternatively, the protective effect of *C. globosum* could be due to the production of antibiotic substances that directly inhibit TMV multiplication (Abdelkhalek *et al.,* 2022b; Singh *et al.,* 2021).

It can be concluded from this study that *C. globosum* application triggers systemic resistance in tomatoes by activating multiple defense pathways, leading to increased plant height, improved chlorophyll content, reduced disease severity, and lower TMV accumulation. Additionally, the application boosted antioxidant enzyme activity (CAT and POD), suggesting *C. globosum*'s potential as a valuable tool for managing plant viral diseases. Future research could test the efficacy of *C. globosum* against a range of tomato viral diseases which will broaden its potential use. Because this study was conducted under controlled conditions, field trials are essential to assess *C. globosum*'s effectiveness under real-world agricultural settings with variable weather and pest pressures.

الملخص

مطرود، عبد النبي عبد الأمير، عبد الحق رحومة، مهند عبد الرضا الوايلي، وحيد أنور ولبنى حاجي هادفي. 2025. تعزيز نمو الطماطم/البندورة وتحفيز المقاومة الجهازية ضدّ فيروس موزاييك التبغ باستخدام الفطر *Cheatomum globousum*. مجلة وقاية النبات العربية، 43(4):570-576.
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أظهرت هذه الدراسة إمكانية استخدام الفطر *Cheatomum globousum*، وهو فطر داخلي، في تعزيز المقاومة الجهازية للأمراض ضدّ فيروس موزاييك التبغ (TMV) في نباتات البندورة/الطماطم. أدى غمر جذور الشتلات بفلتر مزارع الفطر *Cheatomum globousum* تحت ظروف الدفيئة إلى حصاد العديد من التأثيرات الإيجابية على نمو النبات. شملت هذه التأثيرات تحقيق زيادة في طول النبات (55.03 سم) وطول الجذر الأساسي (14.56 سم)، إلى جانب زيادة الوزن الرطب (7.54 و 4.75 غ) والوزن الجاف (3.21 و 2.05 غ) لكلٍّ من المجموعين الهوائي والجذري، على التوالي. بالإضافة إلى ذلك، أظهرت النباتات المعالجة وجود محتوى أعلى من الكلوروفيل الكلي (3.98 مغ/1 غ وزن طازج)، مما يشير إلى حدوث تحسّنٍ في عملية التمثيل الضوئي. علاوةً على ذلك، فإن نشاط إنزيمات الكاتالاز قد بلغ (91.13 وحدة غ$^{-1}$ مل$^{-1}$ دقيقة$^{-1}$) والبيروكسيداز (4.09 وحدة غ$^{-1}$ مل$^{-1}$ دقيقة$^{-1}$)، وهو أمر ضروري للتخلص من جذور الأوكسجين الحرة الضارة، والذي ارتفع بشكل ملحوظ مقارنةً بالشاهد. والأهم من ذلك، أدت المعاملة بفطر *Cheatomum globousum* إلى انخفاض كبير في تراكم فيروس موزاييك التبغ داخل الأوراق المصابة بشكل جهازي (مؤشر شدة المرض = 0.81%). تشير هذه النتائج مجتمعة إلى أن الفطر *Cheatomum globousum* يعدّ عاملاً مزدوج الغرض، حيث يعزز نمو النبات من جهة ويعمل في الوقت ذاته كعامل مكافحة حيوية محتمل لمكافحة الأمراض الفيروسية النباتية.

كلمات مفتاحية: شدة المرض، الفطور الداخلية، معايير النمو، الزراعة المستدامة، المقاومة الجهازية للأمراض، الأمراض الفيروسية.

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