

Original Research Article

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Antifungal Potential of *Chaetomium* Species against *Fusarium oxysporum* f. sp. *lycopersici*

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ABSTRACT

Keywords

Tomato,
Chaetomium spp.
(*Ch. globosum*,
Ch. elatum,
Ch. cochiloides,
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Fusarium
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Tomato (*Lycopersicon esculentum* Mill.) is the most popular vegetable grown across the world. Wilt disease caused by the fungus *Fusarium oxysporum* f.sp. *lycopersici* is one of the most devastating and destructive disease of tomato, resulting in significant yield loss. The pathogen causing wilt disease in tomato was isolated and confirmed its pathogenicity in glasshouse experiment. The effect of fungal biocontrol agent *Chaetomium* spp. was studied against fusarium wilt pathogen under *in vitro* condition. Among the *Chaetomium* spp., *Ch. cochiloides* showed highest inhibition percentage of 64.44 per cent over untreated control against *Fusarium oxysporum* f.sp. *lycopersici*.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely cultivated, popular and important vegetable crops in the world. It is used as a fresh vegetable and can be processed and canned as a paste, juice, sauce,

powder or as a whole (Barone and Frusciante, 2007). Recently, it started gaining more medicinal value because of the antioxidant property of ascorbic acid and lycopene content (Anonymous, 2002). In India, vegetable cultivation reaches 10.3 million hectares with a production of 175 million

tonnes, in which tomato is being cultivated in about 808.5 hectares and the production is 19696.9 metric tonnes (MT) with a productivity of 24.4 MT/ ha (National Horticulture Board, 2017). In Tamil Nadu, the area under tomato cultivation is 38.78 hectares with production of 840.21 MT/ha (National Horticulture Board, 2017). The major tomato growing districts in Tamil Nadu are Dharmapuri (which ranks first in area and production), Krishnagiri, Salem, Theni, Dindigul and Coimbatore (Anonymous, 2009). The main constraints in tomato cultivation are pest and diseases and nematode attack. Among the diseases, bacterial wilt (*Ralstonia solanacearum*), early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), fusarium wilt (*Fusarium oxysporum* f.sp. *lycopersici*) are the most important one which accounts for maximum yield loss globally. Fusarium wilt is caused *F. oxysporum* f.sp. *lycopersici* (Sacc.) Snyder and Hansen is a soil borne pathogen and highly specific to tomato and is worldwide in distribution (Walker, 1969). Symptoms associated with *F. oxysporum* f.sp. *lycopersici* includes yellowing and drooping of the lower leaves. Successive leaves become yellow, wilt and die, often before the plant reaches maturity. Discoloration of the vascular system is characteristic symptom of the disease. Often, infected plants mature earlier and severe infection can lead to a 100 per cent yield loss (Agrios, 2005). It causes significant losses in tomato production both in greenhouse and field-grown tomatoes (Nusret Ozbay and Newman, 2004). It is one of the most prevalent and damaging disease wherever tomatoes are grown intensively because the pathogen can persist indefinitely in the infested soils (Agrios, 1997). Management of soil-borne diseases such as wilt caused by *Fusarium sp.* has always been problematic. Applying chemical fungicides was considered to be the most effective for plant disease management. The excessive

misuse of a wide range of fungicides has led to it being harmful to the environment and increases the resistant pathogenic population (Ozgonen *et al.*, 2001). To overcome this, an alternative method to control the disease had been studied with emphasis on biological control using fungi or bacteria to reduce fungicide application and decrease cost of production. Recently, there have been many reports to control the disease using biological fungicides (Soytong, 1992). Akrami *et al.*, (2011) found that three isolates of *Trichoderma harzianum*, *T. asperellum*, *T. virens* were effective against Fusarium rot of lentil. Srinon *et al.*, (2006) reported that *T. harzianum* WS01 showed efficacies of more than 90% to inhibit conidial production of *F. oxysporum* f.sp. *lycopersici* and *F. oxysporum* f.sp. *cucumerinum* (wilt of cucumber). Keeping all these in view, the main objective of this study was to isolate the pathogenic fungus causing fusarium wilt of tomato from various locations of Tamil Nadu and to evaluate the antifungal potential of fungal biocontrol agent namely *Chaetomium globosum*, *Ch. elatum*, *Ch. cochiloides* and *Ch. bostrychodes* against the isolated pathogen under *in vitro* condition.

Materials and Methods

Survey

A survey was conducted in major tomato growing districts of south Tamil Nadu *viz.*, Madurai, Dindigul, Theni and Coimbatore. Randomly 100 plants were selected in a field and number of plants wilted was counted and the disease incidence was expressed in percentage. The per cent disease incidence was calculated by using the following formula,

$$\text{Percent disease incidence (\%)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants observed}} \times 100$$

Isolation of pathogen

The disease infected samples collected during survey was used for isolation of fusarium wilt pathogen. The wilt infected samples of tomato were collected from different locations of Tamil Nadu during survey. The pathogen was isolated from diseased plants by transferring surface sterilized root tissues onto potato dextrose agar (PDA) medium by using the method of Agrios (1997). Then, single spore isolation was carried out on each isolate to get pure culture and maintained on PDA slants for further studies.

Pathogenicity test

All *Fusarium oxysporum* isolates were tested for its pathogenicity in tomato seedlings using Koch's postulates to confirm pathogenic isolates. Briefly, all isolates were sub-cultured and multiplied on PDA medium and incubated at room temperature approximately 30-32°C for 7 to 10 days. Then spore suspension was prepared and pathogenic inoculum was adjusted to 1×10^7 spores/ml before inoculating to 20 days old tomato seedlings. The roots of tomato seedlings were washed under running sterilized water and cut at five points on the root tips before dipping the roots into a 20ml spore suspension for 15 mins. A control was maintained by dipping seedlings into sterile water. Then, the seedlings were planted in sterilized soil. After 10 days, symptoms of disease development were recorded using the Disease Severity Index (DSI) and rated according to Sibounnavong *et al.*, (2009) as follows: 1 = no symptoms, 2 = 1 to 20% of leaves yellow and wilted, 3 = 21 to 40% of leaves yellow and wilted, 4 = 41 to 60% of leaves yellow and wilted, 5 = 61 to 80% of leaves yellow and wilted, 6 = 81 to 100% of leaves yellow and wilted. The experiment was conducted using a completely randomized block design (CRD) with three replications of each

treatment and the experiment was repeated two times.

Biocontrol

The antagonistic fungi *viz.*, *Chaetomium globosum* 5157, *Ch. elatum* 3322 were obtained from the Indian Type Culture Collection (ITCC) and *Ch. cochiloides* 1019, *Ch. bostrychodes* 2144 were obtained from the Microbial Type Culture Collection (MTCC). These fungal antagonists were sub cultured onto potato dextrose agar (PDA) medium and incubated at 25°C in an incubator. Then these isolates were tested against *Fusarium sp.* under *in vitro* condition.

In vitro evaluation of fungal antagonists against *Fusarium sp.*

The antagonistic fungi were tested against *Fusarium oxysporum* by using the dual culture technique. The antagonistic fungi tested were *Ch. globosum* 5157, *Ch. elatum* 3322, *Ch. cochiloides* 1019, *Ch. bostrychodes* 2144. These isolates were tested to influence their ability to antagonize all the *Fusarium oxysporum* isolates. The test was conducted using the methods of Soyong (1992), Sibounnavong *et al.*, (2009) and Charoenporn *et al.*, (2010). The antagonistic fungi and pathogen were cultured separately on PDA medium at room temperature 30-32°C for 7 days. Then, a sterilized cork borer (0.5 cm diameter) was used to remove agar plugs from the actively growing edge of cultures of the pathogenic fungus and from antagonistic fungi and then, inoculated onto the PDA plates in such a way that an agar plug of the pathogen was placed on one side of the plate and opposite side an agar plug of an antagonistic fungus was inoculated. The plates inoculated with single plug of the pathogen or antagonist acted as control. Then, these plates were incubated at room temperature 30-32°C for 30 days. The

experiment was carried out in a completely randomized block design (CRD) with three replications. The data were recorded based on colony diameter (cm) and number of conidia produced by the pathogen. A haemocytometer was used to count the number of conidia. The colony diameter or conidia of pathogen were measured and percent inhibition was calculated using the following formula,

$$\text{Percent inhibition} = \frac{A - B}{A} \times 100$$

Where, A is the colony diameter or conidial number of pathogen in control plate and B is the colony diameter or conidial number of pathogen in dual plate.

Statistical analysis

All experiments were conducted in a completely randomized block design. Analysis of variance (ANOVA) and the SPSS (Statistical Package for the Social Sciences, version 17.0) were used to analyze the experimental data. The treatment means were compared using DMRT at P = 0.05 (Gomez and Gomez, 1976).

Results and Discussion

Survey

A survey was carried out in four districts of Tamil Nadu. The results betrayed that wilt disease incidence was noticed in all the districts surveyed viz., Coimbatore, Madurai, Dindigul and Theni districts with a range of 32.00 to 51.33 per cent. Maximum wilt incidence was perceived in Checkkanurani village (51.33 per cent) of Madurai district (Fig. 1) and least incidence was observed in Cumbum (32.00 per cent) of Theni district. The data on per cent wilt disease incidence has been presented in the Table 1. Similar observation was reported by Anitha and Rabeeth (2009) on fusarial wilt of tomato at

Coimbatore and Dindigul districts.

Isolation of disease causing agent

Ten isolates of *Fusarium sp.* have been isolated from the infected root portion of the plants collected from various tomato growing areas of Tamil Nadu during survey (Fig. 2). The isolates of *Fusarium sp.* were named as IS(SEV)-1, IS(THO)-2, IS(AYY)-3, IS(SIN)-4, IS(CHE)-5, IS(ACR)-6, IS(USI)-7, IS(MEL)-8, IS(CUM)-9 and IS(SEE)-10 (Table 1). Likewise, Abdel-Salam *et al.*, (2007) justified that the pathogen responsible for wilt (*F. oxysporum* f.sp. *lycopersici*) of tomato was isolated from infected roots showing typical symptoms of wilt.

Assessing the virulence of the pathogenic isolates of *Fusarium sp.*

Isolates of *F. oxysporum* f.sp. *lycopersici* [IS(SEV)-1, IS(THO)-2, IS(AYY)-3, IS(SIN)-4, IS(CHE)-5, IS(ACR)-6, IS(USI)-7, IS(MEL)-8, IS(CUM)-9, IS(SEE)-10] were grouped for pathogenicity according to disease severity index (DSI) as shown in the table 2. The wilt disease severity in the pathogen inoculated tomato plants were ranged from 11.11 to 66.67 per cent. Among the ten isolates tested, the *Fusarium* isolate IS(CHE)-5 collected from Checkkanurani of Madurai district expressed typical symptoms such as yellowing of leaves, wilting of plants, vascular discolouration on inoculated plant after 40 days and showed maximum disease severity of 66.67 per cent wilt incidence followed by 55.56 per cent incidence in the isolate IS(AYY)-3. The remaining isolates of *Fusarium sp.* recorded 44.44 to 11.11 per cent wilt incidence (Table 2). The pathogenicity test performed on tomato seedlings in this study showed that IS(CHE)-5 was the aggressive isolate. Similar work was reported by Charoenporn *et al.*, (2010).

Table.1 Survey on the incidence of *Fusarium* wilt disease incidence in major tomato growing areas of Tamil Nadu

S.No.	Districts	Places	Isolate No.	Latitude & Longitude	Wilt incidence (%)
1	Coimbatore	Sevur	IS (SEV)-1	11 ⁰ 16'35.3"N 77 ⁰ 12'34.9"E	46.67
2		Thondamuthur	IS (THO)-2	11 ⁰ 16'35.3"N 77 ⁰ 12'34.9"E	36.33
3	Dindigul	Ayyalur	IS (AYY)-3	10 ⁰ 28'53.9"N 77 ⁰ 42'26.0"E	41.33
4	Madurai	Singampunarani	IS (SIN)-4	10 ⁰ 1'50.24"N 78 ⁰ 20'24.75"E	35.67
5		Checkkanurani	IS (CHE)-5	9 ⁰ 58'28.7"N 77 ⁰ 56'53.4"E	51.33
6		AC & RI	IS (ACR)-6	9 ⁰ 58'22.1"N 78 ⁰ 12'14.4"E	39.67
7		Usilampatti	IS (USI)-7	9 ⁰ 96'51.1"N 77 ⁰ 78'85.3"E	32.33
8		Melur	IS (MEL)-8	10 ⁰ 03'33.1"N 78 ⁰ 33'59.4"E	42.67
9	Theni	Cumbum	IS (CUM)-9	9 ⁰ 44'43.7"N 77 ⁰ 15'49.6"E	32.00
10		Seelayampatti	IS (SEE)-10	9 ⁰ 58'28.7"N 77 ⁰ 56'53.4"E	46.00

Table.2 Pathogenicity of different isolates of *Fusarium* sp. in tomato

S.No.	Isolate No.	Disease severity (%)
1	IS (SEV)-1	11.11
2	IS (THO)-2	33.33
3	IS (AYY)-3	55.56
4	IS (SIN)-4	22.22
5	IS (CHE)-5	66.67
6	IS (ACR)-6	44.44
7	IS (USI)-7	22.22
8	IS (MEL)-8	44.44
9	IS (CUM)-9	22.22
10	IS (SEE)-10	33.33
11	Control	0.00

Table.3 *In vitro* efficacy of *Chaetomium globosum* against the mycelial growth of different isolates of *Fusarium sp.*

S.No.	Isolates of <i>Fusarium sp.</i>	Mycelial growth of <i>Fusarium sp.</i> against <i>Ch. globosum</i> in(cm)*	Mycelial growth inhibition over control (%)
1	IS (SEV)-1	5.2 ^c	42.22(40.52)**
2	IS (THO)-2	5.4 ^c	40.00(39.23)**
3	IS (AYY)-3	5.3 ^c	41.11(39.88)**
4	IS (SIN)-4	4.6 ^b	48.88(44.36)**
5	IS (CHE)-5	4.5 ^{ab}	50.00(45.00)**
6	IS (ACR)-6	6.4 ^d	28.88(32.51)**
7	IS (USI)-7	4.2 ^a	53.33(46.91)**
8	IS (MEL)-8	5.3 ^c	41.11(39.88)**
9	IS (CUM)-9	5.5 ^c	38.88(38.57)**
10	IS (SEE)-10	6.2 ^d	31.11(33.90)**
11	Control	9.0	-
CD (P= 0.05)		0.32	

*Mean of three replications

Mean followed by a common letter are not significantly different at 5% level by DMRT

**Data in parantheses are arc sine transformed values

Table.4 *In vitro* efficacy of *Chaetomium elatum* against the mycelial growth of different isolates of *Fusarium sp.*

S.No.	Isolates of <i>Fusarium sp.</i>	Mycelial growth of <i>Fusarium sp.</i> against <i>Chaetomium elatum</i> in (cm)*	Mycelial growth inhibition over control (%)
1	FI ₁	5.3 ^{bc}	41.11(39.88)**
2	FI ₂	6.2 ^d	31.11(33.90)**
3	FI ₃	7.1 ^e	21.11(27.35)**
4	FI ₄	6.3 ^d	30.00(33.21)**
5	FI ₅	5.3 ^{bc}	41.11(39.88)**
6	FI ₆	4.2 ^a	53.33(46.91)**
7	FI ₇	6.4 ^d	28.88(32.51)**
8	FI ₈	5.1 ^b	43.33(41.17)**
9	FI ₉	7.2 ^e	20.00(26.57)**
10	FI ₁₀	5.6 ^c	37.77(37.92)**
11	Control	9.0	-
CD (P = 0.05)		0.35	

*Mean of three replications

Mean followed by a common letter are not significantly different at 5% level by DMRT

**Data in parentheses are arc sine transformed values

Table.5 *In vitro* efficacy of *Chaetomium cochiloides* against the mycelial growth of different isolates of *Fusarium sp.*

S.No.	Isolates of <i>Fusarium sp.</i>	Mycelial growth of <i>Fusarium sp.</i> against <i>Chaetomium cochiloides</i> in (cm)*	Mycelial growth inhibition over control (%)
1	FI ₁	5.1 ^c	43.33(41.17)**
2	FI ₂	4.3 ^b	52.22(46.27)**
3	FI ₃	3.3 ^a	63.33(52.73)**
4	FI ₄	4.2 ^b	53.33(46.91)**
5	FI ₅	5.6 ^d	37.77(37.92)**
6	FI ₆	3.3 ^a	63.33(52.73)**
7	FI ₇	3.2 ^a	64.44(53.39)**
8	FI ₈	5.3 ^{cd}	41.11(39.88)**
9	FI ₉	4.4 ^b	51.11(45.64)**
10	FI ₁₀	5.4 ^{cd}	40.00(39.23)**
11	Control	9.0	-
CD (p = 0.05)		0.31	

*Mean of three replications

Mean followed by a common letter are not significantly different at 5% level by DMRT

**Data in parentheses are arc sine transformed values

Table.6 *In vitro* efficacy of *Chaetomium bostrychodes* against the mycelial growth of different isolates of *Fusarium sp.*

S.No.	Isolates of <i>Fusarium sp.</i>	Mycelial growth of <i>Fusarium sp.</i> against <i>Chaetomium bostrychodes</i> in (cm)*	Mycelial growth inhibition over control (%)
1	FI ₁	6.3 ^d	30.00(33.21)**
2	FI ₂	6.2 ^d	31.11(33.90)**
3	FI ₃	6.2 ^d	31.11(33.90)**
4	FI ₄	6.6 ^e	26.66(31.08)**
5	FI ₅	5.7 ^c	36.66(37.26)**
6	FI ₆	6.2 ^d	31.11(33.90)**
7	FI ₇	5.3 ^b	41.11(39.87)**
8	FI ₈	4.5 ^a	50.00(45.00)**
9	FI ₉	6.4 ^{de}	28.88(32.50)**
10	FI ₁₀	5.6 ^c	37.77(37.92)**
11	Control	9.0	-
CD (p = 0.05)		0.31	

*Mean of three replications

Mean followed by a common letter are not significantly different at 5% level by DMRT

**Data in parentheses are arc sine transformed values

Fig.1 Symptoms of Fusarium wilt of tomato **Fig.2** Mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici*



Fig.3 *In vitro* efficacy of *Chaetomium globosum* against the mycelial growth of different isolates of *Fusarium* sp.

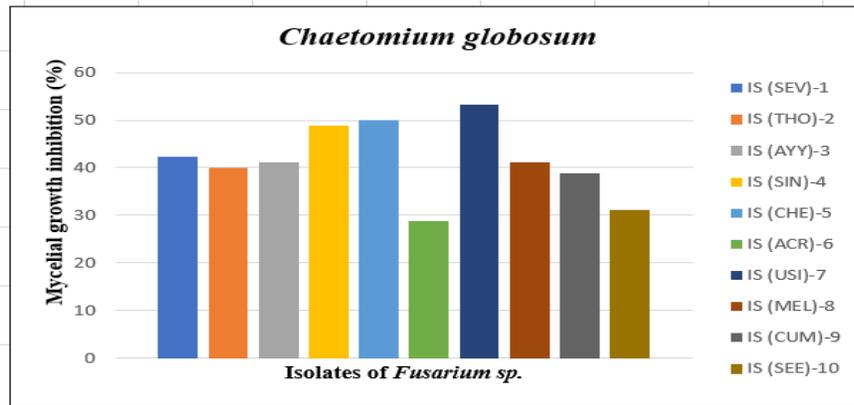


Fig.4 *In vitro* efficacy of *Chaetomium elatum* against the mycelial growth of different isolates of *Fusarium* sp.

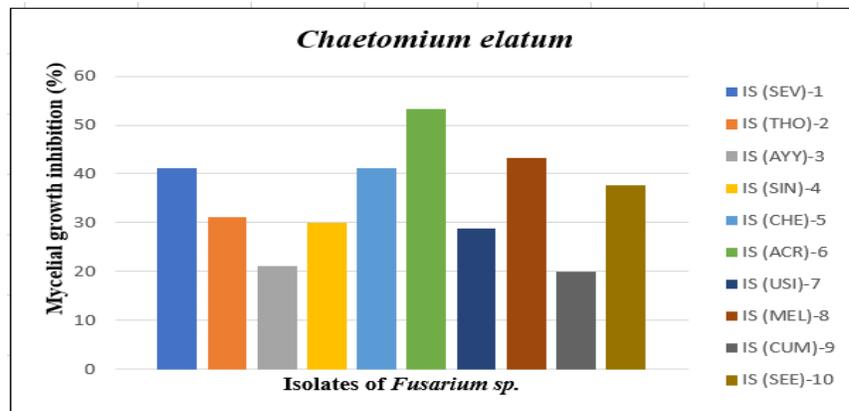


Fig.5 *In vitro* efficacy of *Chaetomium cochiloides* against the mycelial growth of different isolates of *Fusarium sp.*

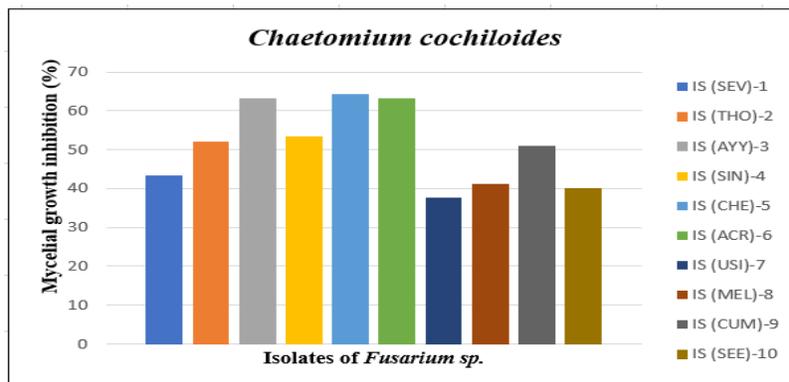
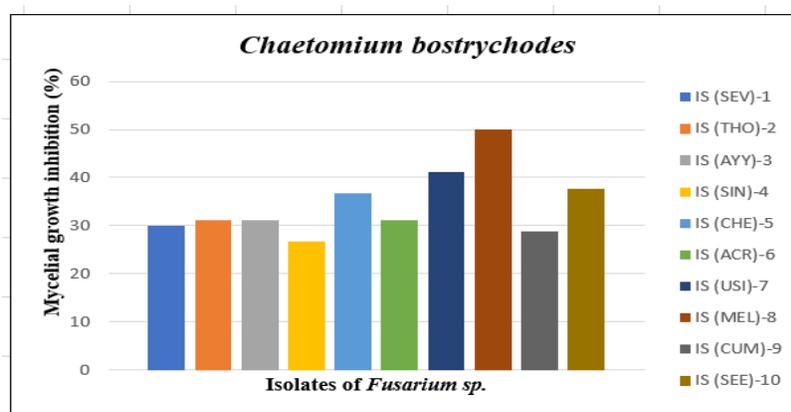


Fig.6 *In vitro* efficacy of *Chaetomium bostrychodes* against the mycelial growth of different isolates of *Fusarium sp.*



Biocontrol

The fungal biocontrol agent *viz.*, *Ch. globosum* 5157, *Ch. elatum* 3322, *Ch. cochiloides* 1019 and *Ch. bostrychodes* 2144 tested against the ten isolates of *F. oxysporum* f.sp. *lycopersici*. Similarly, Charoenporn *et al.*, (2010) brought antagonistic fungi *Ch. globosum*, N0802, *Ch. lucknowense* CLT and *T. harzianum* PC01 from Assoc. Prof. Dr. Kasem Soyotong of King Mongkut of the Institute of Technology Ladkrabang, Thailand and tested the efficacy of the antagonists against wilt fungus under *in vitro*.

In vitro evaluation of fungal antagonists against *Fusarium sp.*

In vitro antagonism of *Ch. globosum* against the mycelial growth of ten isolates of *Fusarium sp.* indicated that, the antagonist was very effective in inhibiting the mycelial growth of isolates of *Fusarium sp.* [IS(USI)-7] to the maximum of 53.33 per cent. This was followed by IS(CHE)-5 with 50.00 per cent, IS(SIN)-4 with 48.88 per cent and IS(SEV)-1 with 42.22 per cent. The least mycelial growth reduction was recorded in the isolate IS(ACR)-6 with 28.88 per cent (Table 3, Fig 3). Correspondingly, Phong *et*

al., (2015) showed that the antagonistic fungi namely *Ch. cupreum* CC3003, *Ch. globosum* CG05 and *Ch. lucknowense* CL01 inhibited NHP-Fusa-2 using bi-culture technique. Then, the fungal antagonist *Ch. elatum* showed the maximum inhibition percentage to an extent of 53.33 per cent against the mycelial growth of *Fusarium sp.* isolate IS(ACR)-6. This was followed by the isolate IS(MEL)-8 which exhibited the mycelial growth reduction of 43.33 per cent over control (Table 4, Fig 4). Similar results were reported by Charoenporn *et al.*, (2010) in tomato against *F. oxysporum* f.sp. *lycopersici*. The fungal biocontrol agent *Ch. cochiloides* was most effective against the isolate of *Fusarium sp.* IS (CHE)-5 to the maximum inhibition percentage of 64.44 per cent (Table 5, Fig 5). The fungal antagonist *Ch. bostrychodes* exhibited the maximum inhibition against the isolate of *Fusarium sp.* IS(MEL)-8 to an extent of 50.00 per cent followed by the isolate IS(USI)-7 with growth reduction of 41.11 per cent (Table 6, Fig 6). The results showed that among the four species of the antagonist tested, *Ch. cochiloides* showed more inhibition percentage of 64.44 per cent against *F. oxysporum* f.sp. *lycopersici* followed by *Ch. globosum*, *Ch. elatum* and *Ch. bostrychodes*. Sibounnavong *et al.*, (2011) studied that *Ch. elatum* significantly inhibited mycelial growth of *F. oxysporum* f.sp. *lycopersici* to 74.95 per cent followed by *Ch. lucknowense* and *Emericella rugulosa*. Phong *et al.*, (2015) reported that *Ch. cupreum* CC3003, *Ch. globum* CG05 and *Ch. lucknowense* CL01 significantly inhibited both mycelial growth and conidial production of *F. oxysporum* NHP-Fusa-2 to the range from 31.69 to 34.03 per cent. The least inhibition was noticed against the isolate IS(USI)-7 (Table 5, Fig 5).

In conclusion this study showed that *Ch. cochiloides* exhibited more inhibition percentage of 64.44 per cent against *F. oxysporum* f.sp. *lycopersici* followed by *Ch.*

globosum, *Ch. elatum* and *Ch. bostrychodes*. These biocontrol agents are better alternative for managing the most devastating disease of fusarium wilt in tomato. The testing of these isolates against the wilt pathogen in tomato is under pipeline.

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