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ORIGINAL PAPER

Chaetomium globosum: a potential biocontrol agent for root rot of date palm seedlings

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ABSTRACT

The date palm has economic importance in the world, especially in the Middle East. In this investigation, the effect of three different *Chaetomium* species on infected date palm plants with different fungal pathogens *i.e., Rhizoctonia solani, Fusarium oxysporum, F. chlamydosporum,* and *F. solani* was studied. Pathogenicity tests of root rot pathogens on date palm seedlings were carried out, *F. oxysporum* was the most virulent isolate which gave the highest percentage of diseases incidence after 45 days (55.5%) while the least virulent isolate was *F. solani* 1 and *F. verticillioides* which gave (11.7 %). Nine isolates of *Chaetomium* spp. suppressed the radial growth of root rot pathogens. The mode of action of *Chaetomium* isolates towards pathogens varied, some isolates suppressed the growth of the pathogens while others showed moderate inhibition of the mycelial growth of pathogens. *C. globosum* isolate No. 2 was chosen depending on the antagonism test that gave a highly significant growth reduction of the pathogen. *C. globosum* isolate No. 2 was the best isolate in controlling root rot disease incidence. Biochemical changes in the treated plants, phenol, indol acetic acid and polyphenol oxidase enzyme (PPO) activity were decreased in infected plants with the tested fungal pathogens, however, were increased in plants treated with *C. globosum* and in plants treated with a mixture of fungi and *C. globosum* compared with control and fungicide treatments. Chlorophyll a and b were increased in plants treated with a mixture of the tested fungi and *C. globosum* compared with all, while carotenoids were increased in infected plants with *F. solani, R. solani,* and *F. oxysporum*, respectively compared with other treatments. CAT enzyme activity was increased in infected plants with any of *F. solani, F. oxysporum* and *R. solani.*

Keywords: *Chaetomium globosum,* date palm root rot , biocontrol

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INTRODUCTION

Date palm *(Phoenix dactylifera),* is considered the tree of life for its nutrient importance; is one of the oldest known cultivated evergreen fruit crop plants in history (Biglari *et al*., 2008). Date fruits are the base of date palm cultivation that is eaten in several stages of maturation (Khlal , Rutub, and Tamar varieties) (Al-nadabi *et al.*, 2020). There are a few agronomic cultivars from more than 5000 recognized cultivars of date palm in the world **(**Ibrahim , 2008). Date fruits are a source of nutrition for people in different parts of the world (Al-Yahyai and Khan 2015) for their high content in carbohydrates (about 70%) and several minerals involving potassium, iron, and calcium (Dayani *et al.*, 2012), Proteins (2-5%), complex of vitamins (A, B1, B2 and C) and oil (0.2-0.5%) (Al-Shahib and Marshal 2003). Date palm trees and offshoots are attacked by several pathogens including bacteria like *Serratia marcescens* which causes pink rot inflorescence disease (Lewaa *et al.*, 2023), *Erwinia chrysanthemi* which causes sudden decline (Abdalla 2001), several soil-borne fungi causing root rot/wilt diseases (*F. oxysporum*, *F. solani*, *Macrophomina phaseolina*, and *Rhizoctonia solani*), Belaat disease (*Phytophthora* sp.), Graphiola leaf-spot

(*Graphiola phoenicis*), Diplodia leaf-base disease (*Diplodia phoenicum*), khamedj (*Mauginiella scaettae*) and Black-scorched (*Ceratocystis paradoxa*) (El-Hassni *et al.*, 2007 and Ahmed 2018). All these diseases cause significant economic losses.

Biological control (BC) is a concept that refers to the control or antagonist of pathognic organisms depending on other organisms. Chaetomium is one of the huge genera in the family *Chaetomiaceae* which includes the most effective agents against many of plant fungal pathogens without causing any damage to plants (Zhang *et al.* 2012). Members of this genus can control the plant fungal pathogens using lytic enzymes and mycotoxins such as chaetomin, xanthenone, chaetoglobins, orsellides, azaphilones, armochaetoglobins A–J and polyhydroxylated steroids (Schlorke *et al.* 2006, Ge *et al.* 2008, Pontius *et al.* 2008, Qin *et al.* 2009 Yamada *et al.* 2011 and Chen *et al.* 2015)

Several studies reported that *Chaetomium* sp. inhibited significantly the growth of *C. paradoxa* **(**Eziashi *et al*., 2007**)** and many phytopathogens belonging to various genera including *Fusarium, Phytophthora, Rhizoctonia,* and *Alternaria* **(**Vitale et al. 2012**).** In addition, many studies revealed that *Chaetomium* sp. reduces plant growth-promoting hormones that increase significantly the growth of many crops, as well as date palm (Perveen and Bokhari 2012).

In this investigation, this work aims to study the effect of *Chaetomium* species as bio-agents to control infected date palm plantlets with identified fungi including: *R. solani*, *F. oxysporum*, and *F. solani*, and the effectiveness of *Chaetomium globosum* on the growth of date palm plantlets.

MATERIALS AND METHODS

The experiments of this study were carried out in the Central Laboratory for Date Palm Research and Development (CLDPRD) and Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

1. Isolation, purification, and identification of fungi associated with date palm root rot:

Diseased samples of date palm exhibiting typical symptoms of root rot were collected from naturally infected date palm trees, from fields at the ARC, Giza goernorate. The infected roots were washed carefully using tap water, and cut into small pieces about 0.5 cm long. These pieces were surface sterilized by immersing in 3.0% sodium hypochlorite solution (with 5% conc.) for two minutes, then rinsed three times in sterilized distilled water. Sterilized samples were dried between two sterilized filter papers then transferred into Petri dishes containing 15 ml of potato dextrose agar (PDA) medium/plate. The plates were incubated at 25°C and examined daily. The isolated fungi were purified using the hyphal tip technique **(**Dhingra and Sinclair, 1985**)** and then transferred into slants of PDA and kept at 5°C for further studies. Fungal isolates were identified at Mycol. Res. and Dis. Survey Dept., Plant Pathol. Res. Insti., Agric. Res. Cent. (ARC) according to their cultural and morphological characteristics on PDA medium as described by Pearson, *et al.* (1986). Identified isolate of *Fusarium solani* was kindly obtained from Dept. of Date-Palm Diseases and Protection, CLDPRD, ARC.

2. Pathogenicity test:

2.1.Preparation of plants:

Sewi cultivar seeds were prepared as follow: the seeds were collected and washed with dH_2O and immersed in Rizolex-fungicide (3gm L^{-1}) solution and immersed in water, changing the water every 2 days (Abd Allah 2018). Then, the seeds were incubated for 50 days at 37ºC/ 50% humidity for planting.

For sterilizing, date seeds and date plants which raised from aged 60 days, they were washed with sterile dH_2O. Finally, the plants were immersed in 3% hypo-chlorite (with 5% conc.) for 10 min and washed with sterile dH_2O. The plants were ready to planting. Finally, plants were planted till at age six months to be ready for pathogenicity test.

2.2. Preparation of the desired fungal inoculum and soil infestation:

Pathogenicity test was carried out in pot experiments under greenhouse conditions (Sallam, *et al*. 2008**)** using six fungal isolates, *i.e., R. solani*, *F. solani* (isolate No.1), *F. solani* (isolate No.2), *F. oxysporum*, *F. chlamydosporum* and *F. verticillioides.* Fungal mass production used for soil infestation was obtained by growing the tested isolates on

sterilized sorghum grain medium in bottles (500 cc) according to Abd El-Khair and El-Mougy (2003); then the inoculated bottles were incubated at 27°C for one week. Pots (10 cm diam) were sterilized by immersing in 5% formalin for 15 minutes and left for a week before use. The experiment was carried out in peat moss clay soil (1:1 w/w) that was sterilized by wet heat in an autoclave at 121°C for 30 min. and left for two days to dry. The soil was then infested individually with the desired fungal inoculum at the rate of 3% (w/w) and watered every two days for a week before sowing. Pots were filled with autoclaved soil mixture free of fungal inoculum to serve as a control treatment. Date palm seedlings were planted in both infested and non-infested soils, one seedling per pot and nine replicates (pots) for each treatment were conducted.

2.3. Disease assessment:

Disease incidence was calculated as the percentage of infected plants. Virulence of the different pathogens isolates was assessed according to diseases incidence estimated at 45 and 90 days after planting, respectively.

Diseases incidence 45 days after planting

$$\%=\Sigma(c- t)/c \times 100$$

Σ= sum**,** c= control (number of healthy number of seedlings in control), t= treatment (number of infected seedlings in infested pots). Diseases incidence after 90 days after plantibg %=Σ(c- t)/c ×100

Σ = sum**,** c = control (number of healthy seedlings in control), t = treatment (number of healthy plants treatment).

3. Source of *Chaetomium* isolates:

Nine isolates representing three *Chaetomium* species (Table 1) were used as bioagents against root rot pathogens. These isolates were obtained from the Mycol. Res. and Dis. Survey Dept., Plant Pathol. Res. Insti., Agric. Res. Cent. (ARC).

4. Effect of different isolates of *Chaetomium* on date palm root rots pathogens (Dual culture testing Petri plates).

A dual culture technique was carried out according to Gao, *et al.,* (2002) with a partial modification. A mycelial disc (5 mm diam) of the desired isolate from *Chaetomium* was taken from the edge of the actively growing colony and placed in Petri plates containing PDA medium. Three days later, a mycelium disc of the root rot pathogen (5 mm in diameter) was placed 6 cm apart from the growing *Chaetomium* sp. in the same PDA plate. Three plates were used for each replicate. PDA plates inoculated with mycelial discs of each pathogen alone were served as control. Inoculated plates were incubated at 27°C. The results were recorded when control plates were fully grown by measuring the linear growth of the growing fungus towards the tested *Chaetomium* sp.

Table (1): *Chaetomium* isolates used in this investigation and their different sources.

Isolate No.	*Chaetomium* isolate	Source
1	*C. nigricolor*	Banana
2	*C. globosum* 1	Citrus
3	*C. globosum* 2	Wood1
4	*C. globosum* 3	Potato
5	*C. globosum* 4	Papper
6	*C. globosum* 5	Wood 2
7	*C. madrasense*1	Citrus
8	*C. madrasense* 2	Soybean
9	*C. madrasense* 3	American cockroach (*periplaneta americana*)

5. Molecular detection

5.1 DNA extraction

Extraction of DNA from the isolated fungi was done according to Arif *et al.,* (2010) with some modifications as follows: About 0.1g of fresh fungi tissue was grounded well using mortar and pestle with 500µl of lysis buffer (100ml/pH 8.0 containing 1.21g tris-base, 0.4g Na_2EDTA, 2.0g CTAB, 8.12g NaCl, and 2.0g PVP). Homogenized tissue was transferred to Eppendorf (1.5ml) and fill the volume to 1ml with lysis buffer and 35µl of SDS (20%) was added. Eppendorf was kept in water-bath at 65ºC/30min. Then, samples were centrifuged at 9500xg/5min and the supernatant was kept at new Eppendorf. An equal volume of chloroform: isoamyl-alcohol (24:1) was added to the supernatant

and Eppendorf and was shaken well and centrifuged at 9500xg/5min. The supernatant was kept in a new Eppendorf and 500µl cold isopropanol and sodium acetate (10% of supernatant, 3M) were added. Eppendorf was centrifuged at 11500xg/10min, and the pellet was washed using 500µl cold ethanol 70% and centrifugation at 7000xg/5min was carried out. Finally, pellet of DNA was resuspended using 50µl of sterilized dH_2O and kept at 4ºC till use.

5.2. PCR Assays

PCR was used for detection of fungi from previous DNA extracted using specific primers. Each 25µl of PCR reaction contained of 2µl of DNA template (~250-400ng), 1µl (10p.mol) of each primer (SMS RS1-F/SMS RS1-R, Fa+7/Ra+6) as listed in Table (2), 12.5µl of TaqMan (*amaR* OnePCR, GeneDireX,Inc) and 6.5µl of dH_2O. The program of PCR for primers SMS RS1-F/SMS RS1-R was 95ºC/ 3min; 30 cycle consist of 95ºc/30s, 57 ºC/30s, and 72ºC/60s; and 72 ºC/5min for the detection of *R. solani*. PCR condition for *F. oxysporum*, that carried out using Fa+7/Ra+6 primers 95ºC/5min; 40 cycles of 94 ºC/1min, 67 ºC/1min, and 72 ºC /1min; and 72 ºC/10min. For confirming the identification of *F. oxysporum* whether it is *F. oxysporum* f.sp. *albedinis* or not, the primer pair TL3/FOA28 was used and the PCR condition was 95ºc/4 min, 30 cycles of 92ºC/30sec, 62 ºC/30sec, and 72ºC/45sec, and 72ºC/15min The previous programs were carried out using thermo-cycler MJ Research (PTC-200). Electrophoresis for the PCR products was carried out using 1.2% agarose gel in TAE buffer at 120V/45min and ethidium bromide (1mg/ml) stain was used to stain gel. The fragments were captured using UV lamp gel documentation (Digimage, Gel Doc Digimage system G15).

Table (2): Primer list of pathogenic fungi.

Pathogen	Primer name	Sequence (5'-3')	Reference
R. solani	SMS RS1-F	5'-AACCTGCGGAAGGATCATTATTG-3'	**Lin *et al.*, 2021**
	SMS RS1-R	5'-GGTGTGATGGATGAAAGAGAAGGT-3'	
F. oxysporum	Fa+7	5'-AACGTCGTCGTCATCGGCCACGTCGACTCT-3'	**Vázquez *et al.*, 2021**
	Ra+6	5'-ACATACCAATGACGGTGACATAGTAGCG-3'	

6. Effect of *Chaetomium globosum*. isolates on date palm root rot *in vivo*

The most virulence isolate of *Chaetomium globosum* was used to study its effect *in vivo* on infection of date palm seedling by root rot pathogens under greenhouse conditions. Inocula of *Chaetomium globosum* isolate were prepared by inoculating sterilized straw and sorghum grain bottles (500 cc) with a disk (5 mm) of *Chaetomium globosum* isolate-2 (7 days-old culture). The bottles were incubated at 27° C for two weeks. The inocula of root rot pathogens (*R. solani*, *F. solani* (isolate No. 2) and *F. oxysporum* (isolate No. 2) were prepared as mentioned before under pathogenicity test. Soil infestation with *Chaetomium* isolates was made at the rate of 3% (w/w) and the pots (10 cm diam) were watered every two days for a week and then pathogen inocula were added also at the rate of 3% (w/w) and watered every two days for a week before sowing. Pots filled with autoclaved soil free of fungal inocula were used as check control and another group of pots were filled with soil infested with the pathogens to serve as infested control.

Date palm seedlings were planted in both infested and non-infested soils, three seedlings per pot, and three replicates were used for each treatment and each replicate had three pots. Date palm seedlings untreated with *Chaetomium globosum* were planted in infested soil with the casuals of root rot to serve as control. Untreated seedlings sown in un-infested soil were used as another control. Disease incidence was determined after 45 and 90 days from

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planting.

7.Biochemical changes

7.1. Biochemical changes determination

Three replicates of each treatment were used for biochemical change determination. One gram of each treatment sample was extracted in 5 of 80% ethanol, thoroughly

Homogenized with apestle in a mortar and stored in the dark for 24 hours at 4°C. Subsequently, at lab temperature and 13000g/5min, the samples were centrifuged. The pellet was disposed of, and the supernatant was transferred to estimate the biochemical changes (including total phenol, total indols, and amino acids).

7.1.1. Total phenol contents:

Total phenol contents were carried out using Folin Ciocalteu's reagent according to Ainsworth and Gillespie (2007) modified by Patel *et al.* (2010) as follows:

One ml of each previously extracted sample was transferred to a tube and 0.5 ml of the Folin-Ciocalteu reagent (F-C) and 5 ml of distilled water were added. The mixture was well mixed by vortexing for 5 minutes. Subsequently, 1.5 ml of 20% sodium carbonate (Na_2CO_3) were added, bringing the total volume to 10 ml. The mixture was then allowed to sit at room temperature at the dark for two hours. At last, the samples on the spectrophotometer (Orion AquaMate 8000) were read at 765nm.

7.1.2 Total indols acetic acid content:

In accordance to Selim *et al.* (1978), the total indols were assayed. After pipetting 1 ml of the extracted aliquot into tubes, 4 ml of the reagent Para di-amino benzoate (PDAB) were added, and the tubes were incubated for 1 hour at 30 to 40 °C. At 530 nm, the samples were finally red on the Orion AquaMate 8000 spectrophotometer.

7.1.3. Total amino acid content:

The procedure was followed as mentioned by Mc.Grath (1972) as follows: In a test tube, 1 ml of each extracted sample was added, and the volume was increased to 4 ml by adding distilled water. Next, all samples were then thoroughly vortexed after 1 milliliter of ninhydrin was introduced as a reagent. After that, each tube was covered and placed in a water bath set at 100°C for 15 minutes to allow the ninhydrin and amino acids to react. Subsequently, the tubes were immersed in cold water to allow them to cool down, and each tube was then completed with 1 milliliter of 50% ethanol (diluent solution). Ultimately, at 570 nm on the spectrophotometer (Orion AquaMate 8000), every sample was read.

7.1.4. Pigments assaying:

Plant pigments (including Chlorophyll a, chlorophyll b and carotenoids) were extracted and assayed according to Wettstein (1957) as follows:

One gram of fresh leaf sample was ground with acetone (85%) using a mortar pestle with an amount of $CaCO_3$ and sterilized sand. Next, the ground was filtered, and the residue was repeatedly washed with acetone until the filtrate lost all color. The extracted solution was completed using acetone to volume (15ml). A spectrophotometer (Orion AquaMate 8000) was used to measure the optical density of this extract at 440, 640, and 660 nm. Using the Weinstein formula, the concentrations of, chlorophyll a,b, and carotenoids were assayed as follows:

- Chlorophyll a = 9.784 X E 660 – 0.99 x E 640 mg/g
- Chlorophyll b = 21.426 X E640 – 4.65 x E 660 mg/g
- Carotenoids = 4.695 X E 440 – 0.268 x C (chl. a + chl. b) mg/g

7.1.5. Polyphenol oxidase (PPO) activity assaying.

Polyphenol oxidase (PPO) enzyme activity was estimated according to Oktay *et al.* (1995) as following:

One hundred microliters of homogenization buffer (crude of grinded plant in phosphate buffer pH7) were added to a quartz cuvette and 600 µl (0.1M) of catechol were added and finally 2.3ml of phosphate buffer (0.1M, pH 6.5) were added. The cuvette was read on the

spectrophotometer (Orion AquaMate 8000) at zero time and at 1 min later at 420nm. PPO activity was assayed using the formula:

$$\text{Activity/ml enzyme} = \frac{\Delta OD/min}{0.001} x \frac{1}{0.1}$$

7.1.6. Catalase (CAT) activity assaying:

The catalase (CAT) enzyme activity was measured in accordance with Chance and Maehly (1955). In cuvette, about 2.9 ml of 15mM H_2O_2 were added to 0.1ml of homogenization buffer and following the decrease of the absorbance at 240 nm for 1min with intervals of 10 sec. CAT activity was measured using formula:

$$\text{Activity (IU/ml enzyme)} = \frac{\Delta OD/min}{\epsilon(40)} X \frac{Test\ volume\ (3)}{1000} X 1000 X \frac{1}{0.1}$$

8. Statistical Analysis

All obtained data were analyzed statistically according to Snedecor and Cochran, (1980) using program SPSS version 23 using one way of analysis of variance ANOVA.

Results and Discussion:

1- Isolation and identification of date palm root rot pathogens:

As shown in Table (3) samples of root-rotted date palm plants were collected from Giza governorate. Fungi associated with diseased plants were isolated and identified at Mycol. Res. and Dis. Survey Dept., Plant Pathol. Res. Insti., Agric. Res. Cent. (ARC). Isolation trials yielded *R. solani, F. solani, F. oxysporum, F. chlamydosporum, F. verticillioides* and *A. alternata.*

Table (3): Fungi associated with rotted roots of date palm plants

Samples	Isolated fungi
Sample 1	*Fusarium verticillioides* Sacc *F.solani* Mart *Alternaria alternate* Keissl.
Sample 2	*F.chlamydosporum* Wollenw and Reinking. *Rhizotonia solani* KÜhn *F. oxysporum* Synd and Hans.

2- Pathogenicity test of the isolated fungi from diseased date palm seedlings

Pathogenicity test was carried out to investigate the virulence of different fungal isolates as the causal of root-rot disease of date palm seedlings. Data in Table (4) show clear variation in disease incidence of date palm seedlings. *F. oxysporum* gave the highest percentage of disease incidence after 45 days (55.5%) followed by *R. solani* (44.4%) while the least virulent isolate was *F. solani* isolate No.1 and *F. verticillioides* which gave 11.7 % disease incidence . In case of the disease incidence after 90 days from planting, data revealed that *F. oxysporum* gave the highest percentage of infection (66.6%) followed by *R. solani* (55.5%),

The symptoms of root rot observed on date palm seedlings inoculated by different root rot pathogens are clear in Fig. (1)

The pathogenicity test of the isolated fungal pathogens revealed that their ability to induce different degrees of disease incidence on date palm seedlings, depends on the isolate. These results are in agreement with many authors either in Egypt or all over the world (Maitlo *et al*., 2013 and EL-Morsi *et a.,l* 2015).

Table (4) Pathogenicity test of different the isolated fungi on date palm seedlings

Isolates code	Locality	Disease incidence % after (days)	
		45	90
F. solani 1	Sample 1	11.7^{ab}	11.7^{ab}
F. verticillioides	Sample 1	11.7^{ab}	11.7^{ab}
R. solani	Sample 2	44.4^{bc}	55.5^{cd}
F. oxysporum	Sample 2	55.5^{c}	66.6^{d}
F. chlamydosporum	Sample 2	33.3^{abc}	33.3^{abc}
F. solani 2	ARC	33.3^{abc}	44.7^{bcd}
Control		0.0^{a}	0.0^{a}

3- Effect of different *Chaetomium* isolates on the linear growth of date palm root rots pathogens: Dual culture test in Petri plates:

The effect of different isolates of *Chatomium* spp. on suppressing mycelial growth of root rot date palm pathogens *i.e R. solani, F. oxysporum, F. solani* and *F. chlamydosporum* was studied. Data in Table (5) show that- the effect of different isolates on inhibiting mycelial growth was varied according to *Chaetomium* spp.

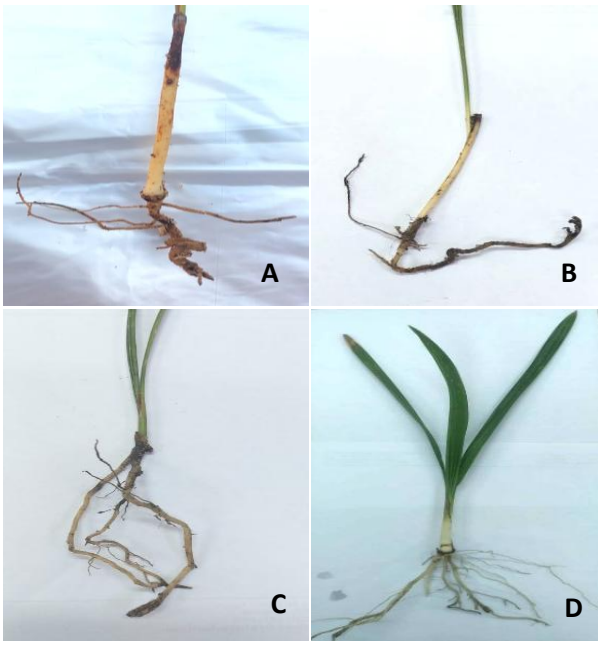

Fig (1): Root rot in date palm Brown-colored, dry roots. (A) *R. solani* symptoms, crown rot and cankers forming around the stem at the soil line, (B) *F. oxysporum*, drying of the leaves and root rot, *F. solani* symptoms, root rot and (D) control seedling

Table (5) Effect of *Chaetomium* spp. on the linear growth of root rot pathogens.

Isolates	*R. solani*		*F. oxysporum*		*F. solani* 2		*F. chlamydosporum*		mean	
	Linear growth (cm)	Growth reduction (%)	Linear growth (cm)	Growth reduction (%)	Linear growth (cm)	Growth reduction (%)	Linear growth (cm)	Growth reduction (%)	Linear growth (cm	Growth reduction (%)
C.nigricolor	4.6^{cde}	48.4^{cde}	4.1^{bc}	54.1^{cd}	4.0^{cd}	55.1^{de}	4.2^{bc}	52.9^{bc}	4.2	52.6
C. globosum 1	4.0^{f}	55.5^{b}	3.1^{e}	65.5^{a}	3.0^{ef}	65.8^{b}	3.4^{d}	61.4^{a}	3.7	62.1
C. globosum 2	3.4^{g}	61.4^{a}	3.4^{de}	61.3^{ab}	2.6^{f}	70.3^{a}	3.4^{d}	62.2^{a}	3.2	63.8
C. globosum 3	4.3^{def}	51.4^{bcd}	3.5^{de}	61.6^{ab}	3.6^{d}	59.2^{cd}	3.5^{d}	61.0^{a}	3.7	58.3
C. globosum 4	4.4^{de}	50.7^{bcd}	3.9^{bcd}	55.9^{bcd}	4.1^{b}	54.4^{e}	4.0^{bc}	54.8^{bc}	4.1	53.9
C. globosum 5	4.3^{e}	52.1^{bc}	4.1^{bc}	54.0^{cd}	3.4^{de}	61.8^{bc}	3.8^{cd}	54.0^{bc}	3.9	55.4
C.madrasense1	5.1^{b}	43.6^{f}	3.8^{cd}	57.3^{bc}	3.9^{cd}	54.0^{ef}	3.8^{cd}	57.3^{ab}	4.1	53.1
C.madrasense2	4.7^{bcd}	47.0^{def}	4.4^{b}	50.7^{d}	4.5^{b}	49.9^{f}	4.5^{b}	49.9^{c}	4.5	49.3
C.madrasense3	5.0^{bc}	44.0^{ef}	4.2^{bc}	52.5^{cd}	4.2^{bc}	52.5^{ef}	4.3^{b}	51.8^{c}	4.4	50.2
Control without pathogen	9.0^{a}	0.0^{g}	9.0^{a}	0.0^{e}	9.0^{a}	0.0^{g}	9.0^{a}	0.0^{d}	9	0

Chatomium globosum isolate No. 2 was the most effective for inhibiting mycelial growth of the different pathogens followed by *C. globosum* isolate No.1 while *C. madrasense* isolate No.3 gave the antagonism least reduction for mycelia growth on the average. Relationship between different *Chaetomium* spp. isolates and root rot pathogens showed by all isolates of *Chaetomium* spp. *C. globosum*

isolate No.2 showed antibiosis. *C. nigricolor* and *C. madrasense* isolate No.1 gave inhibition zone (Fig., 2).

All isolates of *Chaetomium* spp. suppressed the radial growth of the tested pathogens. The mode of action observed in this study was varied. Data obtained indicated that an interaction between *Chaetomium* spp. isolates and phytopathogenic fungi is clear. It was found that some isolates of *Chaetomium* spp. caused severe reduction of the tested fungi. The interaction between biocontrol agents and th pathogenic fungi indicated that the mechanism of *Cheatomium* spp. included more than antagonistic action and inhibition of pathogen growth. These results are in agreement with those recorded by Kanokmedhakul, *et al*., (2006) and Elshahawy and Khattab (2022).

4- Effect of *C. globosum* on date palm root rot *In vivo*.

The most virulent isolate of *Chaetomium globosum* was chosen to study this potentiality as control measures against date palm seedling root rot caused by each of *R. solani, F. oxysporum* and *F. solani*. As shown in Table (6) *C. globosum* (isolate-2) gave the best reduction of disease incidence after 45 days compared to control pathogen only. *Chaetomium globosum* isolate-2 completely suppressed disease incidence at 90 days after planting. Data obtained (Table, 6) illustrate that amendment of soil before cultivation of date palm seedling by *C. globosum* reduced disease incidence after 45 days and increased the number of survived plants in comparison to control when soil was infested with pathogens only. These results are in agreement with those recorded by Shanthiyaa, *et al*. (2014).

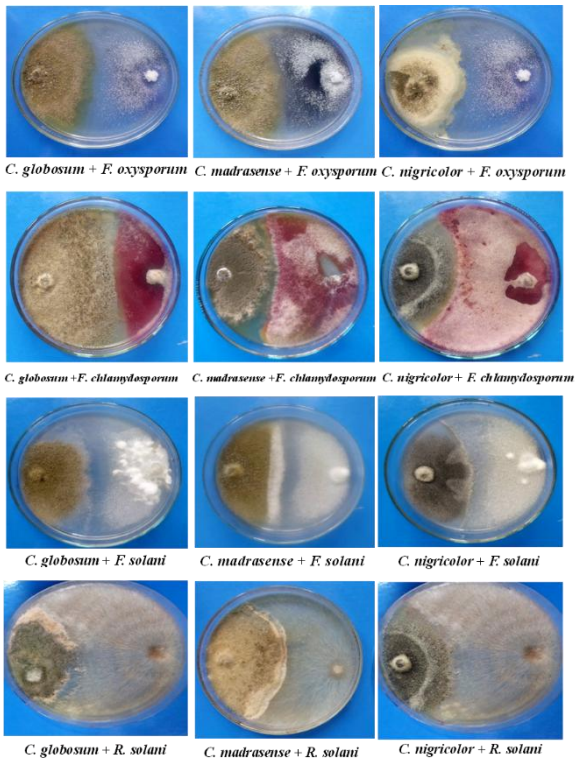

Fig (2): Showing the effect of different isolates of *Chaetomium* spp. on suppressing mycelial growth of pathogens.

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Table (6): Effect of soil infestation with *Chaetomium globosum* isolate (2) on date palm root rot disease *in vivo* under greenhouse conditions

Treatment	Disease incidence, % after 45 days			Disease incidence, % after 90 days		
	R. solani	***F. oxysporum***	***F. solani* (2)**	***R. solani***	***F. oxysporum***	***F. solani* (2)**
C. globosum (2) + pathogen	33.3^{abc}	22.5^{bcd}	11.7^{cd}	33.3^{c}	22.5^{c}	11.7^{b}
Pathogen + fungicide	11.7^{c}	11.7^{c}	0.0^{d}	11.7^{b}	11.7^{b}	0.0^{a}
Control (1) (pathogen only)	55.5^{a}	44.4^{ab}	33.3^{abc}	88.8^{d}	61^{d}	49.9^{c}
Control (2) (without pathogen)	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{a}	0.0^{a}	0.0^{a}

5- Identification of the isolated fungi using molecular detection:

Results from PCR amplification of the isolated fungal DNA using specific primer (Fig.3) show that DNA of fungi in sample (1) was shown at the expected size fragment on agarose gel at 118pb, this confirmed the morphological identification and referred that the sample was *Rhizoctonia solani*. In sample (2), PCR product on agarose gel showed fragment at expected length at 560pb; this result explained that the isolated fungus was *F. oxysporum*. For confirming if the isolated pathogen (*F. oxysporum*) belongs to the forma speciales of *F. oxysporum* f.sp. *albedinis* that causes bayoud disease, the PCR using the specific primer pair of *F. oxysporum* f.sp. *albedinis* showed negative results (not shown).

6- Biochemical changes

The effect of different treatments in this study on biochemical changes was assayed and tabulated in Table (7) and Fig. (4): The obtained data showed that total phenol content was significantly increased in the case of date plant treated with *C. globosum* (61.42mg/g) compared with control (53.89 mg/g) while the other treatments were significantly decreased compared with control and among themselves. Indol acetic acid was estimated in all treatments and the results showed that the level of indol acetic acid was significantly increased in plants grown in soil infested with *C. globosum* (0.793mg/g) compared with control (0.658mg/g), also, plants treated with *C. globosum* and *R. solani*, *F. oxysporum*, and *F. solani* had a significant increase of indol acetic acid level compared with control while the other treatments were significantly decreased in comparison with the control.

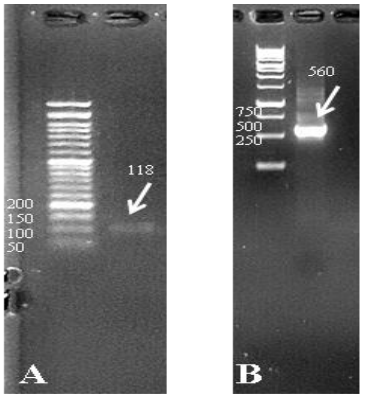
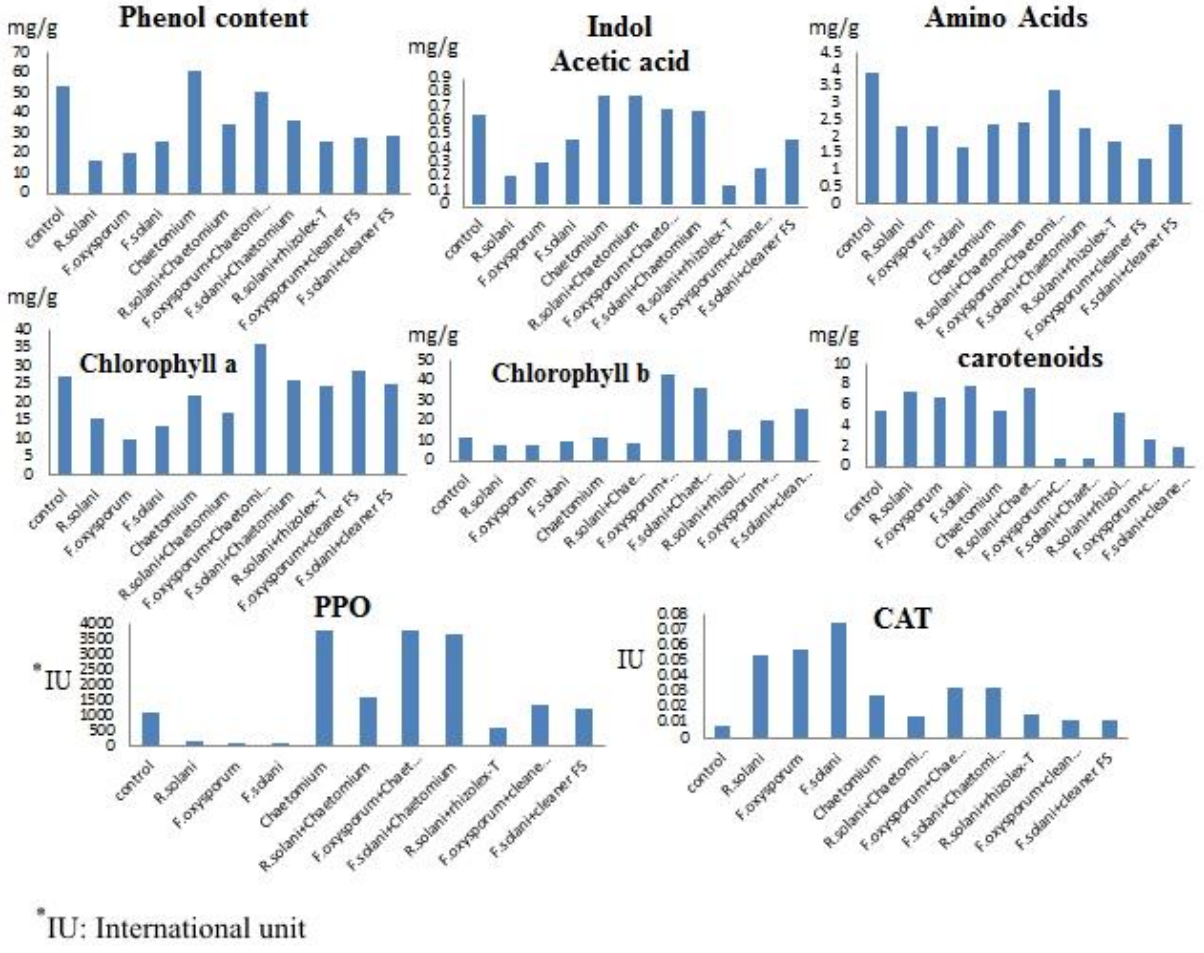


Fig (3): Gel electrophoresis (1.2%) of PCR for identification of the isolated fungi**.**
(A) M1= DNA marker 50pb, L1= Expected size of *R. solani* at ~118pb. **(B)** M2= DNA marker 1Kb, L2= Expected size of *F. oxysporum* at ~560pb.

Table(7): Values of biochemical changes estimated in date palm seedlings due to infection by some root rot pathogens and soil treatment by *C, globosum*.

Treatment	Phenol mg/g	Indol Acetic Acid mg/g	Amino Acid mg/g	Pigments mg/g			PPO IU	CAT IU
				Chl.A	Chl.B	Carot.		
Control	53.892^{b}	0.6587^{d}	3.91^{a}	26.933^{bc}	11.627^{f}	5.451^{bc}	1110^{c}	0.0075^{f}
R. solani	16.129^{j}	0.2141^{i}	2.318^{e}	15.721^{ef}	7.686^{g}	7.270^{a}	150^{e}	0.054^{b}
F. oxysporum	20.1825^{i}	0.3089^{g}	2.295^{f}	9.729^{g}	8.145^{g}	6.825^{ab}	60^{e}	0.0572^{b}
F. solani	25.877^{f}	0.4773^{e}	1.654^{h}	13.511^{f}	9.880^{f}	7.844^{a}	30^{e}	0.075^{a}
C.globosum	61.422^{a}	0.7929^{a}	2.374^{d}	21.713^{d}	11.307^{f}	5.524^{bc}	3770^{a}	0.02715^{d}
R. solani*+ *C.globosum	34.328^{e}	0.6911^{b}	2.419^{c}	16.921^{e}	9.113^{g}	7.723^{a}	1580^{b}	0.0135^{e}
F.oxysporum* + *C.globosum	50.710^{c}	0.6719^{c}	3.373^{b}	36.132^{a}	42.970^{a}	0.783^{ef}	3780^{a}	0.03225^{c}
F.solani* + *C.globosum	36.329^{d}	0.6577^{d}	2.276^{f}	26.327^{bc}	36.188^{b}	0.765^{ef}	3650^{a}	0.033^{c}
***R.solani* + rhizolex**	15.4003^{k}	0.1505^{j}	1.854^{g}	24.412^{cd}	15.312^{e}	5.358^{c}	620^{d}	0.01575^{e}
***F. oxysporum* + cleaner FS**	23.072^{h}	0.2777^{h}	1.327^{i}	28.925^{b}	20.222^{d}	2.717^{d}	1330^{bc}	0.012^{ef}
***F.solani* + cleaner FS**	23.709^{g}	0.4732^{f}	2.374^{d}	24.805^{c}	25.840^{c}	1.922^{de}	1240^{bc}	0.01125^{ef}
P. value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
L.S.D	0.57	0037	0.0202	2.98	1.57	1.44	358.37	0.0057



Fig(4): Biochemical changes in date palm plants due to using different treatments on date palm

In the case of amino acids, the level of amino acids was significantly decreased in all treatments compared with the control (3.91mg/g). Pigments (chlorophyll a, b, and carotenoids) were assayed. Results showed that values of chlorophyll a were increased significantly in plants treated with a mix of *F. oxysporum* and *C. globosum* compared to other treatments as well as control except the mix of *F. solani* and *C. globosum* showed significant defrences with control plants. In chlorophyll b, plants treated with a mix of *F. oxysporum* and *C. globosum* and those treated with the mix of *F. solani* and *C. globosum* had a significant increase of chlorophyll b (42.97 and 36.18mg/g, respectively) compared to other treatments and control. Results concerning the level of carotenoids showed that infected plants with *R. solani, F. solani* and mix of *R. solani* with *C. globosum* had significant increase in level of carotenoids comparing with control. Activity of polyphenol oxidase (PPO) was estimated, the results indicated that activity of PPO in plants treated with any of *C. globosum*, mixture of *F. oxyoprum* and *C. globosum*, and mixture of *F. solani* and *C. globosum* was increased significantly compared to control and other treatments. Catalase enzyme activity was also assayed. The obtained results showed that activity of catalase was significantly increased in plants infected with *F. solani* then *R. solani* and *F. oxysporum*. Then, the activity was decreased in other treatments. Results of the present study showed that *C. globosum* is considered a factor for improving plant growth and helping the infected plants to control the causal pathogens. *C. globosum* plays a role to increase phenol level in date palm plants by increasing the growth of whole date palm by secretion some phytohormones. Bomke and Tudzynski, (2009) and Khan *et al.,* (2012**)** mentioned that *C. globosum* produces some of phytohormones like Gibberellins and Indole acetic acid (IAA) that play essential role to increase growth of plants. That reflects increasing of phenol level comparing to the control, also the levels of phenols were increased in plants treated with mixture of *R. solani* and *C. globosum*, mixture of *F. oxyoprum* and *C. globosum* and mixture of *F. solani* and *C. globosum* because of presence of *C. globosum* that can control the causal agents by secretion some of anti-fungal like chaetoglobosins and provide plants with basic requirements to grow like phytohormones as revealed by Soytong (2015). Yan *et al.,* (2019) reported that *C. globosum* provides plants with IAA as a phytohormone that affects plants to extend beneficial plant growth. Because of the activation of the metabolism of date plants that treated with *C. globosum*, the level of chlorophyll was increased as mentioned by Khan *et al.,* (2012). PPO enzyme was decreased in infected date plants compared with control because all metabolisms of date plants were degraded, similar to the results by Izadi and Shahsavar (2015) and Abd Allah (2018). While in CAT enzyme activity was increased in infected plants compared with control because of that consider catabolism enzymes that help to resist the causal agents by degrading the infected cells.

Some parameters of biochemical changes in infected plants with *F. oxyoprum* and *F. solani* were increased like Indole acetic acid and chlorophyll, this could be because *Fusarium* spp. produce some phytohormones like IAA (Junaidi and Bolhassan, 2017 and Lestiyani *et al.,* 2021) and Gibberellic acid (Ben Rhouma *et al.,* 2020 and Lestiyani *et al.,* 2021).

CONFLICTS OF INTEREST:

The authors declare that they do not have any actual or potential conflict of interest.

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