



Management of Tomato Root Rot Disease Using *Chaetomium globosum* Formulations under Greenhouse and Field conditions

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ABSTRACT

The effects of three formulations of *Chaetomium globosum* applied as seed bet treatments on root rot disease of tomato plants were studied under greenhouse and field conditions. Using bi-culture techniques, three isolates of *Chaetomium globosum* (CG) (1,2,3) were investigated in vitro for their inhibitory efficacy against RS and FORL. The results showed that the most sever isolate is CG1 which reduced the growth area by 81.1 and 78.0% for RS and FORL respectively. Other isolates showed moderate effect. The results revealed that all formulations of *Chaetomium globosum* (CG) i.e. spore suspension (CGSS), pellets(CGP) and spore suspension + pellets (CGSS+CGP) controlled tomato root rot under greenhouse and field conditions. Data showed that all formulations reduced disease incidence and severity. The most effective formulation is CGSS+CGP which inhibited the disease incidence by 80.2 and 71.2 % for RS and FORL respectively. The same trend was observed with disease severity. All of the formulations increased in enzyme activities. As for RS the formulations CGP and CGSS+CGP produced the greatest increase in enzyme activity which increased PO and CH more than 127.3 and 129.4% respectively. As for FORL the same formulation increased PO and CH more than 152.2 and 122.2 % respectively. Furthermore, during two growing seasons in the field, the most effective formulation is CGSS+CGP which inhibited disease incidence and severity. Followed by CGP treatments, meanwhile, CGSS has satisfactory impact. As for tomato yield, the most effective formulation is CGSS+CGP which significantly increased tomato yield. Followed by CGP treatment.

Keywords: Tomato, root rot, biological control, enzyme activity, yield

1. Introduction

Tomatoes (*Solanum lycopersicum* L.) is attacking by several soilborne diseases i.e. Fusarium crown and root rot (FCRR), caused by *Fusarium oxysporum* f.sp. *radicis lycopersici* (FORL). It is becoming more common in tomato cultivation in greenhouses. The disease affects tomato plants in both greenhouses and fields around the world, resulting in major losses in tomato production (Hibar *et al.*, 2006; Bakeer *et al.*, 2016).

Also Rhizoctonia root rot disease caused by *Rhizoctonia solani* (Kuhn) was the most extensively produced tomato cultivars. This fungus cause collar and root rots, as well as the death of severely infected plants.

We wanted to find ecologically friendly, long-lasting, and effective methods for plant disease management, such as biological control (Curtis *et al.*, 2010).

Chaetomium fungus is one of genera in the *Chaetomiaceae* family, with over 100 species (Fatima *et al.*, 2016). A common saprophyte, *Chaetomium globosum* is found in soil, plant rhizospheres, and phyllospheres. This species successfully controlling several plant diseases (Zhang *et al.*, 2010).

According to Elshahawy and Khattab (2022), treatments with *Chaetomium globosum* (Chg-1) in pots considerably decreased late wilt and promoted the growth of the two cultivar of maize plants. Chg-

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1 caused induction of antioxidant enzymes (PO, CH, PPO) chlorophyll, phenols, and flavonoids. Comparing to the untreated control in the field, it not only decreased the symptoms of late wilt but increasing yield.

Gaining greater effectiveness against target pathogens in a number of ways requires an understanding. *Chaetomium* strains have been discovered to exhibit varying antifungal potencies both in vitro and in vivo (Vitale *et al.*, 2012). According to Tomilova and Shternshis (2006), *Chaetomium* species controlled black pepper, citrus root rot, and sugar beetroot damping off disease.

In addition to increasing agricultural productivity and quality, the use of helpful microbes for plant disease prevention lowers the need for chemical pesticides (Bonanomi *et al.*, 2018). The need for sustainable agriculture-based alternative disease management techniques has arisen (Elshahawy *et al.*, 2018; Degani 2022; Mohamed and Elshahawy 2022).

Numerous investigations have demonstrated that endophytic fungi promote plant development, boost disease enhance plant resilience to biotic stressors, and recycle nutrients (Madbouly et al 2020). According to Soyong *et al.*, (2001), *Chaetomium globosum* identified as an endophyte fungus in soil, organic compost, and living plant tissues. Numerous gymnosperms, dicots, and monocots have been found to have it as an endophyte (Naik *et al.*, 2009, Longoni *et al.*, 2011), and Li *et al.*, 2014).

Numerous plant diseases have been demonstrated to be antagonistic towards some *Chaetomium* species (Charoenporn *et al.*, 2010; Sibounnavong *et al.*, 2012). One endophytic biocontrol agent that has shown promise among them is *C. globosum* (Abou Alhamed and Shebany 2012; Zhao *et al.*, 2017). Hung *et al.*, (2015) showed the endophytic *C. globosum* shows strong antifungal action against several pathogenic fungi, also, against *Botrytis cinerea*, according to Pan *et al.* (2016). Fierro-Cruz *et al.* (2017) found that *Fusarium oxysporum*'s growth was greatly suppressed by the endophyte *C. globosum*.

Endophytic *Chaetomium* species suppressed *Rhizoctonia* root rot of cucumber plants, according to Huang *et al.*, (2020). Arunkumar *et al.* (2022), reported that *C. globosum* Cg-6 showed the highest potential for inhibition *R. solani*, the (black scurf of potato), out of 40 fungal isolates examined in vitro. Research on using endophytic fungi in maize to control late wilt disease was reported by Qi *et al.* (2023). Microencapsulation can improve the stability and longevity of biocontrol species or strains while also being environmentally friendly (Ma *et al.*, 2015). Microcapsules is low cost, favourable conditions, simplicity of use, and short priming time (Holme *et al.*, 2008).

The food, pharmaceutical, and agricultural sectors have shown a great deal of interest in alginate, a natural copolymer, because of its biocompatibility, low toxicity, affordability, and mild priming conditions (Islam *et al.*, 2010). In the traditional extrusion generation method, a syringe needle or nozzle drips the sodium alginate solution into a CaCl₂ solution to produce spherical, uniform calcium alginate hydrogel beads. These microcapsules are the most commonly utilised formulation due to their homogeneous shape, tiny size, and better encapsulation characteristics, which result in a relatively high survival of the encapsulated BCAs (Ma *et al.*, 2015).

Therefore, the objectives of this study is evaluating the efficacy of different formulations of *Chaetomium globosum* by soil introduction of alginate beads-based *Chaetomium globosum* or spore suspension alone or in combinations for controlling root rot disease of tomato plants under greenhouse and field conditions.

2. Material and Methods

Root rot pathogens and Bioagent

Rhizoctonia solani (RS) and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) obtained from Plant Pathology Department, National Research Center, Egypt.

Chaetomium globosum no. 1 (accession number MN689080.1) and other two isolates of *Chaetomium globosum* (2 and 3) were obtained from Dr. Ibrahim E. Elshahawy, Plant Pathology Department, NRC, Egypt.

Evaluation of different isolates of *Chaetomium globosum* on the growth area of pathogenic fungi

Three isolates of *Chaetomium globosum* (CG) (1,2,3) were tested against growth area of RS and FORL in vitro using bi-culture techniques (Hung *et al.*, 2015). On PDA plates, disc (6-mm-diam.) from the active developing region of a 5-day-old colony of RS and FORL was either positioned next to a mycelial disc from one isolate of CG (9 cm diameter) or alone (as a control). Following that, all plates were kept at 28°C in the dark, and the pathogens' growth area was measured when the average growth

area was determined after the control plates had completed full growth. Five duplicates of each treatment were used in the two repetitions of the experiment.

Greenhouse experiment

Efficacy of different formulations of *Chaetomium globosum* on tomato root rot disease

The efficacy of three formulations of *Chaetomium globosum* (CG) consists of spore suspension (GCSS), pellets (CGP) and spore suspension + pellets (CGSS+P) on root rot of tomato plants caused by RS and FORL was tested

Preparation of *Chaetomium globosum* spore suspension

Chaetomium globosum isolate no.1 was used in this study. It was proved as highly antagonistic to pathogenic fungi in vitro experiments. Sterilized Erlenmeyer flask (1L) holding 500 milliliters of sterile PDB, Difco Laboratories, Detroit, MI, EUA) was used to cultivate the *Chaetomium globosum* isolate. A Lab-Line Orbit Environ-shaker was used to incubate the inoculated flasks for two weeks at $28 \pm 2^\circ\text{C}$ and 150 rpm.

A sterile three-layered muslin cloth was used to filter the shake culture suspension after it mixed for 30s on low speed in a coffee blender. After adjusting the spore population to 108 CFU/ mL. Spore solution was utilized to create an alginate-based formulation (Locatelli *et al.*, 2018).

Preparation of alginate pellets-based *Chaetomium globosum*

In accordance with Knudsen *et al.* (1990), alginate pellets containing *Chaetomium globosum* were made by thoroughly combining 50 mL of the spore suspension (106 spore/mL), 20 g of starch, and 100 ml of 2% (wt/vol) aqueous sodium alginate (Sigma Chemical Company, St. Louis, Mo.). The mixture was applied in drops to 0.25 M aqueous CaCl_2 . Entrapped fungal conidia were found inside tiny beads with a mean diameter of 2 mm. To get normal solid beads, the beads were placed in the CaCl_2 solution at room temperature for an additional hour or two. Pellets dry at 22 to 25°C using filter paper after the CaCl_2 solution was squeezed off. (Shaban and El-Komy 2000).

Treatments

Tomato seeds (cv. Super Strain B) were sown in transplant foam trays containing peat-moss soil mixed with following formulations of *Chaetomium globosum* at 15 days before sowing: - Spore suspension (CGSS) at rate 50 ml / kg soil; Pellets (CGP) at rate 50 g / kg soil and Pellets + Spore suspension (CGP+SS) at rate 50g or 50 ml/kg soil respectively.

Applications

Each test treatment was produced as previously described, and all prior treatments were used as seed bed treatments.

Preparation of fungal inocula and soil infestation

The pathogen was cultured on (PDB medium) in 200 ml Erlenmeyer flasks for 15 days at $25 \pm 2^\circ\text{C}$ to create the FORL inoculum. A hemocytometer was used to adjust the conidial suspension to 106 spores/ml after the culture was filtered to eliminate mycelia (Hibar *et al.*, 2006). One kilogramme of soil was infested with 50 millilitres of the conidial suspension (106 spores/ml). R. solani's inoculum consisted of the top solid mycelium layers that were cultivated on Potato Dextrose Agar (PDA) medium after being cleaned and allowed to air dry using layers of sterile filter paper. The distilled water was mixed with the air-dried mycelium to create 1-2 mm inoculum pieces. The rate of soil inoculation was 2.0 g of dry mycelium/kg of soil (Al-Mahareeq 2005).

Planting of treated tomato seedlings in pots artificially inoculated with root rot pathogens

Open greenhouse in NRC, Egypt the experiment, the lowest and maximum temperatures were 20°C and 30°C , were used. Plastic pots (30 cm-diam.) containing 5 kg sandy loam soil with a pH of 7.2, 133.1 ppm of available nitrogen (N), 22.4 ppm of available phosphorus (P), and 308.1 ppm of available potassium (K) were used for the experiment. As previously stated, soil that had been artificial infested with each of the both pathogens was placed in plastic pots. Tomato seedlings (45 days old, cultivar Super Strain B) which treated individually with three formulation of *Chaetomium globosum* as

mentioned before were planted at a rate of four seedlings per pot in plastic pots, with five replicates for each treatment, using check treatment (soil that had not been inoculated).

Assessment of root rot disease

Forty-five days after planting, the root rot disease (incidence and severity) were assessed. Disease incidence, percentage of infected plants were recorded.

Rhizoctonia root rot severity

The disease severity was estimated using a 0–5 scale that was based on the extent of root browning throughout the entire root system: 0 = no symptoms, 1 = 0–25% of root browning, 2 = 26–50%, 3 = 51–75 %, 4 = 76–100% of root browning, and 5 = plant death (Abdeljalil *et al.*, 2016).

Fusarium crown and root rot severity

According to Rowe (1980), the severity of the disease was assessed 45 days following transplantation using a rating system of 0–5, where 0 denoted neither root discoloration nor leaf yellowing. 1=1-25% yellowing of one leaf or discoloration of the roots 2 = 26–50% root discoloration or several fading leaves, three indicates 51–75% root discoloration with one wilted leaf, four indicates up to 76% root discoloration with many wilted leaves, and five indicates entirely dead plants.

Enzyme activities

Extraction of enzymes

Twenty days following transplantation, enzyme activity was assessed. Plant leaves (g) were homogenised at a rate of 1/3 w/v using 0.1M sodium phosphate buffer (pH 7.1) (Goldschmidt *et al.*, 1968).

Peroxidase assay

Peroxidase activity was measured at 470 nm (Abeles *et al.*, 1971). Peroxidase activity was assay using increasing in absorbance at 470 nm/gram fresh weight/1 minute using a spectrophotometer (Spectronic 20-D).

Chitinase assay

The process described by Ried and Ogryd-Ziak (1981) was used to transform chitin powder into the substrate, colloidal chitin. Tubes were placed in a water bath at 37 °C for 60 minutes, cooled, and then centrifuged before being tested. One millilitre of the supernatant's reducing sugar content was measured using dinitrosalicylic acid. To stop the process, the tubes were heated to 100 °C for five minutes. After the tubes cooled, three millilitres of distilled water were added before the assay. The Spectronic 20-D spectrophotometer was used to measure the optical density at 540 nm. Chitinase activity was measured as mM N-acetylglucosamine equivalent released/gram fresh weight tissue/60 minutes.

Field experiment.

Efficacy of different formulations of *Chaetomium globosum* on tomato root rot disease

The efficacy of three formulations of *Chaetomium globosum* (CG) i.e spore suspension (GCSS), pellets(CGP) and spore suspension + pellets (CGSS+P) on tomato root rot disease and yield under filed conditions was applied.

This experiment was conducted in two growing seasons in 2023 and 2024 in a field that was naturally infested tomato root rot fungi. The field is located in Eldeer village, Toukh Centre, Qalyubia Governorate, Egypt, and has a light loamy texture with natural infestation.

Treatment of tomato seedlings

As previously noted, each formulation of *Chaetomium globosum* was combined separately with transplant production foam trays filled with peat-moss soil before tomato seeds (cv. Super Strain B) were planted. Three plots, each measuring 4 5 m, were used as replicates for each treatment. There were 100 tomato transplants in each duplicate. The identical fertilizer and watering schedule were applied to each tomato transplant. As a comparison, the fungicide Topsin M 70% WP (Thiophanate Methyl),

manufactured by Sumitomo Corporation Company in Cairo, Egypt, was used. As previously stated, after 75 days of post-planting, disease incidence and severity were noted, and the fruit weight per plant (kg) was calculated.

3. Results

Effect of different isolates of *Chaetomium globosum* on the growth area of both fungi

Three isolates of *Chaetomium globosum* (CG) (1,2,3) were tested against RS and FORL growth area in vitro using bi-culture techniques. Results in Fig (1) show that all isolates decreased the growth area of both fungi. The most sever isolate is CG1 which decreased the growth area by 81.1 and 78.0% for RS and FORL respectively. Other isolates showed moderate effect.

Greenhouse experiment

Efficacy of different formulations of *Chaetomium globosum* on tomato root rot disease

The efficacy of three formulations of *Chaetomium globosum* (CG) i.e. spore suspension (GCSS), pellets (CGP) and spore suspension + pellets (CGSS+P) on tomato root rot was tested. Data in Table (1) indicate that all formulations decreased disease incidence and severity. The most effective treatment is CGSS+ CGP which suppressed the disease incidence by 80.2 & 71.2 and severity by 87.5 & 81.8 % for RS and FORL respectively. Followed by CG P while, CGSS has satisfactory impact. The same trend was observed with disease severity.

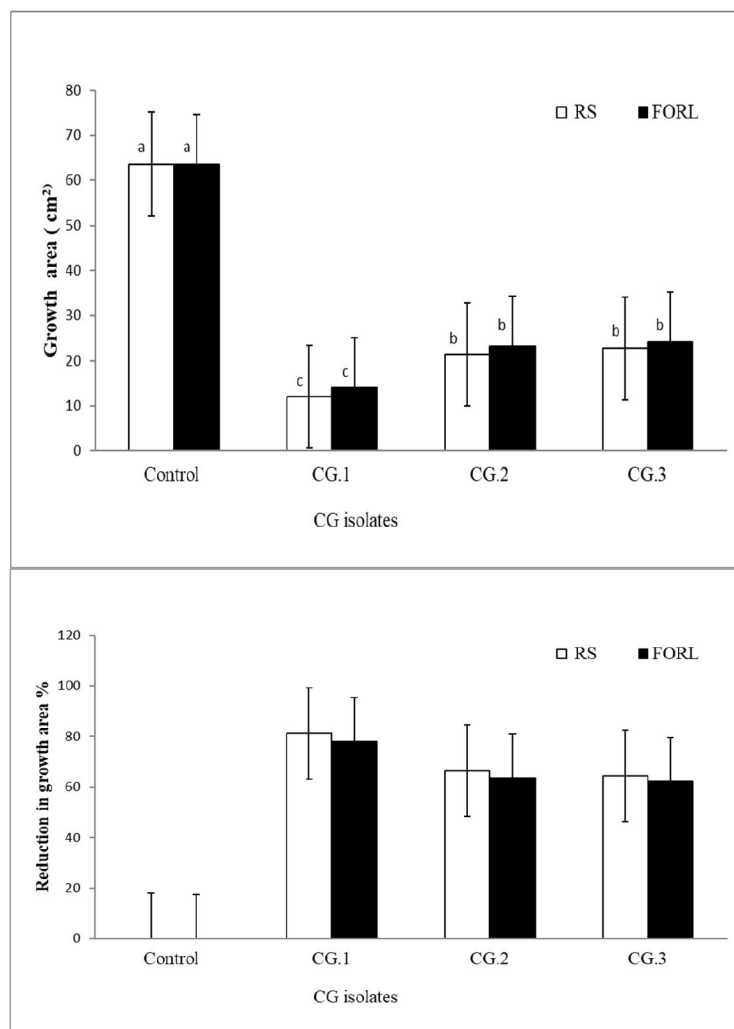


Fig. 1: Impact of *Chaetomium globosum* on growth area and reduction% of pathogenic fungi

Table 1: Impact of different *Chaetomium globosum* formulations as seed bed treatment on tomato root rot disease

Formulation of <i>Chaetomium globosum</i>	Root rot disease							
	<i>Rhizoctonia solani</i>				<i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>			
	Disease incidence	Reduction %	Disease severity	Reduction %	Disease incidence	Reduction %	Disease severity	Reduction %
CGSS	22.5b	57.5	1.0b	58.3	25.0b	41.9	1.2b	45.5
CGP	16.0c	69.8	0.7c	70.8	18.0c	58.1	1.0b	54.5
CGSS + CGP	10.5d	80.2	0.3 d	87.5	12.4 d	71.2	0.4dc	81.8
Control	53.0a	0.0	2.4 a	0.0	43.0 a	0.0	2.2 a	0.0

Means followed by the same letters are not significantly different (P= 0.05)

Impact of different formulations of *Chaetomium globosum* on enzyme activities of tomato plants

The efficacy of three formulations of *Chaetomium globosum* (CG) i.e. spore suspension (GCSS), pellets (CGP) and spore suspension + pellets (CGSS+CGP) on enzyme activities of tomato plants was determined. Results in Fig (2 and 3) show that the enzyme activity were considerably raised by all formulations. As for RS the greatest rise in enzyme activity was obtained with formulations of CGP and CGSS+CGP which increased PO and CH more than 127.3 and 129.4% respectively. While, as for FORL the same formulations increased PO and CH more than 152.2 and 122.2 % respectively. The moderate increased was obtained with CGSS.

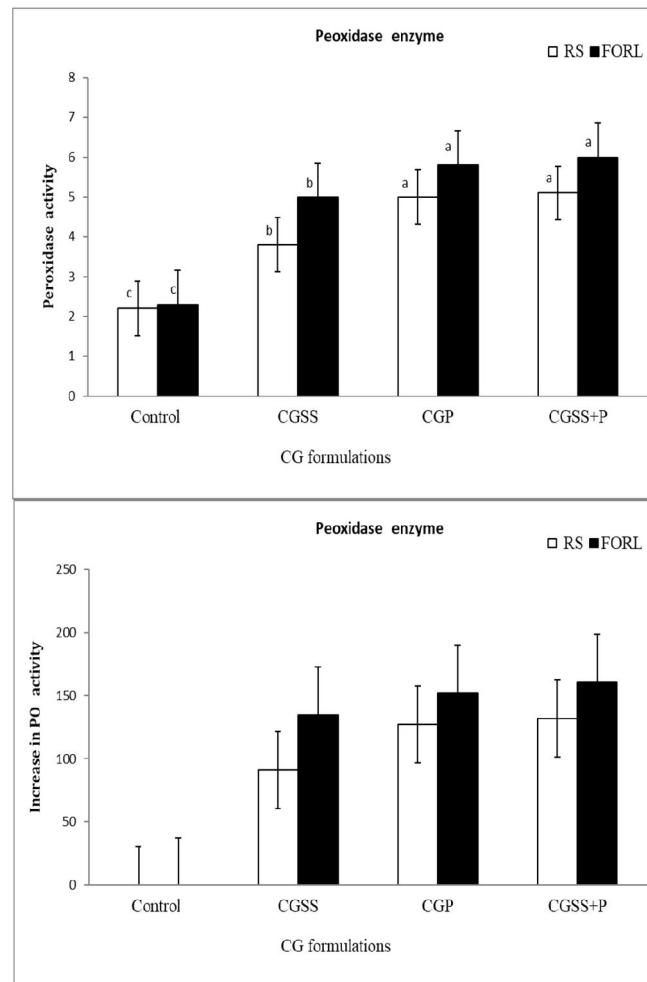


Fig. 2: Effect of *Chaetomium globosum* formulations applied as seed bed treatment on peroxidase activity and increase of tomato plants

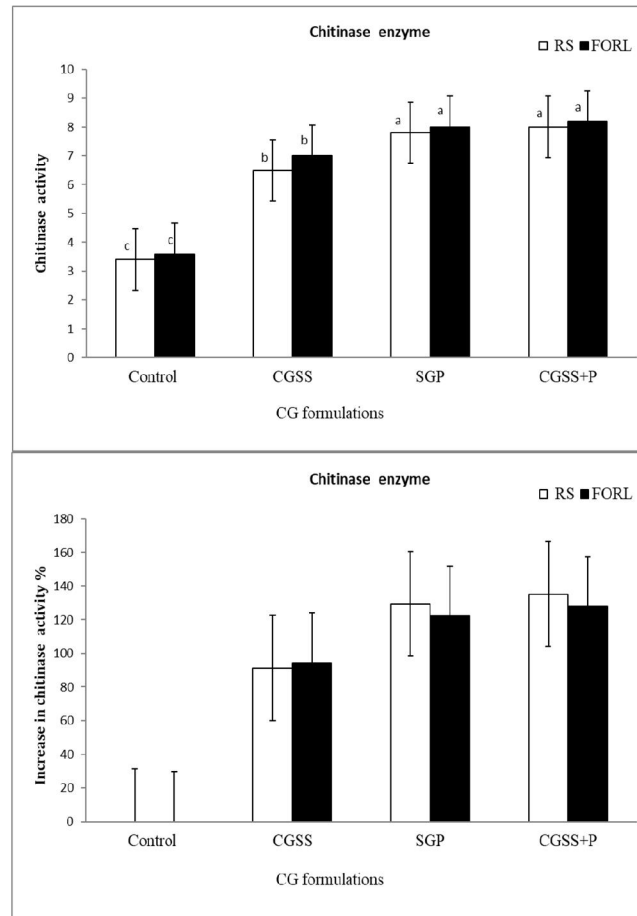


Fig. 3: Effect of *Chaetomium globosum* formulations applied as seed bed treatment on chitinase activity and increase of tomato plants

Field experiment

Efficacy of different formulations of *Chaetomium globosum* on tomato root rot disease

The efficacy of three formulations of *Chaetomium globosum* (CG) i.e. spore suspension (GCSS), pellets (CGP) and spore suspension + pellets (CGSS+CGP) on tomato root rot and yield in field was applied.

Effect on disease incidence

All CG formulations considerably decreased disease incidence over the course of two growing seasons, according to the results in Table (2). Formulation of CGSS+CGP which suppressed disease incidence by 78.3 and 75.5 %. Followed by CGP which suppressed disease incidence by 62.5 and 60.8 % during first and second growing seasons respectively. Meanwhile, CGSS has satisfactory impact.

Table 2: Impact of different formulations of *Chaetomium globosum* on tomato root rot incidence

Formulation of <i>Chaetomium globosum</i>	First growing season (2023)		Second growing season (2024)	
	Disease incidence	Efficacy %	Disease incidence	Efficacy %
CGSS	24.0 b		25.5 b	
CGP	18.0 c	62.5	20.0 c	60.8
CGSS+CGP	10.4 d	78.3	12.5 d	75.5
Rhizolex-T 3	12.0 d	75.0	13.0 d	74.5
Control	48.0 a	00.0	51.0 a	00.0

Means followed by the same letters are not significantly different (P= 0.05)

Effect on disease severity.

Results in Table (3) reveal that all CG formulations decreased disease severity. The most effective formulation is CGSS+CGP which suppressed disease severity by 76.0 and 71.4 % during first and second seasons respectively. Followed by CGP treatment. While, CGSS has satisfactory impact.

Table 3: Effect of different formulations of *Chaetomium globosum* on tomato root rot severity

Formulation of <i>Chaetomium globosum</i>	First growing season(2023)		Second growing season(2024)	
	Disease severity	Efficacy %	Disease severity	Efficacy %
CGSS	1.0 b	60.0	1.1b	60.7
CGP	0.9 c	64.0	0.9 c	67.9
CGSS+CGP	0.6 d	76.0	0.8c	71.4
Rhizolex-T 3	0.7 d	72.0	0.8c	71.4
Control	2.5 a	0.0	2.8 a	0.0

Means followed by the same letters are not significantly different (P= 0.05)

Effect on tomato yield.

Results in Fig (4) indicate that all formulation of CG significantly increased tomato yield . Formulation of CGSS+CGP increased tomato yield by 50.0 and 59.4 % during first and second seasons respectively. Followed by CGP. Meanwhile, CGSS has satisfactory impact..

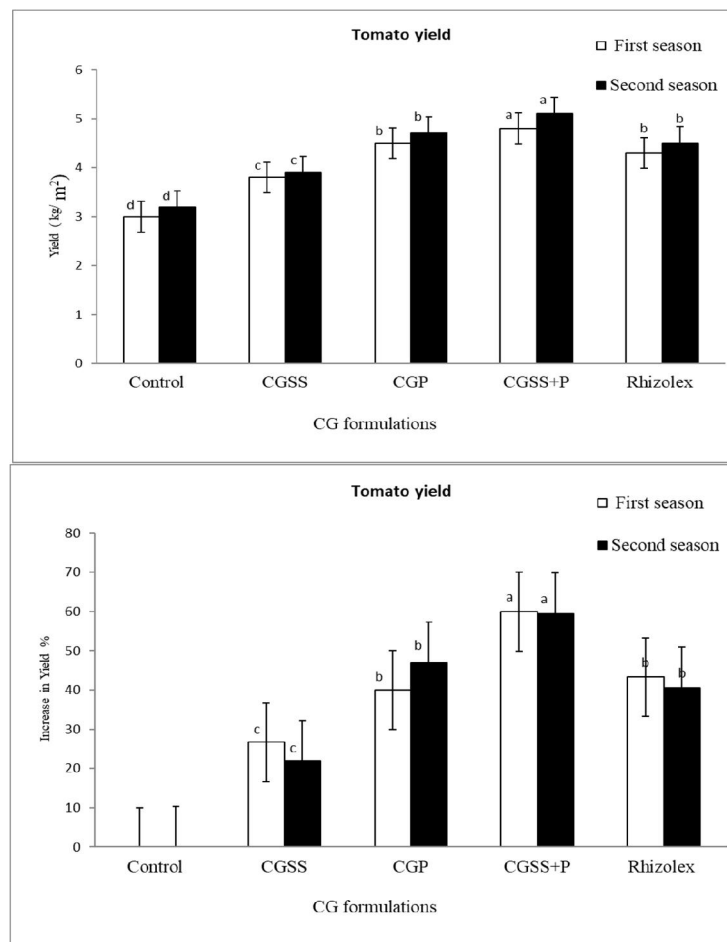


Fig. 4: Tomato yield (kg/m²) in response to different formulations of *Chaetomium globosum* under field conditions.

4. Discussion

In the current research, results showed that all isolates of CG decreased the growth area of both fungi. The most sever isolate is CG1 which decreased growth area by 81.1 and 78.0% for RS and FORL respectively. Other isolates showed moderate effect.

In this regards, several reports about mode action of *Chaetomium* species, including azaphilones (Yamada *et al.*, 2011), polyhydroxylated steroids (Qin *et al.*, 2009), armochaetoglobins (Chen *et al.*, 2015), orsellides (Schlörke *et al.*, 2006), xanthenone (Pontius *et al.*, 2008), and chaetoglobins (Schlrke *et al.*, 2006; Ge *et al.*, 2008). Some of these compounds had biological activities such as cytotoxicity, enzyme inhibition, antifungal, and antibacterial properties (Li *et al.*, 2016). In vitro and in vivo, several strains of *Chaetomium* spp. exhibited different antifungal potencies against a range of phytopathogens (Soytong *et al.*, 2001 and Vitale *et al.*, 2012). According to Tomilova and Shternshis (2006), *Chaetomium* spp. controlled black pepper, citrus root rot, and sugar beetroot damping off disease. However, a number of parameters, including soil temperature, moisture content, and pH, should be taken into account. Antibiotics, competition, induction of resistance, chitinase, β -1-3-glucanase, β -1-4-glucanase, and the deactivation of pathogen-produced enzymes during infection are some of the mechanisms of action that *C. globosum* uses to control plant pathogens, according to earlier research (Pieterse *et al.*, 2014; El-Naggar *et al.*, 2016).

In the current research, results showed that for RS, formulations of CGP and CGSS+CGP increased PO and CH more than 127.3 and 129.4% respectively. While, as for FORL the same formulation increased PO and CH more than 152.2 and 122.2 % respectively. Moreover, in the field, all CG formulations decreased disease incidence and severity. Formulation of CGSS+P suppressed disease incidence and severity. Followed by CGP treatments, meanwhile, CGSS has satisfactory impact. Furthermore, the most effective formulation is CGSS+CGP which increased tomato yield. Followed by CGP. In this respect according to a recent study by Daroodi *et al.*, (2021), *Acrophialophora jodhpurensis* (*Chaetomium jodhpurense* Lodha) activates tomato defence responses, including the induction of antioxidant enzymes, which has indirect effects on the *R. solani*. As a result of increased H₂O₂ generation, elevated PO and PPO activities (Vance *et al.*, 1980). Wei *et al.*, (2019) found that treated cotton plants with the endophytic fungus pre inoculated with *Fusarium solani* increased lignin accumulation and decreased disease progression. However, by hydrolysing their cell walls, chitinase enzymes aid plants in protecting themselves from fungus.

According to Zhang *et al.* (2010), CG is good species in this area, developing as a typical soil coloniser as well as a saprophytic (Zhang *et al.*, 2010). Tomato wilt was significantly decreased *C. globosum* according to Charoenporn *et al.*, (2010). The results of Charoenporn *et al.*, (2010), bio-fungicides from *C. globosum* N0802 resulted in reducing disease incidence of tomato wilt and increasing yield.

In field tests, Shanthiyaa *et al.*, (2013) found that employing *C. globosum* increased tuber yield by decreased late blight disease. The results of this investigation are also corroborated by the work of several other reports (Zhang *et al.*, 2013; Hung *et al.*, 2015a, b b; Huang *et al.*, 2020). However, another investigation validated this endophyte's ability to produce plant hormones (Khan *et al.*, 2012). Additionally, they asserted that plant hormones including gibberellins (GAs) and indole acetic acid (IAA), *C. globosum* can be utilized to increased pepper plant development and yield in the field.

In order to create sufficient defence responses to biotic or abiotic challenges, these hormones enhanced soil nutrient, transduce signals amongst plant (Vessey, 2003; Ghanashyam and Jain 2009).

Through intricate control of plant hormone biosynthesis and metabolism, Tian *et al.*, (2022) recently discovered colonization events of *C. globosum* strain ND35 increased cucumber growth. Tian *et al.*, (2022) recently discovered that the infection and colonization events of *C. globosum* strain ND35 enhanced cucumber growth.

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