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Research Article

The Effect of *Clonostachys rosea* (sch.) Schroers and Samuels Against Verticillium wilt (*Verticillium dahliae* Kleb.) and Early Blight [*Alternaria solani* (Ell. and G. Martin) Sor.] Diseases in Tomato Plants

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Abstract: The effectiveness of *Clonostachys rosea* against Verticillium wilt (*Verticillium dahliae*) and early blight (*Alternaria solani*) diseases, as the two most important problems in tomato cultivation with significant economic losses, was determined. It was determined that *C. rosea* was effective on *A. solani* and *V. dahliae* and suppressed mycelial growth. Also, the *C. rosea* on wheat grains inoculated to plants at 20 g, 30 g, and 40 g concentrations before and after pathogens inoculation. Then, fungal discs (2 mm in diameter) from *V. dahliae* growing colonies were inoculated on the host plant root zone. *A. solani* was also inoculated ($1x10^6$ conidia ml^{-1}) by spraying the foliar parts of the plants. Results showed that *V. dahliae* caused 76.0% disease severity in control plants, while the disease severity indices were 58.3%, 55.3%, and 25.3% at 20 g, 30 g, and 40 g *C. rosea* application, respectively. In *A. solani* x *C. rosea* treatments, the disease severities were determined as 96.6%, 63.3%, 43.6% and 46.6% in control, 20 g, 30 g, and 40 g application of *C. rosea*, respectively. The pathogen suppression rates by *C. rosea* at 30g application dose was 54.8% against *A. solani* and at 40 g application dose was 66.6% against *V. dahliae*. The effects of *C. rosea* on plant growth parameters were also determined. Results showed that *C. rosea* had a positive effect on the morphological parameters in tomato plants.

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1. Introduction

Tomato (*Solanum lycopersicum* L., Solanaceae), with a cheap and plentiful source of vitamins, is one of the most widely grown vegetables worldwide. The global tomato production is over 187 million tons (FAO, 2020). China ranked first in production (64.8 million tons), followed by India (20.6 million tons) and Turkey (13.2 million tons) (FAO, 2020). It is suffered from different plant pathogens, especially fungal ones that cause significant economic losses (Yaviç et al., 2020 Gül, 2021). Among them, *Alterneria solani* (Ellis and Martin) Sorauer causes "early blight" disease, and *Verticillium dahliae* causes Wilt" disease in tomato fields' worldwide (Jones et al., 1991; Yiğit, 1993; Shinde et al., 2018).

Alternaria solani causes early blight of tomato, which is a serious problem in warm, humid climates and semiarid locations with frequent and prolonged night dew. Early blight (EB) decreases photosynthetic area and can defoliate plants under suitable environmental conditions. It causes yield losses if no preventive measures are taken during the leaf blight stage, which is the most crucial stage in the disease's growth (Foolad et al., 2000; Koike et al., 2010). *V. dahliae*, a soil-borne fungus, is responsible for more than half of all crop losses. The disease affects the quality and quantity and can even kill the whole plant (Bletsos et al., 2003; Coşkun et al., 2021).

Regarding control strategies, chemical treatment is often suggested for *A. solani*, while for *V. dahliae*, resistant cultivars or other control measures were recommended (Demir et al., 2015; Shaban et al., 2018). The negative effects of using chemicals on different economic, social, and ecological aspects have increased using of alternative methods especially biological control techniques (Bora, 2002). Using of biological control agents on different plant diseases, including *A. solani* and *V. dahliae* has been reported in several studies (Boyno et al., 2020; Benouzza et al., 2021; Poveda and Baptista, 2021), which indicated the great importance of this technique (Naik et al., 2020; Karthika et al., 2020; Boyno et al., 2022).

Clonostachys rosea (formerly *Gliocladium roseum)*, was first described by Bainier (1907). However, Schroers et al. (1999) found that the morphology, ecology, teleomorph, and DNA sequence data of *G. roseum* were quite different from other *Gliocladium* species, so reclassified *G. roseum* as *C. rosea*. This species has been used as a biological agent against several pathogens such as *Alternaria dauci*, *A. radicina, Botrytis cinerea, B. aclada, Bipolaris sorokiniana, Drechslera teres, F. graminearum, F. verticillioides, F. croohvellense, F. culmorum, H. solani, Moniliophthora roreri, Phytophthora palmivora, Rhizoctonia solani, Rhynchosporium commune* and *S. sclerotiorum* (Krauss and Soberanis, 2001; Jensen et al., 2004; Yohalem et al., 2004; Aydın and Turhan, 2009; Kosawang et al., 2014; Schöneberg et al., 2015; Sun et al., 2015; Jensen et al. et al., 2016; Lysøe et al., 2017; Samsudin et al., 2017). The mechanisms used by *C. rosea*'s proposed to be releasing of cell wall degrading enzymes (CWDE), producing secondary metabolites, including antibiotics and toxins, as well as inducing plant resistance (Chatterton and Punja 2009; Fatema et al., 2018). The study on the effectiveness of *C. rosea* against *A. solani* and *V. dahliae* as well as its efficacy on plant growth parameters were the main objectives of this study.

2. Material and Methods

The tomato variety FDR 8516 (Seminis Tohum-Monsanto) was used in this study as the plant material. *A. solani* EAb1 (As) (Boyno, 2019) and *V. dahliae* Vd11 (Vd) (Erdoğan et al., 2014) as the pathogen isolates were provided by Mycology laboratory's culture collection, Department of Plant Protection, Faculty of Agriculture, Van Yuzuncu Yil University, and their pathogenicity had been verified by previous investigations. The biological control agent *C. rosea* MF536537 (Cr) was also obtained from the Şanlıurfa-GAP Agricultural Research Institute.

2.1. Effect of *C. rosea* against *A. solani* and *V. dahliae* at *in vitro* conditions

The inhibition rates of *C. rosea* against *A. solani* and *V. dahliae* were studied *in vitro.* The study was carried out using dual culture technique with 10 replicates. A mycelial disc (5 mm in diameter) from the margin of the one-week old *C. rosea* and pathogens (*A. solani* and *V. dahliae*) colonies were cultured at the opposite sides of the PDA plates at an equal distance (6 cm) (Figure 1). The plates with only fungal pathogen cultures were used as the controls. Then, plates were incubated at 24±2^{0}C. The fungal colonies were measured on 7th, 9th, 14th, and 21st days. The growth inhibition rate of *A. solani* and *V. dahliae* colonies were determined using the formula proposed by Royse and Ries, 1978 (Equation 1):

$$RI = [(r1 - r2) \div r1]x100 \quad (1)$$

Where RI: Inhibition Rate (%), r1: Fungal pathogen colony diamatere, r2: Fungal pathogen colony diametere in the direction of the biological control agent (Fig. 1).

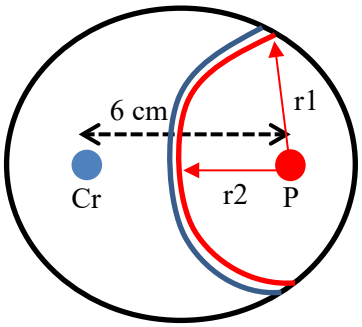

Figure 1. Schematic representation of the dual culture method. Cr: *C. rosea,* P: Pathogens (*A. solani* or *V. dahlia*), r1: Colony radius of the pathogen, r2: Growth radius of the pathogen in the direction of the biological control agent.

2.2. Preparation of *C. rosea* stock culture

C. rosea was first cultured in PDA plates Then, the sterilized wheat seeds (3 kg) were inoculated by a 5 mm diameter mycelial discs of *C. rosea* cultures. For stimulation of fungal growth, 50 g glucose was dissolved in 300 ml of sterile water and poured into the storage containers, and kept at 25 °C until the fungus covered the wheat seeds. The *C. rosea* fungus was then inoculated in plants before and after the pathogens inoculation with different doses (20, 30 and 40 g).

2.3. Effects of *C. rosea* against *A. solani* and *V. dahliae* at *in vivo* conditions

The study was carried out in a growth chamber (for weeks at 24±2 ^{0}C, 60-70% RH, 16 hours light, and 8 hours dark). The mixture of Peat: perlite (1:1) and vermiculite was used as the seedling growing medium. The growing seedlings were transplanted after 2 weeks (observing the emergence of the first leaves) into pots containing 3 kg of sterilized soils and different concentrations of *C. rosea* Then, 5 days later, Pathogens inoculation was carried out. For this purpose, a 2 mm disc of *V. dahliae* colony was inoculated in the root zone of each plant. The *A. solani* was also inoculated to the foliar parts of the plants (50 ml spore suspension containing $1x10^{6}$ conidia ml^{-1}) by hand sprayer. Ten days after the pathogens' inoculation, again, the *C. rosea* was applied, similar to the first inoculation. The study was carried out in a completely randomized design (CRD) experiment with 10 replicates for 9-weeks up to host plants fully developed.

2.3.1. Disease severity index assessment

Disease symptoms were examined 3th, 4th, 5th and 6th weeks after both pathogens' inoculation. For measuring the disease severity index in *V. dahliae*, a 0-4 scale was used for wilting symptoms in leaves (Zeise and Tiedemann, 2002) as well as a 0-3 scale was used for symptoms in stem (Erwin et al., 1976). For *A. solani* disease severity index, a 0-4 scale was used (Devanathan and Ramanujam, 1995). The measured scale values were then converted to disease severity (DS) by using the Townsend Heuberger formula (Townsend, 1943) as follows:

$$DS\ (\%) = [\sum(S \times L) \div (M \times Smax)] \times 100 \qquad (2)$$

Where: S = scale value, L = the number of plants evaluated in the scale (number of leaves for *A. solani*), M = the total number of the plant (number of total leaves for *A. solani*), and Smax = the highest scale value.

2.3.2. Plant growth parameters Assessment

The experiment was finished after nine weeks, and some growth parameters of the plants, including shoot dry and fresh weight (g), root dry and fresh weight (g), total plant length (cm), and stem diameter (mm), were also measured. The seedlings were cut from the root collar for the shoot and fresh root weights, and the upper parts were weighed directly after washing with tap water. Then were dried

at 70°C for 48 hours for measuring dry weights. The stem diameter was also determined with a digital calliper (Insize-1112-150, Germany). The total plant height was recorded by measuring with a ruler.

2.4. Statistic analyses

The recorded data were statistically analyzed using IBM SPSS v21 (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp) program (SPSS, 2012). The mean values were also compared using the DUNCAN multiple range test at 5%. The data values measured based on studied times were also converted into graphs using the Microsoft Excel program, and the means were calculated.

3. Results and Discussion

3.1. The Effect of *C. rosea* against *A. solani* and *V. dahliae in vitro* conditions

The *C. rosea* inhibited the growth of *A. solani* and *V. dahliae* fungal pathogens on 7th, 9th, 14th, and 21st days compared to controls (Cr, As, and Vd). Also, in Vd+Cr treatment, the inhibition rates were 34.05% and 25.48% on the 9th and 14th days of postinoculation, respectively (Figure 2).

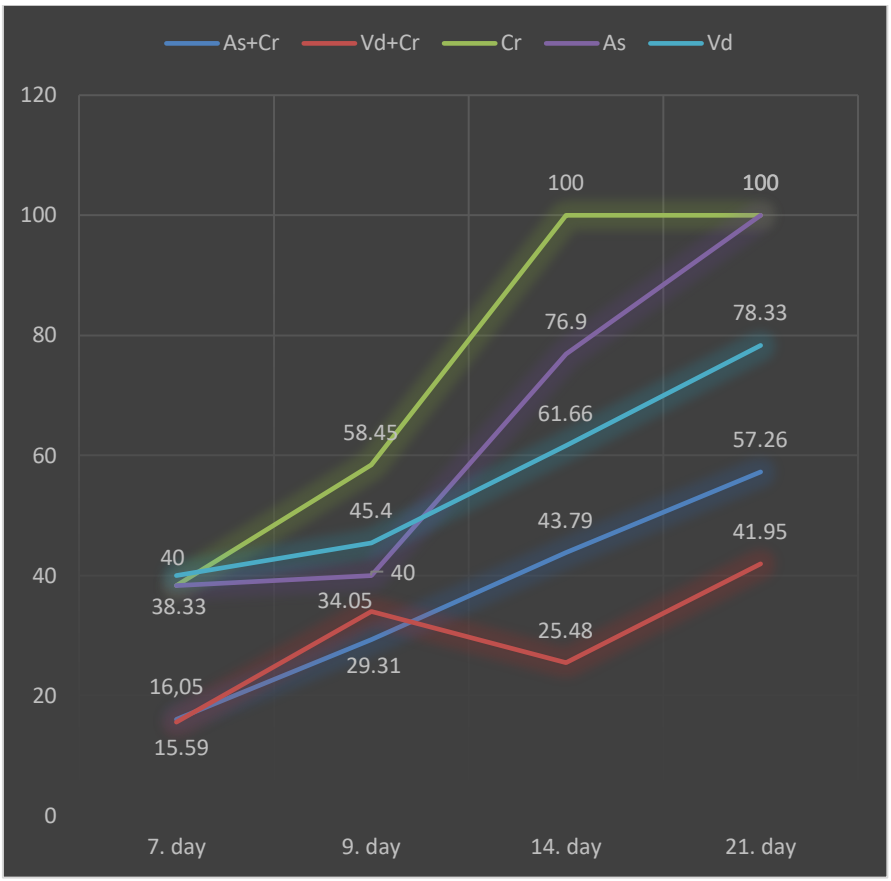

Figure 2. The growth inhibition of *A. solani* (As) and *V. dahliae* (Vd) fungal pathogens by *C. rosea* (Cr).

Generally, Cr decreased fungal pathogen growth (As+Cr and Vd+Cr) compared to control treatments (Cr, As, Vd). *C. rosea* is a highly potential mycoparasitic fungus with biological control ability against several plant pathogenic fungi (Roberti et al., 2008; Nygren et al., 2018; Sun et al., 2020). However, there are no reports on the effect of *C. rosea* against the *V. dahliae in vitro.* In a study conducted by Flores et al. (2015) under *in vitro* conditions, it was reported that *C. rosea* had a significant antagonistic effect on *Fusarium oxysporum*, *A. solani,* and *Botrytis cinerea*.

3.2. The Effect of C. rosea on the A. solani-infected plants growth parameters

The effects of *C. rosea* (20 g, 30 g, and 40 g) on the growth parameters of *A. solani*-infected plants were statistically significant ($p<0.05$). The As treatment decreased significantly the total plant height (12.34 cm) compared to the NK treatment (26 cm). The other plant growth parameters decreased compared to control treatments but not significantly (Table 1). *A. solani is* reported to cause damages on plants in all growth stages (Faheed et al., 2005). Furthermore, it has been found that this fungus affects plant growth as well as host plant yield by decreasing photosynthesis and pigment contents(Agamy et al., 2013). Results showed that different doses of Cr, especially Cr_{40} had a significant increase on all plant's growth parameters in both NK and As treatments. Also, Cr_{40}, Cr_{40}+As, and Cr_{30} treatments significantly improved the stem diameters (1.23 cm, 1.01 cm, 1.00 cm, respectively). All doses of *C. rosea* (20 g, 30 g, and 40 g) were also promoted plant growth in As-infected plants. The highest amounts of total plant height (68.42 cm), shoot dry weights (48.53 g), and root dry weights (4.94 g) could be observed in Cr_{40}+ AS treatment (Table 1).

Using of *C. rosea* increased the growth parameters of both infected and uninfected plants with *A. solani* (Table 1). Generally, it is reported that biological control agents promote plant growth parameters (Murphy et al., 2003; Harman, 2006; Woo et al., 2006). Goh et al. (2020) reported that the leaf area, stem diameter, and plant height were significantly increased 5 months after inoculation by *C. rosea* compared to the controls. It has also been reported that some biological control agents increase the growth parameters of plants infected with *A. solani* (Fritz et al., 2006; Chowdappa et al., 2013; Boyno et al., 2020; 2022). Lahlali and Peng (2014) was also determined that *C. rosea* stimulates the growth of pathogen-infected plants and induced plant resistance

Table 1. The effects of *C. rosea* on the morphological growth parameters of the plants infected with *A. solani*

Treatments	Plant total length (cm)	Stem diameter (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
NC*	26.00±3.46^{d}**	0.46±0.05^{de}	6.07±1.99^{e}	2.59±0.52^{e}	1.12±0.11^{f}	0.13±0.06^{d}
As	12.34±1.52^{e}	0.27±0.01^{e}	2.88±0.13^{e}	0.15±0.03^{e}	0.93±0.25^{f}	0.06±0.04^{d}
Cr_{20}	66.33±3.21^{b}	0.86±0.05^{bc}	186.41±6.70^{b}	47.12±3.98^{b}	42.35±2.00^{b}	5.47±0.48^{ab}
Cr_{30}	65.01±5.00^{b}	1.00±0.30^{ab}	191.39±14.50^{b}	41.29±2.72^{c}	40.46±3.28^{bc}	3.19±0.27^{c}
Cr_{40}	80.66±9.01^{a}	1.23±0.20^{a}	212.49±7.73^{a}	58.77±3.83^{a}	48.83±3.66^{a}	5.78±0.33^{a}
Cr_{20}+As	48.66±3.51^{c}	0.60±0.17^{cd}	145.41±12.86^{d}	34.01±4.01^{d}	32.27±2.13^{e}	3.32±0.60^{c}
Cr_{30}+As	54.33±4.04^{c}	0.73±0.05^{bcd}	161.16±7.19^{c}	36.99±2.95^{cd}	35.20±1.36^{de}	3.72±0.26^{c}
Cr_{40}+As	68.42±3.64^{b}	1.01±0.11^{ab}	164.30±6.12^{c}	48.53±3.44^{b}	37.51±2.29^{cd}	4.94±0.47^{b}

*NC: Negative Control, As: *Alternaria solani*, Cr_{20}: 20 g dose of *C. rosea*, Cr_{30}: 30 g dose of *C. rosea*, Cr_{40}: 40 g dose of *C. rosea*.
**Values are significantly based on Duncan's multiple test range at $p < 0.05$.
Data in the table indicated as mean ± SD.

3.3. The effect of *C. rosea* on the *V. dahliae*-infected plants growth parameters

The effects of *C. rosea* (20 g, 30 g, and 40 g) on the growth parameters of the plants infected with *V. dahliae* were found to be statistically significant ($p<0.05$). Results showed that Vd treatment reduces (not significantly) all growth parameters compared to NC treatment except for root fresh (not significantly) and dry weight (significantly), which increased (Table 2). It is found that the hyphae of *V. dahliae* colonize the internal tissues and spread systemically in the plant (Robb, 2007). It has also been demonstrated that vascular wilt is generally observed in plants susceptible to the disease (Veronese et al., 2003), which decreases plant height and fresh/dry weight values (Veronese et al., 2003; Robb, 2007; Demir et al., 2015).

Results showed that all Cr treatments significantly increased all the plant growth parameters except of stem diameter and root dry weight (Table 2). There are significant increases in stem diameter parameters among the Cr_{20} (0.86 cm), Cr_{30} (1.00 cm), Cr_{40} (1.23 cm), and Cr_{40}+Vd (0.73 cm) treatments with NC and Vd treatments. While the Cr_{20} (5.47 g), Cr_{40} (5.78 g), and Cr_{40}+Vd (4.09) treatments increased root dry weight significantly more than Vd (2.60 g), all other treatments increased this parameter significantly compared to NC. Also, it was found that Cr_{40} treatment had the highest values of all growth parameters. All doses of *C. rosea* (20 g, 30 g, and 40 g) enhanced total plant height, shoot

fresh/dry weight, and root fresh weight in Vd-infected plants; however, only Cr_{40}+Vd treatment significantly increased stem diameter and root dry weight parameters (Table 2).

C. rosea was observed to promote the growth parameters of plants infected or uninfected with *V. dahliae* (Table 2). The previous studies showed that *C. rosea* promoted plant growth parameters infected by different pathogens (Lahlali and Peng, 2014; Goh et al., 2020). Although there is no information regarding the effect of *C. rosea* on plants infected with *V. Dahliae,* it has been reported that different biological control agents may increase plant growth parameters infected by *V. dahliae* (Demir et al., 2015; Gómez-Lama Cabanás et al., 2018).

Table 2. The effects of *C. rosea* on the morphological growth parameters of the plant infected with *V. dahliae*

Treatments	Plant total length (cm)	Stem diameter (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
NC*	26.00±3.46^{e}**	0.46±0.05^{e}	30.07±1.99^{e}	12.59±0.52^{f}	1.12±0.11^{e}	0.13±0.06^{e}
Vd	20.34±1.02^{e}	0.40±0.10^{e}	28.12±3.36^{e}	10.27±2.37^{f}	3.69±0.51^{e}	2.60±0.52^{cd}
Cr_{20}	66.33±3.21^{b}	0.86±0.05^{bc}	186.41±6.70^{b}	47.12±3.98^{b}	42.35±2.00^{b}	5.47±0.48^{a}
Cr_{30}	65.01±5.00^{b}	1.00±0.30^{ab}	191.39±14.50^{b}	41.29±2.72^{c}	40.46±3.28^{b}	3.19±0.27^{c}
Cr_{40}	80.66±9.01^{a}	1.23±0.20^{a}	212.49±7.73^{a}	58.77±3.83^{a}	48.83±3.66^{a}	5.78±0.33^{a}
Cr_{20}+Vd	34.33±3.52^{d}	0.47±0.05^{e}	102.30±11.47^{d}	24.36±2.94^{e}	22.73±2.81^{d}	2.34±0.48^{d}
Cr_{30}+Vd	45.45±4.32^{c}	0.56±0.05^{de}	123.03±9.09^{c}	30.82±2.42^{d}	26.57±2.04^{cd}	2.80±0.26^{cd}
Cr_{40}+Vd	50.57±1.49^{c}	0.73±0.06^{cd}	112.42±9.23^{cd}	39.49±0.65^{c}	29.85±0.51^{c}	4.09±0.16^{b}

*NC: Negative Control, Vd: *Verticillium dahliae*, Cr_{20}: 20 g dose of *C. rosea*, Cr_{30}: 30 g dose of *C. rosea*, Cr_{40}: 40 g dose of *C. rosea*.
**Values are significantly based on Duncan's multiple test range at $p < 0.05$.
Data in the table indicated as mean ± SD.

3.4. Effect of *C. rosea* on disease severity index

The effects of *C. rosea* (20 g, 30 g, and 40 g) on the disease severity index were significant ($p<0.05$). The positive control treatments (PC) in both fungal pathogens were found to have the maximum disease severity rates (Table 3).

A. solani causes serious diseases in tomatoes if no control measure is made (Grigolli et al., 2011; Gannibal et al., 2014; Shinde et al., 2018). The Cr_{30} and Cr_{40} treatments decreased significantly the disease severity index (43.66% and 46.67%, respectively) rather than *A. solani* positive control. Also, the Cr_{20} treatment was effective against the disease compared to the control treatment, with a disease severity rate of 63.33% and a suppression rate of 34.72% rather than *A. solani* positive control treatment. All doses of *C. rosea* (20 g, 30 g, and 40 g) were significantly effective on *A. solani* (Table 3).

Table 3. The effect of *C. rosea* on disease severity and suppression rates of *A. solani*

Treatments	*A. solani*		*V. dahliae*	
	Disease severity (%)	Suppression rates (%)	Disease severity (%).	Suppression rates (%)
PC*	96.67±3.05^{c}**	-	76.00±5.29^{c}	-
Cr_{20}	63.33±4.63^{b}	34.72	58.33±10.4^{0b}	23.25
Cr_{30}	43.66±11.15^{a}	54.83	55.34±5.50^{b}	27.18
Cr_{40}	46.67±15.27^{ab}	51.72	25.33±6.11^{a}	66.67

*PC: Positive Control, Cr_{20}: 20 g dose of *C. rosea*, Cr_{30}: 30 g dose of *C. rosea*, Cr_{40}: 40 g dose of *C. rosea*.
**Values are significantly based on Duncan's multiple test range at $p < 0.05$.
Data in the table indicated as mean ± SD.

It was reported that the *V. dahliae* infected tomato plants had a 91.25% disease severity index after 12 weeks (Ait-Rahou et al., 2020). This vascular pathogen with a wide host range was distributed worldwide and could be able to survive in the soil for several years (Acharya et al., 2020). In this study, the most effective treatment against *V. dahliae* was Cr_{40}, with 25.33% disease severity index and 66.67% suppression rate. However, the Cr_{20} and Cr_{30} treatments were also effective, with 58.33% and 55.34% disease severity indices and 23.25% and 27.18% suppression rates, respectively. On the other hand, it

was found that all doses of *C. rosea* (20 g, 30 g, and 40 g) were significantly effective on *V. dahliae* (Table 3).

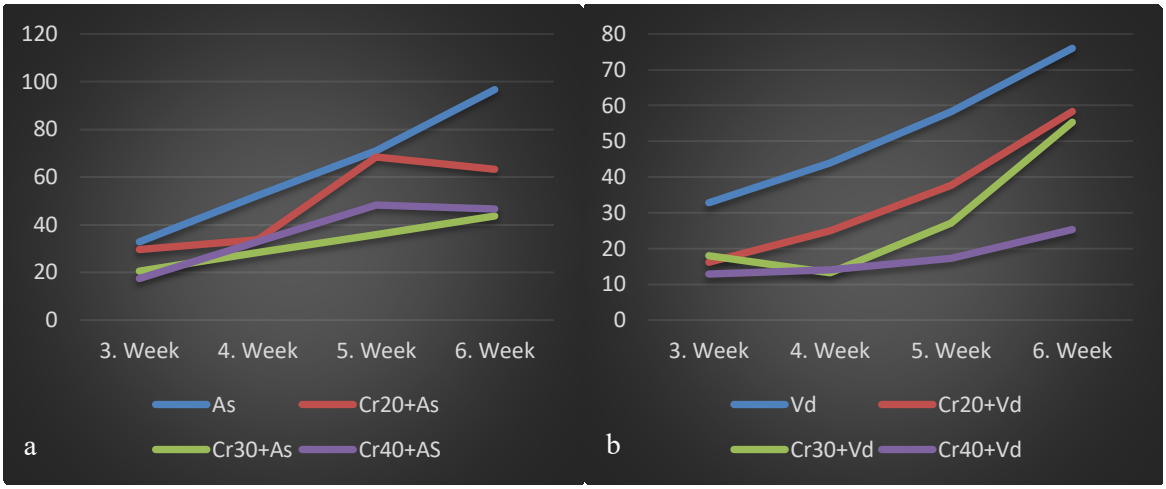

Figure 3. The effect of *C. rosea* (Cr) on disease severity indices of the plants infected with (a) *A. solani* (As) and (b) *V. dahliae* (Vd) during the times.

The disease severity indices were at high rates in As inoculated plants at different times (3rd, 4th, 5th, and 6th) (Figure 3a). Boyno et al. (2022) showed that the disease severity index exceeded 50% and reached the highest level five weeks after inoculation by *A. solani.* All doses of *C. rosea* (20 g, 30 g, and 40 g) were observed to reduce disease severity indices compared to As treatment. The Cr_{40}+As treatment was the most effective treatment against the disease at all times (Figure 3a). It has been observed that *C. rosea* has an endophytic nature in plant tissues, colonizes plants quickly, and works against a variety of fungal plant pathogens (Jensen et al., 2004; Sun et al., 2020; Silva et al., 2021). During the recognition of the biocontrol agent by the host plant, several defense mechanisms such as different hormone and enzyme activities were activated and reached the maximum level during this period (Azcón-Aguilar and Barea, 1996; Morandi, 1996). Although the mechanisms and their activation are unknown, it is proposed that lignification, formation of hydroxyproline-rich cell walls, hypersensitive reactions, antifungal enzyme production, and activated physical barriers, which occur as a result of localized rapid biochemical defense mechanisms, are effective (Demir, 2005). Silva et al. (2021) reported that *Clonostachys* species significantly reduced the disease severity index of *A. solani* in potatoes. Also, it was reported that *C. rosea* suppresses disease by activatingthe defense mechanisms of plants against many fungal plant pathogens such as *A. dauci, A. radicina,* and *Botrytis cinerea*, as well as *A. solani* (Jensen et al., 2004; Sun et al., 2020).

Vd inoculated plants had the highest levels of a disease severity index in all studied times (3rd, 4th, 5th, and 6th weeks). All doses of *C. rosea* (20 g, 30 g, and 40 g) reduced disease severity rates compared to Vd positive control treatment. Also, Cr_{40}+Vd was the most effective treatment against the disease in different studied times (Figure 3b). The biological control agents are quite effective against pathogens (Amin et al., 2010; Tapwal et al., 2011; Sun et al., 2018; Boyno et al., 2020). The *C. rosea* has been reported to be effective against several soil-borne pathogens, especially *Fusarium graminearum, F. verticillioides, F. crookwellense, F. culmorum, Phytophthora palmivora, Rhizoctonia solani,* and *Sclerotinia sclerotiorum* (Krauss and Soberanis, 2001; Yohalem et al., 2004; Kosawang et al., 2014; Schoneberg et al., 2015; Lysøe et al., 2017; Samsudin et al., 2017). Although there is no study on the effect of *C. rosea* on *V. dahliae*, it was found that it inhibited the germination of pathogen microsclerotia in the soil (Keinath et al., 1991; Varo et al., 2016). In addition, it is supposed that *C. rosea* inhibits mycelial growth and microsclerotia formation with its non-volatile secondary metabolites (Rodriguez et al., 2011).

4. Conclusion

In conclusion, it was observed that *C. rosea* could be an effective biological control agent due to its positive effects on both the disease severity index as well as promoting plant growth. Therefore, this biological control agent could be used as an alternative Plant Growth Regulator. However, it is supposed that further studies are needed to fully elucidate its mechanisms.

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