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Impact of *Chaetomium globosum* on Growth, Yield and Quality of Cucumber Plants in Relation to Damping-off Disease under Greenhouses

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Abstract: Cucumber (*Cucumis sativus* L) considers one of the most important vegetable in the world. Damping-off disease under climate of Egypt is causing severe losses in the cucumber yield. In the light of need for safe agriculture by reduce the use of fungicides this study aimed to investigate the effect of different methods of supply of *Chaetomium globosum* fungus as a biological control and promoting plant growth on cucumber damping-off caused by *Rhizoctonia solani in vitro* and *in vivo* as well as crop yield and crop quality. Four isolates of *R. solani* were tested and found to be pathogenic to cucumber plants; *R. solani* No.4 gave the highest percentage of pre-emergence damping off. Twenty-one tested isolates of *C. globosum* were suppressed the radial growth of *R. solani*, *C. globosum* No.6 was the best isolate in reduction *R. solani* linear growth of pathogen. Two greenhouse experiments were carried out during the two successive seasons of 2021 and 2022, at the Experimental Farm, Kaha, Horticulture Research Institute (HRI), Agriculture Research Center (ARC), El-Kaluobia, Governorate, Egypt, to study the effect of different methods of supply of *C. globosum* No.6. The experiment included 4 treatments with the different supplying ways of *C. globosum* (drench before transplanting, soil drench after transplanting inoculation in peat moss and foliar application before flowering). Results indicate that, the best way to supply *C. globosum* was with inoculation in peatmoss followed by foliar application before flowering which recorded the tallest cucumber plants, number of leaves, maximum of leaves area, chlorophyll content, fruits weight and length. Moreover, gives the heaviest early and total yield of cucumber as well as increment of chemical fruits composition (N, P and K) in the two greenhouse seasons. Data also confirmed the capability *C. globosum* in reducing of damping off incidence during the greenhouse seasons especially when supply as inoculation in peatmoss. In this respect, data also indicated that, *C. globosum* treatment can be stimulated defense-related marker genes including salicylic acid (NPR1 and PR1), jasmonic acid (LOX1 and PR3) and Phenylalanine ammonia-lyase (PAL) which obtained by using real-time PCR (qPCR) methods.

Key words: Cucumber · *Chaetomium* · Yield · Quality · Damping-off · Greenhouses

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an important vegetable and one of the most popular members of the cucurbitaceae family. Damping-off is one of the most serious diseases infecting and causing severe losses in the cucumber yield. In light of increasing the use of natural resources or ecologically friendly agriculture adopted around the world, it had to be advisable to use a safe agriculture practice for cucumber production [1]. Using beneficial microorganisms for plant disease control not only improves yield and quality but also reduces the use of chemical pesticides and ensures food and environmental safety; thus, using beneficial

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microorganisms is an environment friendly technology for sustainable agricultural development [2]. Kaewchai *et al.* [3] reported that fungal biofertilizers and biofungicides have been recommended for agricultural use due to their ability to suppress plant diseases and increase crop production. *Chaetomium* is considered as bio-fungicide and applied to plant disease control for protective and curative effects in controlling plant disease and promoting plant growth. Moreover, in various studies, endophytes fungi have been shown to stimulate plant growth, increase disease resistance, improve plant resistance to environmental stresses and recycle nutrients [4, 5]. Endophytes fungi also are resources for plant production and biocontrol agents. The symbiotic relationship between plants and endophytes fungi, it is the plant's helps endophytes fungi by providing nutrients and shelter, while, endophytes can produce plant hormones, increase of antioxidant enzymes activity and promote the growth of host plants under stress conditions [6]. Song and Soytong [7] found that *Chaetomium* spp. safe for humans and the environment and can be developed as a biofertilizer to increase plant growth and yield in several economic plants, also *Chaetomium* spp. produce antibiotic substances and ergosterol which increase soil fertility and affect plant growth parameters and yield. Sibounnavong *et al.* [8] indicated that Chaetomium-bioproduct increased the yield of tomatoes compared to untreated control. On the other hand, *C. globosum* is considered as endophytic fungus, showing antagonistic activities against several pathogenic fungi *in vitro*, their mechanisms in inhibit the pathogen growth was depended on the antibiosis [9-11]. *C. globosum* can be used as a biofertilizer for field crops and woody plant seedlings, where it could colonize the plants, promote plant growth and protect plant from the biotic and abiotic stresses [12, 13]. In this respect, *C. globosum* fungal biofertilizer significantly influenced growth, increased yield and improved the quality of strawberries [14]. Moreover, when cucumber seedlings inoculated with the *C. globosum* and transferred to soil, the plants exhibited faster growth and earlier flowering that due to *C. globosum* has colonized cucumber and increase their growth due to promoting the metabolic pathways which involved in plant growth. Tian *et al.* [15] showed that, interaction between the cucumber and endophytic *C. globosum* strain ND35 can contribute significantly to plant growth.

Methods of *C. globosum* biofertilizer plant growth-promoting fungi (PGPF) can be applied to the plant as a seed treatment, suspension to soil, or a combination between them [15]. The application methods have significantly variable results for promoting growth when applied by a combination of supply methods show high efficiency in promoting wheat growth [16]. *C. globosum* increased the shoot growth and all growth characteristics of pepper plants. The plant had higher chlorophyll content, shoot biomass and leaf area [17]. Radhi *et al.* [18] showed that the plant growth parameters such as plant height, yield, chlorophyll content and some of plant metals content are affected by using Dix N10 and *C. globosum* either together or alone. Also, Singh *et al.* [19] reported that *C. globosum* enhances tomato plant growth.

Therefore, our present study investigates the effect of different ways of addition of *Chaetomium globosum* fungus addition vegetative growth, yield, quality on cucumber plants grown under greenhouse as well as damping-off disease caused by *Rhizoctonia solani in vitro* and *in vivo*.

MATERIALS AND METHODS

Source of Isolates: Four isolates from *Rhizoctonia solani* according to Sneh *et al.* [20] and twenty-one from *C. globosum* according to Ames [21] isolated from different sources (Tables 1 & 2) were obtained from Mycology Research & Diseases Survey Dept., Plant Pathology Research Institute, Agriculture Research Center.

Table 1: Sources of isolates *R. solani*

Isolate No.	Isolate	Source
1	*R. solani*1	Potato
2	*R. solani*2	Pepper
3	*R. solani*3	Bean
4	*R. solani*4	Cucumber

Table 2: Sources of *Chaetomium* isolates

Isolate No.	*Chaetomium* isolate	Source
1	*Chaetomiumglobosum1*	Citrus 1
2	*Chaetomiumglobosum2*	Papper
3	*Chaetomiumglobosum3*	Broccoli
4	*Chaetomiumglobosum4*	Rice
5	*Chaetomiumglobosum5*	Ornamental Plant 1
6	*Chaetomiumglobosum6*	Citrus3
7	*Chaetomiumglobosum7*	Ornamental Plant 2
8	*Chaetomiumglobosum8*	Barley
9	*Chaetomiumglobosum9*	Soybean
10	*Chaetomiumglobosum10*	Papper
11	*Chaetomiumglobosum11*	Insect
12	*Chaetomiumglobosum12*	Tomato
13	*Chaetomiumglobosum13*	Citrus2
14	*Chaetomiumglobosum14*	Insect
15	*Chaetomiumglobosum15*	Potato
16	*Chaetomiumglobosum16*	Strawberry
17	*Chaetomiumglobosum17*	wood
18	*Chaetomiumglobosum18*	Potato
19	*Chaetomiumglobosum19*	Drasena
20	*Chaetomiumglobosum20*	Insect
21	*Chaetomiumglobosum21*	Tomato

Pathogenicity Test of Isolated Microorganism

Preparation of Pathogen Inoculum and Soil Infection: Pathogenicity test was carried out in pot experiments under greenhouse conditions using four isolates of *Rhizoctonia solani*. Fungal mass production used for soil infection was obtained by growing the tested isolates on sorghum grain medium in bottles (500 cc) according to Abd El-Khair and El-Mougy [22], then bottles were incubated at 27°C for a week. The experiment was carried out in sterilized sandy clay soil (1:1 w/w). The soil was infested individually with the pathogenic fungal cultures at the rate of 3% (w/w) according to Muhanna *et al*. [23] and watered every two days for a week before sowing. Another group of Pots was filled with autoclaved soil free of fungal inoculum as a control treatment. Three seeds per pot were planted and five replicates (pots) for each treatment were conducted.

Disease Assessment: Disease incidence was calculated as the percentage of infected plants. Virulence of the different pathogens isolates was assessed according to pre-and post-emergence damping-off estimated at15 and 30 days after planting, respectively. All of the described procedures were repeated five times and the average percentages were calculated.

Pre-emergence damping off % = Σ (c- t)/c ×100

Σ = sum, c= control (number of healthy emergent seedlings in control), t= treatment (number of emergent seedlings in infested pots).

post-emergence damping-off % = Σ (c- t)/c ×100.

Effect of Different Isolates of *C. globosum* on Cucumber Damping-Off Pathogen

Dual Culture Test on Petri Plates: The antagonistic potentiality of 21 from different *C. globosum* isolates were tested for their ability to inhibit the growth of *R. solani* 4, the causal agents of pre- and post-damping off diseases using a dual-culture technique carried out according to Gao *et al*. [9]. The linear growth area of *R. solani* 4 was measured to determine the most effective antagonistic isolate among the tested *C. globosum* isolates for further studies in the greenhouse. Percentages of the fungal growth reductions (R) were calculated using the following formula by Singh and Balodi [24]. The results were recorded when pathogens control plates were fully grown by measuring the linear growth of the pathogens growing towards *Chaetomium*.

R = (G1- G2 / G1) x 100.

where:

R = reduction of fungal growth (%).
G1 = linear growth of the pathogen grown in control plate (cm).
G2 = linear growth of the pathogen towards the tested bio-agent (cm).

Effect of *C. globosum* on Cucumber Damping of Diseases under Greenhouse Condition: The highest virulence isolate of *C. globosum* (No. 6) was used to study their effect on damping off pathogens under greenhouse conditions. Inocula of *C. globosum* 6 were prepared by inoculating sterilized straw and sorghum grain bottles (500 cc) with a disk (5 mm) of each *C. globosum* 6 isolate (7 days-old culture). The bottles were incubated at 27°C for three weeks. Then *C. globosum* 6 was infested with the rate of 3% (w/w) for the treatments conducted as shown above. Disease incidence was determined as the percentage of pre- and post-emergence damping off.

Expression of Defense-Related Genes via RT-qPCR:

Total RNA Isolation and cDNA Synthesis: The total RNA was isolated from frozen cucumber leaves (control and treatments) using TRIZOL reagent following the recommended procedures (Thermo Fisher, USA), then the RNA was treated using RNase-free DNase I (Thermo Fisher, USA) to remove DNA contamination. RNA presence and integrity were checked on a 1.0% agarose gel electrophoresis and quantified by Nano Drop 2000 Spectrophotometer (Thermo Scientific, MA, USA). The first strand of cDNA was synthesized from 1.2µg of total RNA with the M-MLV Reverse Transcription Kit (Promega Corporation, United States) was used to synthesize cDNA, which was then diluted to 1:10 before quantitative PCR (qPCR) analysis. The qPCR-specific primers were designed using the Primer Quest Tool (Table 3).

Quantitative PCR (qPCR) Analysis: The expression level of all samples was validated through qPCR analysis using the Solg™ 2X Real-Time PCR Smart mix (SYBR Green Mix; Sol Gent Co., Korea) on an Agilent Stratagene Mx3005p real-time PCR detection system, following the manufacturer's instructions. For expression analysis, a total volume of 20 µl was used, comprising 1 µL of diluted cDNA, 10µL of 2 × SYBR Green PCR Master Mix and 0.3 µL of each forward and reverse primer (10 µm). Amplification was performed using the following program:

Table 3: The sequences of used primers

Gene	Sequence
CS_NPR1-F	TTACTGATAAGGGCAAGAAGGCC
CS_NPR1-R	AAAGTTCACAAAGAGCAGGATGG
CS_PR1-F	GTGCCTTGTGATGAAGTAGG
CS_PR1-R	CCACACTAGAGGAGGTTGAT
CS_PAL1-F	GCAGTGCCACTTATCCATTA
CS_PAL1-R	GGCGTTCTTCTCATCTTCTC
CS_PR3-F	TTCCGACATCGAAGCTTTAC
CS_PR3-R	GGTTGGAAGAACTTGAGAAATAAG
CS_Actin-F	GGCCGTTCTATCACTGTATG
CS_Actin-R	GAGCATAACCCTCGTAGATTG

95°C for 15 min, followed by 40 cycles at 95°C for 3 s and 60°C for 40 s. To ensure the specificity of qPCR products, a melting curve analysis was performed from 65°C to 95°C to verify the specificity of the qPCR products. The Actin gene was used as the internal reference gene for normalization. All reactions were conducted in triplicate and relative expression levels were calculated using the 2-°Ct method Schmittgen and Livak [25].

Field Experiments: Two field experiments were carried out during the two successive seasons of 2021 and 2022 at the Experimental Farm, Kaha Vegetable Research Farm, El-Kaluobia Governorate, Horticulture Research Institute, Agriculture Research Center (ARC), Egypt. The experiments aimed to study the effect of different methods of addition of *C. globosum* fungus on yield, quality and its relationship to infection with damping-off of cucumber plants under the greenhouse. A complete randomized block design with three replicates was adopted. The soil of the experimental farm was clay loam in texture with a pH of 7.98. The cucumber seeds of Sina 2 Hybrid F1were sown in 84 cell trays (cell diameter 9 mm) containing 26.5 cm^3 of commercial peat moss substrate. At January 24th and 26th during the 2021 and 2022 seasons respectively. The seedlings containing two true leaves, were transplanted in the greenhouse on February19th and 22nd in the first and second seasons, respectively. Each experimental plot was, two sides of the ridge with 18 m length and 1.20 cm width and transplants were spaced 0.5 m between plants. The experiment included 4 treatments with different ways of *C. globosum* (1×10^8 spores/mL) addition as follows:

T1: *C. globosum* trays drench before transplanting at the greenhouse.
T2: *C. globosum* soil drench after transplanting at the greenhouse.
T3: *C. globosum* peat moss inoculation.
T4: *C. globosum* foliar application before flowering.
T5: Control (without *C. globosum*).

All agriculture practices were done according to the recommendation by the Ministry of Agriculture for the cucumber crop.

Studied Characteristics:

Vegetative Growth Characteristics: Vegetative growth characters were recorded after 60 days from transplanting, in samples of five plants randomly chosen from each plot and the following data were recorded:

Plant Height (cm): It was measured as the average length in centimeters of five random plants. The measurement started from the ground surface to the plant stem apex.

Number of Leaves/Plant: It was determined after 60 days from planting as the average the leaves number of five random plants.

Leaf Area (cm^2): It was expressed as the mean leaf area in cm^2 using the dry weight method. The mature leaf was cleaned from dust and then weighted to the nearest 0.001g. Then 20 disks of known area were separated as weight.

$$\text{Leaf area } (cm^2) \frac{\text{Dry weight of mature}}{\text{Dry weight of 20 disk}} \times 20 \times \text{the area of disk}$$

where, the area of a disk is about 1.0 cm

Chlorophyll Content: The youngest fully expanded mature ten leaves were used per plant as mentioned by Westerman [26]. It was measured in SPAD unit, where SPAD= 10mg chlorophyll/gm fresh weight using digital chlorophyll meter (model Minolta chlorophyll Meter SPAD- 501).

Plant Survival Percentage (%): It was calculated as percentage for the survived plants after 10 days after planting.

Disease Incidence (%): It was calculated as percentage for the survived plants was determined after 60 days from planting.

$$\text{Disease incidence} = \frac{\text{Control - Treatment}}{\text{Control}} \times 100$$

Yield and its Components

Early Yield /Plant: It was determined as the weight of the first four harvested fruits.

Total Yield /Plant: It was calculated using plot yield and plot area all over the season then fruit yield per plant was calculated.

Average Fruit Weight (g): Ten fruits from each experimental plot were weighted and average fruit weight was calculated.

Physical Characteristics of Fruits: A random sample of 10 fruits from each experimental plot was taken to determine length and diameter of fruit.

Chemical Characteristics

Mineral Content: A half gram of dried samples was digested using the H_2SO_4 and H_2O_2, then grinded to fine particles and used to determine chemical contents such as minerals content, (K) potassium were measured using flame photometer method as described by Brown and Lillil [27]. Total nitrogen was determined using the modified micro Kjeldahl method. Phosphorus was determined using the colorimetric method following the procedure described by Cottenie *et al*. [28].

Statistical Analysis: The obtained data were subjected to statistical analysis by the method of Duncan's multiple range test as reported by Gomez and Gomez [29]. All statistical analysis was performed with SAS computer software.

RESULTS AND DISCUSSION

Pathogenicity Test: A pathogenicity test was carried out to investigate the virulence of different fungal isolates as the cause of root-rot disease of cucumber plants (Fig. 1). Data in Table (4) show a significant variation between *R. solani* isolates clearly in incidence of pre and post-emergence damping-off of cucumber plants. In this regard isolate of *R. solani* No.4 gave the highest percentage of pre-emergence damping off (55.5 %) followed by No.1 (44.4%) while the lowest virulent isolate was No.2 (22.2 %).

Antagonistic Effect of Different Isolates of *C. globosum*: The antagonistic effect of 21 isolates of *C. globosum* on suppressing of *R. solani* isolate No. 4, was shown in Table (5) and Fig. (2) which clearly showed that, there are a variation between *C. globosum* isolates in their effect on mycelial suppressing of *R. solani* 4. The data also found that, all *C. globosum* significantly decreased the linear growth as well as growth reduction% for *R. solani* compared with the control. In this respect, *C. globosum* No.6 recording the best growth reduction (54.4%) with *R. solani*4 followed by No.2 and No.*7,* while No.3gave the least reduction for mycelia growth.

Fig. 1: Lesions at the soil line, rot symptoms sunken reddish-brown lesions develop on stems below the soil line caused by *R. solani*

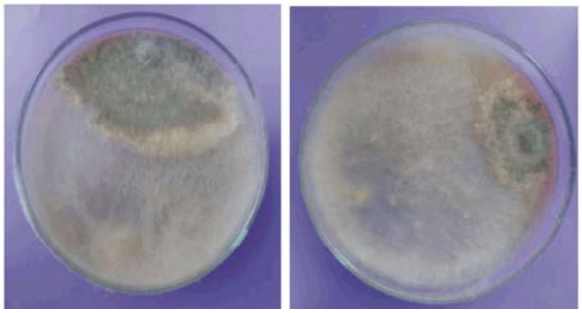

Fig. 2: The effect of *C. globosum* on suppressing mycelia growth of *R. solani* 4

Effect of *C. globosum* No.6 on Cucumber Damping-off Disease under Greenhouse Condition: The effect of *C. globosum* No. 6 to control of *R. solani* 4 which gave the highly incidence of damping-off were study and found that, *C. globosum* No. 6 reduced pre-and post-emergence damping-off compared with control treatment (without *C. globosum*), while fungicide treatment was the best Table (6).

Expression of Defense-Related Genes via RT-qPCR: cucumber leaves which have infection symptoms were collected for carrying out a quantitative Real-time PCR (qPCR) expression study of defense-related marker genes including salicylic acid (NPR1 and PR1), jasmonic acid (LOX1 and PR3) and Phenylalanine ammonia-lyase (PAL). The expression level of NPR1 was significantly up-regulated under *C. globosum* (Ch), *R. solani* with fungicides (R+Rhizolex) and *C .globosum* with *R. solani* (Ch+R)by 10, 7 and 5-fold, respectively, compared to plant treatment with *R. solani* (R), which showed a slight up-regulation compared to the control by 3-fold.Consequently, the expression levels of PR1 and PR3 were significantly upregulated under Ch by 8 and 64-fold,

Table 4: Pathogenicity test of different isolates on cucumber plants (cv. Sina 2) recorded as pre-emergence and post-emergence damping off

Isolates code	Pre-emergence damping-off %	Post-emergence damping-off %
R. solani1	44.4 a	33.3 ab
R. solani2	22.2 ab	55.5 a
R. solani3	33.3 ab	66.6 a
R. solani4	55.5 a	77.7 a
Control	0.0 b	0.0 c

Recorded at 15* and 30** days after sowing.

Table 5: Effect of different isolates of *Chaetomium globosum* on the linear growth and growth reduction of *R. solani* No. 4

Isolate No.	*Chaetomium* isolate	Linear growth	Growth reduction (%)
1	*C. globosum*1	4.7 fgh	47.7 abcd
2	*C. globosum* 2	3.8 i	51.4 ab
3	*C. globosum* 3	5.9 b	37.7 d
4	*C. globosum* 4	4.2 ghi	48.4 abcd
5	*C. globosum* 5	5.7 bc	42.5 bcd
6	*C. globosum* 6	3.7 i	54.4 a
7	*C. globosum* 7	4.7 fg	51.0 ab
8	*C. globosum* 8	4.2 ghi	45.1 abcd
9	*C. globosum* 9	5.5 bcd	40.7 bcd
10	*C. globosum* 10	5.5 bcd	39.2 cd
11	*C. globosum* 11	5.2 cdef	42.9 bcd
12	*C. globosum* 12	5.3 bcde	41.0 bcd
13	*C. globosum* 13	4.86 ef	44.4 abcd
14	*C. globosum* 14	5.4 bcde	39.2 cd
15	*C. globosum* 15	5.0 def	43.6 abcd
16	*C. globosum* 16	5.4 bcde	41.8 bcd
17	*C. globosum* 17	4.73 fg	45.5 abcd
18	*C. globosum* 18	5.1 def	44.7 abcd
19	*C. globosum* 19	5.2 cdef	40.7 bcd
20	*C. globosum* 20	5.4 bcde	43.6 abcd
21	*C. globosum* 21	4.1 hi	49.2 abc
22	Control *R. solani*4	9.0a	0.0e

Table 6: Effect of *C. globosum*6 on cucumber damping-off disease *in vivo* recorded at 15* and 30** days after sowing

Treatments	Pre-emergence damping-off %*	Post-emergence damping-off %**
Control	55.5 a	66.6 a
*R. solani*4+ *C. globosum*6	33.3 ab	22.2 ab
*R. solani*4+ Rhizolex	11.1 b	0.0 b
*C. globosum*6 without pathogen	0.0 b	0.0 b
Control without pathogen	0.0 b	0.0 b

respectively and by 10 and 48-fold, respectively, under Ch+R. On the other hand, their expression levels did not reveal significant values under R+Rhizolex. The expression level of PAL was significantly up-regulated under R- by 6-fold compared to Ch+R, Ch and R+Rhizolex by 5, 4.5 and 3-fold respectively.

Significantly *C. globosum* can reduce pre and post-emergence damping off disease incidence caused by *R. solani*. Also, these treatments significantly increase growth parameters and yield components compared with the control (without *C. globosum*). Huang *et al.* [30] reported that *R. solani* causes root rot seedling disease, which occurs before and after seedling emergence, causing bud and root rot, seedling damping-off and stem canker. Growth and disease severity of cucumber seedlings were significantly different with different treatments of cucumber with endophytic fungal (*C. globosum*) strains and *R. solani* also; endophtic affected promoting seedling growth and gives the highest efficacy in control damping off disease. These results are in agreement with Tian *et al.* [15] who showed that endophytic *C. globosum* can promote the growth of the cucumber plant in terms of radical length, plant height, root length, fresh weight and dry weight, through efficient colonization in the cucumber root system and enhancing the biological process of phytohormone homeostasis,

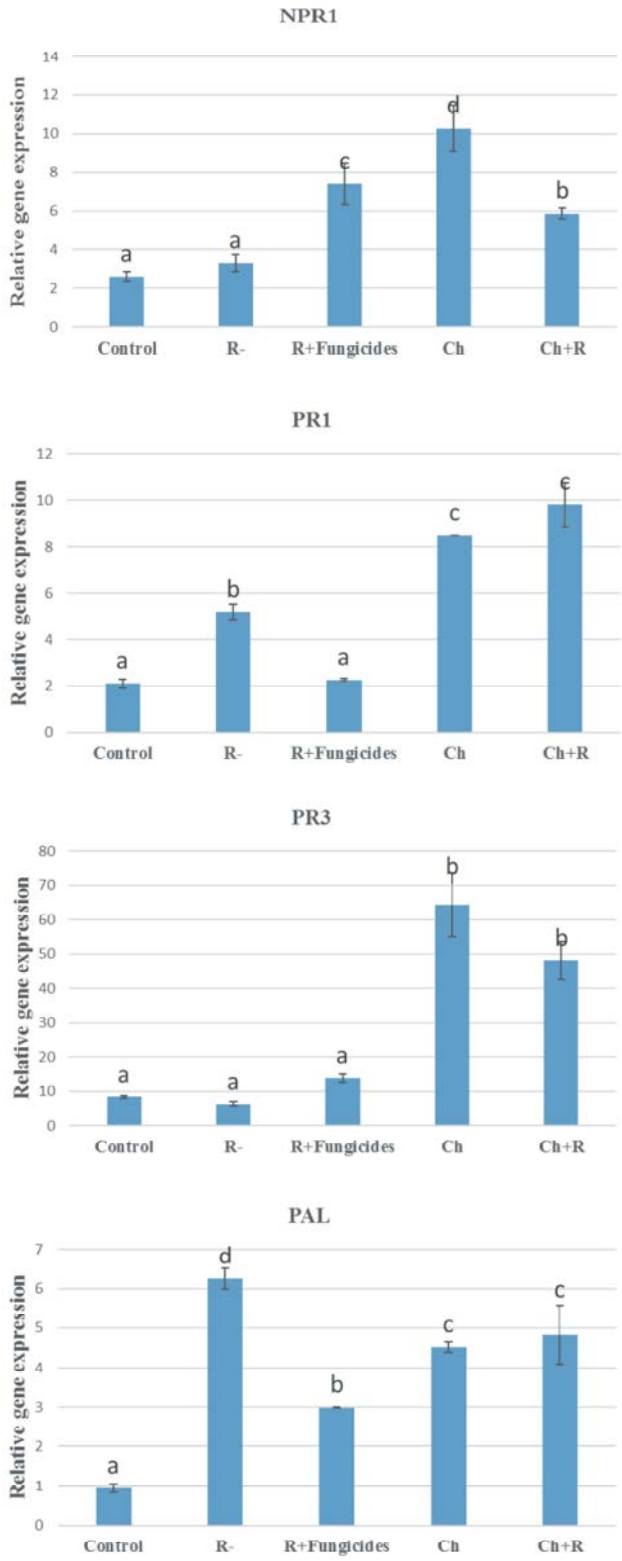

Fig. 3: Expression of defense-related genes (NPR1, PR1, PR3 and PAL)
Control:- plant without any treatment, R:- plant with pathogen *R. solani,* R+Fungicdies :- plant with *R. solani* and Rhizolex, ch:- plant with *C. globosum,* Ch+R:- plant was infested with *C. globosum* and *R. solani*.

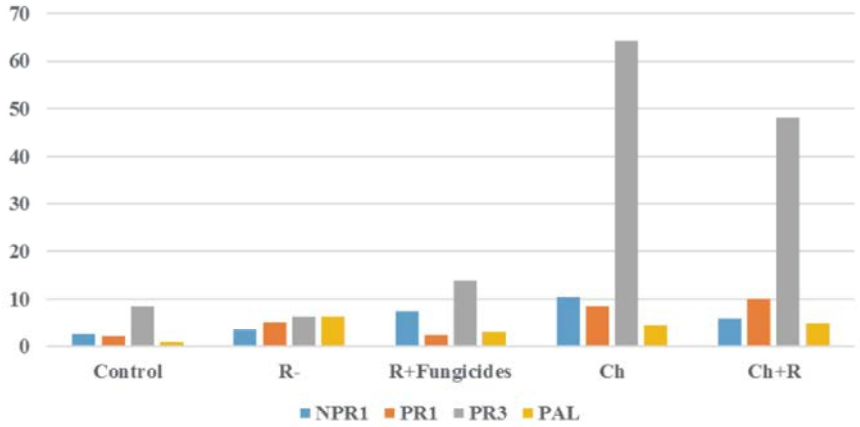

Fig. 4: Effect of different treatments on NPR, PR1, PR3 and PAL.
whereas: Control: plant without any treatment, R: plant with pathogen *R. solani,* R+ Fungicdies: plant with *R. solani* and Rhizolex, ch: plant with *C. globosum,* Ch+R: plant was infested with *C. globosum* and *R. solani.*

antioxidant activity, phenylpropanoid biosynthesis, as well as producing phytohormones, especially gibberellins and IAA [31, 32]. Moreover, the higher concentrations of ergosterol can increase plant growth and development by up-regulating the expression of genes involved in the JA/ET signaling pathways in tomato plants [33, 34]. Dong and Lin [35] reported that the expression of many genes related to the phenylpropane pathway was gradually up-regulated in cucumber radicals treated with *C. globosum* during the development of cucumber seedlings, thus lignin content and the activity of PAL, 4CL, CAD and POD were increased in the seedlings. *C. globosum* could be reinforced host cell walls by the formation of cell wall appositions (CWAs) or papillae, the formation of CWAs or papillae may be attributed to enhancement of phenylpropane metabolic activity. Phenylpropanoid metabolism is enhanced under the regulation of diverse phytohormone signal pathways, such as IAA, JA, GA and ethylene (ET). So phytohormone can increase lignin deposition by activating the expression of PAL, cinnamate 4-hydroxylase (C4H), 4CL, caffeoyl-CoA O-methyl transferase (CCoAOMT) and CAD [35].

Tain *et al.* [15] revealed that endophytic *C. globosum* increased host endogenous hormone content such as IAA, GA, zeatin (ZT), SA and JA and up-regulation of gene expressions related to those hormones and the changes of endogenous hormone may increase lignin content and the activity of key biosynthetic enzymes, such as phenylalanine ammonia lyase (PAL), 4-coumarate-CoA ligase (4CL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD). Mallory *et al.* [36] and Zhang *et al.* [37] indicated that, the *C. globosum* has activated host plant defense signaling pathways and phytohormone signal pathways in the process of establishing a symbiotic system with host plants, to promote plant growth and enhance host plant resistance to biotic and abiotic stress. Also, plant hormones, such as IAA, GA, CTK, abscisic acid (ABA), SA and JA function as essential endogenous signal molecules and play an important role in regulating plant development and response to biotic and abiotic stresses in plants.

Field Experiments

Effect of Supplying Methods of *C. globosum* No. 6 on Cucumber Damping-Off Disease under Greenhouse: The presented data in Table (7) showed that all tested treatments were increased the percentage of survival plants compared to control treatment during the two greenhouse seasons 2021 and 2022. As for disease incidence, results indicated that all treatments significantly decrease of diseases incidence during the two experimental seasons compare to control treatment. In this respect, the highest percentage of survival plants were recorded when *C. globosum* applied as a foliar treatment. This is due to the ability of the *C. globosum* to activate plant defense, beside their role in direct inhibition of pathogen growth, ultimately cucumber plants can be easily growing up in field conditions [35, 38].

Effect of Supplying Methods of *C. globosum* No. 6 on Plant Height and Leaf Number of Cucumber Plants under Greenhouse: Results in Table (8) showed that, there are significant differences in plant height and leaves number between all tested treatments and untreated control in the two tested seasons. The tallest plant height

Table 7: Effect of different supplying methods of *Chaetomium globosum* fungus on plant survival % and disease incidence of cucumber plants during 2021 and 2022 seasons

	Survival plant (%)		Disease incidence (%)	
Treatments	2021	2022	2021	2022
T1	92.50 ab	97.50 a	7.50 b	2.50 b
T2	92.50 ab	96.67 a	7.50 b	3.33 b
T3	95.83 a	97.50 a	4.17 b	2.50 b
T4	98.33 a	98.17 a	1.67 b	1.83 b
T5	78.34 c	81.67 b	21.66 a	18.33 a

T1: *Chaetomium* trays drench T2: *Chaetomium* soil drench T3: *Chaetomium* peat moss inoculation T4: *Chaetomium* foliar application T5: Control

Table 8: Effect of different methods of addition of *Chaetomium globosum* fungus on plant height and number of leaves of cucumber plants during 2021 and 2022 seasons

	Plant height (cm)		Number of leaves/ plant	
Treatments	2021	2022	2021	2022
T1	267.78 ^{c}	268.33 ^{b}	91.10 ^{c}	89.11 ^{b}
T2	275.63 ^{bc}	273.83 ^{b}	88.32 ^{cd}	87.22 ^{b}
T3	286.94 ^{a}	297.67 ^{a}	112.84 ^{a}	109.0 ^{a}
T4	286.22 ^{ab}	286.5 ^{ab}	101.82 ^{b}	94.50 ^{ab}
T5	245.22 ^{d}	221.5 ^{c}	85.86 ^{d}	82.72 ^{b}

T1: *Chaetomium* trays drench; T2: Chaetomium soil drench; T3: *Chaetomium* peat moss inoculation; T4: *Chaetomium* foliar application; T5: Control.

Table 9: Effect of different methods of addition of *C. globosum* fungus on leaves area and chlorophyll content of cucumber plants in the two studied seasons

	Leaves area (cm^2)		Clorophyll content (SPAD)	
Treatments	2021	2022	2021	2022
T1	2297.5 c	2297.0 b	37.77 c	38.86 a
T2	2305.8 c	2307.40 b	38.13 bc	38.33 a
T3	2609.2 a	2771.7 a	40.86 a	37.90 a
T4	2429.3 b	2424.7 ab	40.17 ab	36.06 a
T5	1928.8 d	1786.1 c	37.50 c	40.30 a

T1: *C. globosum* trays drench; T2: *C. globosum* soil drench; T3: *C. globosum* peat moss inoculation; T4: *C. globosum* foliar application; T5: Control.

and the maximum number of leaves were found when *C. globosum* inoculation in peat moss but without significant differences with the foliar treatment in the two growing seasons. This is due to the ability of the *C. globosum* to improvement plant growth. Similar results obtained by Latz *et al*. [4]; Al-Taie *et al.* [16]; Madbouly *et al.* [5]; Ortega *et al.* [17]; Radhi *et al.* [18] and Tian *et al.* [15].

Effect of Supplying Methods of *C. globosum* No. 6 on Leaves Area and Chlorophyll Content of Cucumber Plants under Greenhouse: Significant variation was observed in leaf area and chlorophyll contents with *C. globosum* fungus treatments in Table 9. The maximum percentage of leaves area and chlorophyll content were found when *C. globosum* inoculation in peat moss. On the other hand, in the second season there are no significant differences in chlorophyll content of cucumber leaves. Theses agreement with those of Latz *et al*. [4]; Al-Taie *et al.* [16]; Madbouly *et al.* [5]; Ortega *et al.* [17]; Radhi *et al.* [18] and Tian *et al.* [15]. While Schulz and Boyle [38], explain that, the endophyte fungi (*Chaetomium* sp.) attributed to stimulate the growth of cucumber plants by producing auxins that can stimulate plant growth and root development. Hamayun *et al*. [39] obvious that *Chaetomium* fungi also produce phytohormones, vitamins and solubilizing minerals besides. Endophytic mutualism can extend beneficial growth regulatory effects on host plants under normal as well as extreme environmental conditions. Plants treated with endophytes are often healthier than those lacking such interaction, which may be attributed to the endophytes excretion of phytohormones such as IAA and GAs.

Effect of Supplying Methods of *C. globosum* No. 6 on Fruit Characters of Cucumber Plants under Greenhouse: Data in Table (10) revealed that, the fruit weight, length and diameter of cucumber plants gives a significant variation with *C. globosum* fungus treatments

Table 10: Effect of different methods of addition of *C. globosum* fungus on fruit weight, length and diameter of cucumber plants during 2021 and 2022 seasons

	Fruit weight (g)		Fruit length (cm)		Fruit diameter (cm)	
Treatments	2021	2022	2021	2022	2021	2022
T1	136.87 b	135.67 b	15.58 b	15.75 ab	3.50 a	3.51 a
T2	138.48 b	135.50 b	15.50 b	15.17 bc	3.48 a	3.44 a
T3	148.96 a	148.94 a	16.87 a	16.89 a	3.55 a	3.55 a
T4	145.68 a	142.56 ab	16.55 a	16.28 ab	3.54 a	3.54 a
T5	129.11 c	121.22 c	14.66 c	14.00 c	3.52 a	3.53 a

T1: *C. globosum* trays drench; T2: *C. globosum* soil drench; T3: *C. globosum* peat moss inoculation; T4: *C. globosum* foliar application; T5: Control.

Table 11: Effect of supplying methods of *C. globosum* No. 6 on Early and total yield of cucumber plants during 2021 and 2022 seasons

	Early yield (g/ plant)		Total yield (kg/plant)	
Treatments	2021	2022	2021	2022
T1	918.9 b	911.66 b	2.759 b	2.735 c
T2	922.3 b	914.66 b	2.766 b	2.744 b
T3	1028.3 a	1048.94 a	3.111 a	2.834 a
T4	1022.7 a	1042.56 ab	3.086 a	2.834 a
T5	908.4 c	902.22 c	2.723 b	2.708 d

T1: *C. globosum* trays drench; T2: *C. globosum* soil drench; T3: *C. globosum* peat moss inoculation; T4: *C. globosum* foliar application; T5: Control.

Table 12: Effect of supplying methods of *C. globosum* No. 6 on N, P and K % of cucumber plants during the two tested seasons

	N %		P %		K %	
Treatments	2021	2022	2021	2022	2021	2022
T1	1.67 b	1.81a	0.16bc	0.16 cd	3.61a	3.74 c
T2	1.73 b	1.67 b	0.16 bc	0.17c	3.60 a	3.91 bc
T3	2.21 a	2.50 a	0.17b	0.19 b	3.93a	4.30 a
T4	1.95 ab	2.40 a	0.19a	0.21a	3.83a	4.12 ab
T5	1.65 b	1.57 b	0.15c	0.15 d	2.85b	2.85 d

T1: *Chaetomium* trays drench; T2: *Chaetomium* soil drench; T3: *Chaetomium* peat moss inoculation; T4: *Chaetomium* foliar application; T5: Control.

compared with untreated control, the maximum value of fruit weight and fruit length were found when *C. globosum* inoculated in peat moss followed by foliar application in the two greenhouse seasons. On the other hand, no significant differences were found in fruit diameter among all treatments in the two tested seasons.

Effect of Supplying Methods of *C. globosum* No. 6 on Early and Total Yield: Results in Table (11) showed that, there is a significant variation effect of different supplying methods of *C. globosum* fungus on the early and total yield of cucumber plants under the greenhouse. The results showed also that, the maximum early and total yield of cucumber was found when C. *globosum* inoculated in peatmoss followed by foliar application without significant differences between them compared with control. Similar results was also observed by Kaewchai *et al.* [3]; Sibounnavong *et al.* [8]; Xin [14]; Song and Soytong [7] and Radhi *et al.* [18].

Effect of Different Methods of Addition of *C. globosum* No. 6 Fungus on Cucumber Fruit Chemical Composition: Data in Table (12) revealed that the maximum N in cucumber fruits was 2.21and 2.50 percent in the first and second seasons respectively, which was recorded from the *C. globosum* inoculation in peat moss followed by foliar application without any significant between them compared with other treatment in the two studied seasons.

Concerning the effect of P percentage, data in Table (12) stated that, the maximum P in cucumber fruits was 0.19 and 21percentin the first and second seasons respectively, which was recorded in when *C. globosum* inoculation in peatmoss.

Regarding to the K content, data in Table (12) explained that, all treatments gave high percentage without any significant between supply treatment except untreated control in the first season which gave the lowest value of K. Moreover, the maximum K percentage

in cucumber fruit was found when *C. globosum* inoculation in peatmoss compared with untreated control in the two tested seasons.

The presented data confirmed that, soil health can be maintained by avoiding excessive use of chemicals, ensuring adequate amounts of organic matter in the soil and a rich microbial population in the soil. The use of biofertilizers and biopesticides is the best alternative to agrochemicals. whereas this results in line with those obtained by Al-Taie *et al.* [16].

CONCLUSION

Under clay loam soil results commended inoculating peatmoss with *C. globosum* before transplanting and spraying cucumber plants with *C. globosum* as foliar application before flowering to enhance the vegetative growth and maximize early and total fruit yield and increasing fruit quality.

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