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ANTIMYCOTIC POTENTIAL ASSESSMENT OF *TRICHODERMA* SPECIES AND FUNGICIDES FOR SUSTAINABLE MANAGEMENT OF *SCLEROTINIA TRIFOLIORUM* CAUSING STEM AND CROWN ROT OF *TRIFOLIUM ALEXANDRINUM* L.

[a]Anjum Faraz, [a]Imran Ul Haq, [b]Siddra Ijaz, [a]Shahbaz Talib Sahi, [c]Imran Khan

[a] *Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.*

[b] *Center of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan.*

[c] *Department of Agronomy, University of Agriculture Faisalabad, Pakistan.*

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ABSTRACT

Sclerotinia trifoliorum, the fungal plant pathogen first reported in 2021 from Pakistan on *Trifolium alexandrinum* L. (Egyptian clover; an annual winter fodder crop), causing Stem and crown rot disease. About 46% to 55% incidence of this disease was recorded on *E. clover* cultivated in the irrigated tract of the country in 2018-19. This disease is subjecting significant crop losses and drastically reducing growth. An integrated disease management approach employing biological and chemical control was adopted to manage this wide-spreading fungal pathogen. The fungal antagonists, including *Trichoderma harzianum*, *T. longibrachiatum,* and *T. asperellum* Moreover, fungicides, including Thiophanate Methyl, Tebuconazole, Tubeconazole+Emdachloprid, Chlorothalonil+cymoxanil, Azoxystrobin, Pyraclostrobin+Metiram, and Mancozeb+Metalaxyl were tested under *in vitro* and field conditions. Among *Trichoderma* species, the best response was achieved by *T. harzianum* with 80.61% inhibition compared to control. Among concentrations of *T. harzianum*, the best response was achieved on 1/10 (1.24 cm) with 2.4 average No. of Sclerotia and 66% inhibition. Under filed condition experiments, the data regarding Disease severity in *T. harzianum* treated trays was 51.7% compared to untreated/control 73.5%. Besides disease control, the application of *T. harzianum* showed a significant increase in green and dry fodder weight (851 grams with 87 grams of dry weight) than untreated/control (561 grams with 55 grams of dry weight) in the fourth cut. For chemical evaluation, seven fungicides tested at three concentrations under *in vitro* trials among these Thiophanate methyl (0.5 cm) with 90.7% inhibition were found more effective. Thiophanate Methyl's application significantly reduced the disease severity compared to control plants with disease severity in fungicide-treated trays was 28.7% compared to untreated/control 73.5% and significant increase green and dry fodder weight (931 grams with 92 grams of dry weight) than untreated/control (561 grams with 55 grams of dry weight) in the fourth cut.

Corresponding Author: Imran Ul Haq
Email: Imran_1614@yahoo.com

INTRODUCTION

Livestock is an integral part of agriculture in Pakistan, contributing 60.6 percent to agriculture and 11.9 percent to the national GDP. The livestock's gross added value was 1,466 billion in 2019-20 (Economic survey of Pakistan, 2019-20). The growing livestock industry requires low-cost and nutritious fodder and feed, but on

the contrary, forage cultivation is decreasing (Burki *et al.*, 2005). *Trifolium alexandrinum* (Egyptian clover) is an annual, multicut winter fodder that belongs to order Fabales and family Febbacae. (Virender and Narwal, 2000; Amanullah *et al.*, 2005). It plays a vital role in the biodegradation of heavy metals such as lead (Pb), zinc (Zn), copper (Cu), and cadmium (Cd) present in soil (Ali *et al.*, 2012). It can be ensiled with cereals at low pH, high lactic acid, and a lower percentage of nitrogen (Mustafa and Seguin, 2003). The feeding of *E. clover* fodder supplemental concentrates to the lactating dairy animals may produce 10-15 litter milk per day (Naeem *et al.*, 2006). Egypt, India, Pakistan, Australia, Afghanistan, Southern Europe, the USA (California), Turkey, and South Africa are the significant *E. clover* cultivated countries (Knight, 1985). Pakistan ranks third with 0.71 Million hectares under *E. clover* cultivation after India (2 Million hectares) and Egypt (1.1 Million hectares) (Muhammad *et al.*, 2014). Various diseases report that attack and reduce the E. clover production. Amongst, Stem and crown rot disease caused by *Sclerotinia trifoliorum* is a widely spread and severely crop-damaging disease in Pakistan and other countries worldwide (Faraz and Ijaz, 2021; Faraz *et al.*, 2022). *S. sclerotiorum* is a fungal pathogen affecting about 500 host plants worldwide. *Sclerotinia* rot is a major hindering to oilseed brassica crop worldwide (Sharma *et al.*, 2016). The *Sclerotinia* spp. are reported to cause rots in a wide variety of host plants such as *Phaseolus vulgaris* L. is a bean plant affected by *S. sclerotiorum* (de Figueiredo *et al.,* 2010; Shaat *et al.*, 2011), *Brassica oleracea* L. (Cabbage) is a vegetable commonly cultivated worldwide affected by white mold disease caused by *S. sclerotiorum* (Lib.) de Bary (Elif *et al.*, 2016). *S. sclerotiorum* is a necrotrophic fungus causing diseases, including stem rot, head rot, white mold, and crown rot on different commercial crops (Kamal *et al.*, 2016).

S. sclerotiorum causes disease stem rot of soybean in central regions of USA (Mueller *et al.*, 2002). Once the pathogen is established, their management is challenging; integrating cultural, biological, chemical, and resistant cultivars approaches the best management strategy to control this disease (Sharma *et al.*, 2016). There are many reports on the management of *Sclerotinia* spp causing various diseases on various crops. As far as biological management is concerned, *Trichoderma* sp. had the potential to antagonize the *Sclerotinia* spp under *in-vitro* and *in vivo* conditions. (Figueirêdo *et al.*, 2010) However, these fungi' efficacy had limited consistency when applied to control sclerotinia stem rot incidence in canola fields as sustainable management (Kamal, 2016). *T. erinaceum*, *T. koningiopsis,* and *T. asperellum* are also reported as antagonists that entirely inhibit the mycelial growth of *S. sclerotiorum* (Boat *et al.*, 2018). Elif *et al.* (2016) proved different strains of *B. subtilis* (TV-17C, TV-6F, and TV-12H) as biocontrol agents against *Sclerotinia.* For chemical management, a scenario of fungicides has been tested to control *Sclerotinia* spp. such as Iprodione, Carbendazim, and Thiophanate Methyl showed promising results (Figueirêdo *et al.*, 2010; Lehner *et al.*, 2015). Fludioxonil had a positive effect on the morphology and physiology of *S. sclerotiorum*. (Duan *et al.*, 2013). Sumida *et al.* (2015) analyzed that procymidone inhibits the growth of *Sclerotinia*. This research study adopted biological and chemical control strategies to devise the appropriate Stem and crown rot disease control strategy.

MATERIALS AND METHODS

In vitro evaluation of antagonistic fungi against the *Sclerotinia trifoliorum*

Evaluation of different antagonistic fungi (Table 1) against confirmed fungal pathogen associated with stem and crown rot of E. clover was done using dual culture technique in *in vitro* conditions. Fungal cultures of antagonists and pathogenic were acquired from FMB-CC-UAF culture collection and revived on PDA culture medium. Seven to ten-day-old 5 mm diameter bits of both antagonistic and pathogenic fungi were placed on a fresh PDA plate opposite axenic conditions. A 5 mm bit of pathogenic fungi were placed on PDA containing Petri plate kept as a control for comparison. Culture plates were incubated in the incubator under 12 hours alternate light and dark periods. The diameter of fungal cultures was recorded daily to evaluate the inhibitory effect. Antagonism effect of *Trichoderma* spp. was evaluated by using the categories described by Bell *et al*., 1982 against *S. sclerotiorum,* and inhibition efficiency were also calculated by using the formula percent inhibition (Faraz *et al.*, 2020).

$$\text{Percentage Inhibition (P. I.)} = \frac{\text{C} - \text{T}}{\text{C}} \times 100$$

Where, C = Growth radius in control plate; T = Growth radius inhibited by antagonistic fungi in a dual culture plate.

Table 1. Fungal antagonistic Fungi acquired from FMB-CC-UAF culture collection used for evaluation against *S. trifoliorum.*

Sr.	Antagonistic Fungi	FMB-CC-UAF culture collection Number	Genbank accession Number
1	*Trichoderma harzianum*	FMB 0012	MG029259
2	*Trichoderma longibrachiatum*	FMB 0031	MF767445
3	*Trichoderma asperellum*	FMB 0032	MF767444

In vitro evaluation of culture filtrate of antagonistic fungi against the *S. trifoliorum*

Most effective antagonistic fungi *T. harzianum* in dual culture tests were used to evaluate their culture filtrate at different concentrations. The antagonistic fungus was grown on PDA culture medium at 28ºC for seven days to sporulate. Two milliliter Spore suspension (1×10^7 spores/mL concentration) of fungus was inoculated in 300mL potato dextrose broth (PDB) and incubated in a shaking incubator (I 4000, IRMECO, Germany) at 28 ℃ with 180 rpm. Culture filtrate was collected from culture medium after three days of incubation by initially filtered through gauze (8 layers) and a 0.22 µm pore size filter. Culture filtrate was further diluted into three different concentrations 1/10, 1/100, and 1/1000 dilutions of original filtrates in PDA culture medium and poured in Petri plates. PDA culture medium having no culture filtrates kept as a control for comparison. A 5mm disc of pathogenic fungi was placed in the Petri plate center and then incubated at 20±2 ℃ for four days. Readings of colony diameters in test plates and control plates were recorded and calculated using the formula described by Küçük *et al.* (2003) and Faraz *et al.* (2020). After ten days of incubation number of sclerotia were also recorded. The experiment was performed thrice, and five replicates of each treatment were used.

In vitro evaluation of different fungicides against the *Sclerotinia trifoliorum* associated with stem and crown rot of Egyptian clover

At different concentrations of 100 PPM, 150 PPM, and 200 PPM (Table 2), seven different fungicides were evaluated using poised food technique under i*n vitro* condition to found the most effective fungicide against the fungal pathogen of Stem and Crown rot of E. Clover. Each active ingredient of fungicides was prepared in sterile distilled water and incorporated in PDA medium and poured in sterilized Petri plates under axenic conditions. A 5 mm pathogenic fungal culture was placed in the center of fungicides containing PDA Petri plates, and a bit of culture was placed in the PDA plate without fungicide kept as a control for comparison. Plates were incubated at 18±2 ℃ under alternate light and dark periods. The fungal cultures' diameter was recorded daily to evaluate fungicides' inhibitory effect (Haq *et al.*, 2021). The inhibition efficiency of fungicides was calculated by using the formula described by Vincent (1947).

$$\text{Percentage Inhibition (P. I.)} = \frac{C - T}{C} \times 100$$

Where, C = Diameter of mycelial growth of pathogenic fungal culture in the plate without any fungicide; T = Dimeter of mycelial growth of pathogenic fungal culture in fungicides containing PDA Petri plate.

Table 2. Fungicides used against Stem and Crown rot of Egyptian Clover at different concentrations.

Sr.	Treatments	Active Ingredient	Trade Names	Dose
1	T1	Tebuconazole	Top Guard 30% SC	100 PPM, 150 PPM, and 200 PPM
2	T2	Tubeconazole+Emdachloprid	Hombra 37.25 FS	do
3	T3	Mancozeb+Metalaxyl	Mexal 72 % WP	do
4	T4	Chlorothalonil+cymoxanil	Cosmos 36 % WP	do
5	T5	Pyraclostrobin+Metiram	Cabriotop 60%WDG	do
6	T6	Azoxystrobin	Micoguard 25 % SC	do
7	T7	Thiophanate Methyl	Topsin-M 70 % WP	do

Evaluation of fungal antagonist (selected in i*n vitro* evaluation) against stem and crown rot of Egyptian clover under field conditions

The most effective fungal antagonist evaluated under *in-vitro* conditions was used to evaluate in the field conditions at their best concentration level. The most susceptible Agaiti cultivar of E. clover evaluated in the varietal response experiment in FMB research area

Department of plant pathology, UAF was used for evaluation. Fungal inoculum of *T. harzianum* (1×10^7 spores/mL concentration) with 1/10 dilution was prepared on PDB culture medium and mixed with artificial sick trays (one year post inoculation of *Trichoderma*) soil containing *S. trifoliorum E. clover* crop was established in trays, and stem inoculation was done in FMB research area Department of plant pathology, UAF. The crop was established without antagonistic fungi kept as a control for comparison. Disease severity was recorded according to key described by Dixon and Doodson (1974). Three replicates were used in experiments under complete randomized design (CRD) and repeated twice.

Evaluation of fungicide (selected under *in vitro* evaluation) against the stem and crown rot of Egyptian clover under field conditions

Most effective fungicides evaluated under *in vitro* conditions at their best concentration level were used to evaluate field conditions as seed treatment and spray. A most susceptible cultivar of Egyptian clover evaluated in the varietal response experiment in FMB research area Department of plant pathology, UAF was used for evaluation. Agaiti cultivar's seed was treated with Thiophanate Methyl at Dosage 200 PPM and sown in artificial sick trays soil containing *S. trifoliorum* FMB, research area Department of plant pathology, UAF. Aerial applications of Thiophanate Methyl were also made. Disease assessment was done using the key of Dixon and Doodson (1974) and Nagarajan *et al.* (1983). Three replicates were used in experiments under complete randomized design (CRD) and repeated twice.

The disease assessment key described by Dixon and Doodson (1974):

0 for Healthy,
1 for Slight symptoms,
2 for Moderate symptoms
3 for Severe symptoms

$$\text{Disease index} = \left[\frac{\{(1\times X)+(2\times Y)+(3\times Z)\}}{3\times N}\right]\times 100$$

Where, N is the total number of plants assessed, and X, Y, Z are numbered in each category

RESULTS

***In Vitro* Evaluation of Antagonistic Fungi (*Trichoderma* Species) Against *S. trifoliorum* Causing Stem and Crown Rot**

According to Bell *et al.* (1982) categorization *T. harzianum* and *T. longibrachiatum* in class 2 (Trichoderma grows and covers 2/3 of the medium surface) and *T. asperellum* in class 3 (*Trichoderma* and *Sclerotinia* colonize each one, half of the medium surface, and none seems to dominate the other). Table 3 shows that the colony growth of all the treatments significantly different from each other. Among *Trichoderma* species, the best response was achieved by *T. harzianum* (1.13 cm) with 80.61% inhibition, followed by *T. longibrachiatum* (1.17 cm) and *T. asperellum* (2.13 cm) with 69.63% and 63.46% inhibition in comparison to control (Figure 1). The colony growth in the control plate was 5.83 cm.

***In Vitro* Evaluation of Culture Filtrate of *T. harzianum* Against the *S. trifoliorum* Causing Stem and Crown Rot**

Table 4 shows that the mean mycelial growth of all concentrations was significantly different from each other. The best response was achieved among different concentrations on 1/10 (1.24 cm) with 2.4 average No. of Sclerotia and 66% inhibition followed by 1/100 (1.86 cm) and 4.6 average No Sclerotia 49% inhibition. 1/1000 concentration/dilution was least effective against *S. trifoliorum* with 2.16 cm mycelia growth, 8.4 average No. of Sclerotia, and 41% inhibition compared to control. The control plate's colony growth was 3.64 cm with 12.4 average No. of Sclerotia (Figure 2).

Table 3. Mean Mycelia growth of *S. trifoliorum* in the presence of antagonistic fungi *Trichoderma* species.

Codes	Treatments	Mean mycelia growth in cm
T1	*Trichoderma harzianum*	1.13 d
T2	*T. longibrachiatum*	1.77 c
T3	*T. asperellum*	2.13 b
T4	Control	5.83 a
	LSD value	0.108

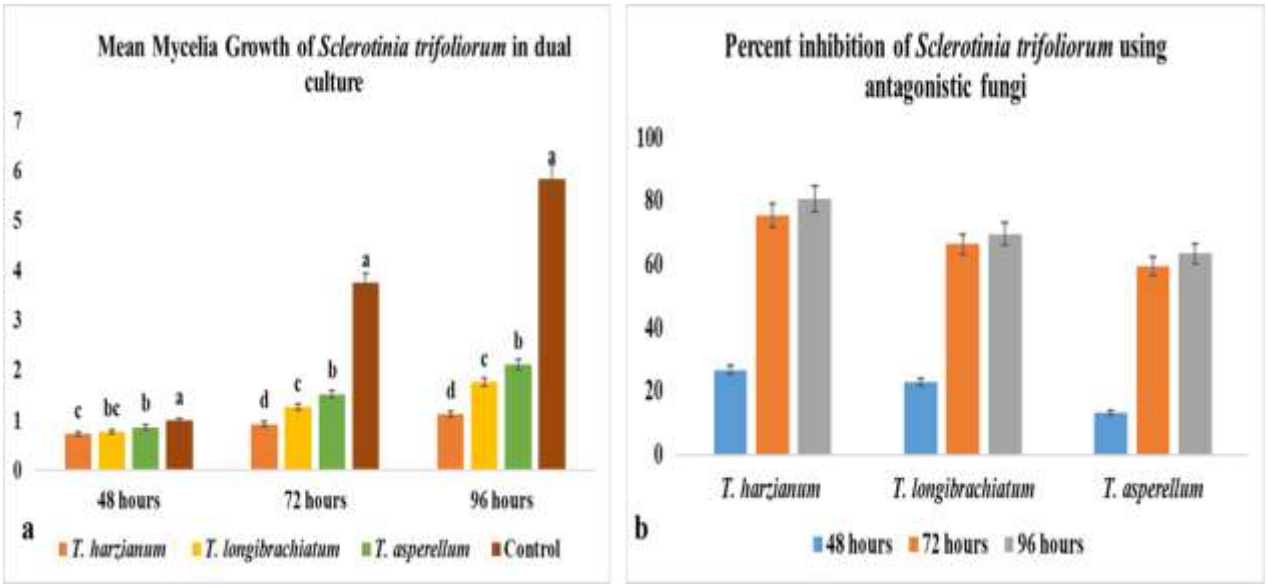

Figure 1. Mean Mycelia growth of *S. trifoliorum* in the presence of antagonistic fungi *Trichoderma* species at a different time interval (a) Efficacy of antagonistic fungi *Trichoderma* species against *S. trifoliorum* (b).

Table 4. Mean Mycelia growth of *S. trifoliorum* and average No. of Sclerotia at different concentration of culture filtrates of *T. harzianum.*

Dilutions of *T. harzianum*	Mean Mycelia Growth in cm	Average No. of Sclerotia
1/10	1.24 d	2.4 d
1/100	1.86 c	4.6 c
1/1000	2.16 b	8.4 a
Control	3.64 a	12.4 a
LSD Value	0.07	0.73

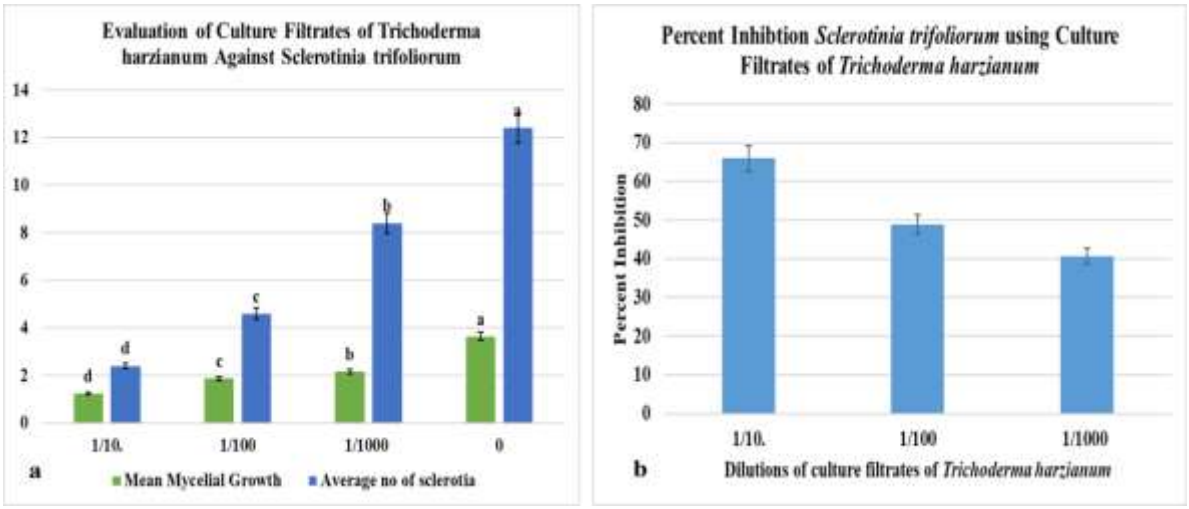

Figure 2. Mean Mycelia growth of *S. trifoliorum* and average No. of Sclerotia at different concentration of culture filtrates of *T. harzianum* (a) Efficacy of different concentrations/dilutions of *T. harzianum* against *S. trifoliorum* (b).

In Vitro* Evaluation of Different Fungicides Against *S. trifoliorum

The percent inhibition on different concentrations were significantly different from each other. The data presented in the table 5 shows among different fungicides, the best response was achieved by

Thiophanate Methyl (0.5 cm) with 90.7% inhibition followed by Tebuconazole (1.0 cm), Tubeconazole + Emdachloprid (1.3 cm), Chlorothalonil + cymoxanil (1.6 cm), Azoxystrobin (1.8 cm), Pyraclostrobin (2.0 cm) and Mancozeb + Metalaxyl (2.1 cm) with 83.3, 78.2, 72.4, 69.0, 66.4 and 63.2 percent inhibition respectively as compared to control (Figure 3). Mean mycelia growth in the control plate was 5.8 cm.

Table 5. Mean Mycelia growth of *S. trifoliorum* in the presence of fungicides.

Treatments	Mean Mycelial Growth (cm)
Tebuconazole	1.0 g
Tubeconazole+Emdachloprid	1.3 g
Mancozeb+Metalaxyl	2.1 b
Chlorothalonil+cymoxanil	1.6 e
Pyraclostrobin	2.0 c
Azoxystrobin	1.8 d
Thiophanate Methyl	0.5 h
Control	5.8 a
LSD Value	0.13

Evaluation of Fungal Antagonist (Selected in i*n Vitro* Evaluation) Against Stem and Crown Rot of Egyptian Clover Under Field Conditions

Egyptian clover seeds were treated with the most effective fungal antagonist *T. harzianum,* and its culture filtrates at 1/10 dilution mixed in soil artificially infested with *S. trifoliorum* in trays in which *E. clover* was grown. The disease severity index was recorded weekly. Green fodder weight and dry weight were also recorded after each cut of *E. clover*. Table 6 shows that the *T. harzianum* significantly reduced the disease severity as compared to control plants. Disease severity in *T. harzianum* treated trays was 51.7% as compared to untreated/control 73.5%. Table 7 shows green and dry fodder weight significantly increased by the application of *T. harzianum*. Green fodder weight of *T. harzianum* treated trays was 851 grams with 87 grams of dry weight than untreated/control 561 grams with 55 grams of dry weight in the 4th cut.

Evaluation of Fungicide (Selected in *in Vitro* Evaluation) Against Stem and Crown Rot of Egyptian Clover Under Field Condition

Table 8 shows that Thiophanate Methyl's application significantly reduced the disease severity compared to control plants. Disease severity in fungicide-treated trays was 28.7% as compared to untreated/control 73.5%. Table 9 shows green and dry fodder weight significantly increased by the application of Thiophanate Methyl. Green fodder weight of fungicide-treated trays was 931 grams with 92 grams of dry weight compared to untreated/control 561 grams with 55 grams of dry weight in the 4th cut.

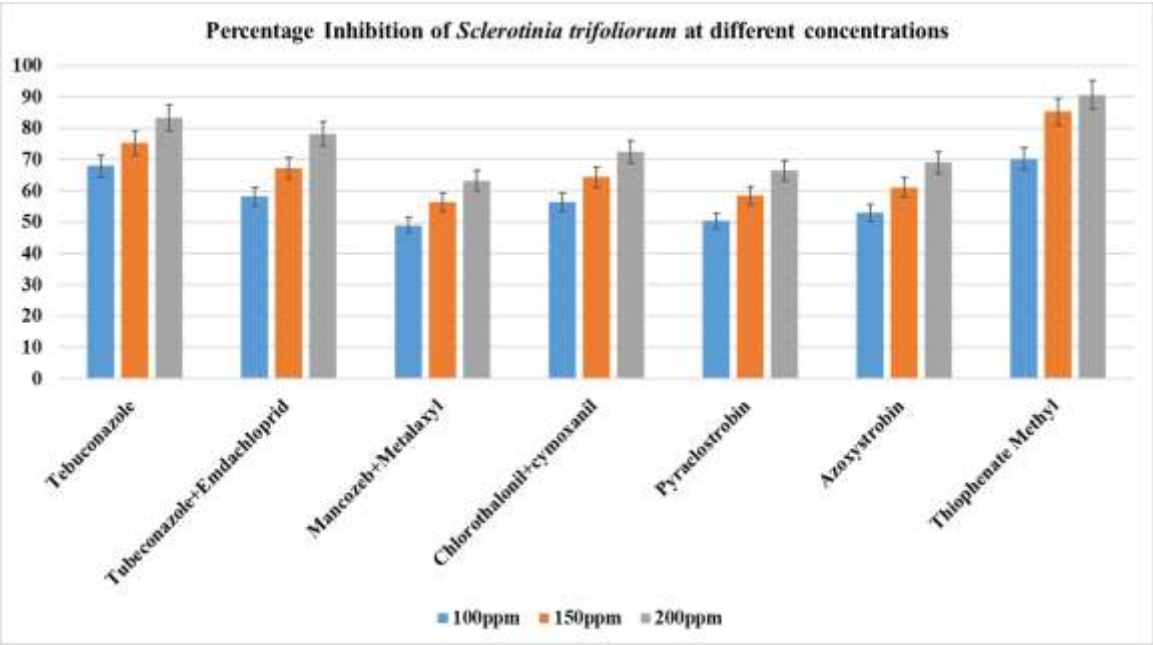

Figure 3. Efficacy of different fungicides at different concentrations against *S. trifoliorum.*

Table 6. Stem and Crown Disease severity index of Egyptian clover (*T. harzianum* over control).

Treatments	Disease severity index	
	T. harzianum	control
Week 1	3.1 p	6.1 p
Week 2	6.3 o	10.4 o
Week 3	9.3 n	15.1 n
Week 4	12.5 m	19.4 m
Week 5	16.1 l	24.1 l
Week 6	19.3 k	28.6 k
Week 7	20.3 j	33.3 j
Week 8	21.6 i	37.5 i
Week 9	28.9 h	42.4 h
Week 10	32.4 g	46.6 g
Week 11	35.6 f	51.3 f
Week 12	36.7 e	55.6 e
Week 13	38.7 d	60.4 d
Week 14	45.4 c	64.5 c
Week 15	48.4 b	69.2 b
Week 16	51.7 a	73.5 a

Table 7. Green and dry fodder weight of Egyptian clover (*T. harzianum* over control).

Treatment	Control		*T. Harzianum*	
	Fresh weight in gram	Dry weight in gram	Fresh weight in gram	Dry weight in gram
1st cut	610 c	63 c	810 d	75 d
2nd cut	964 a	95 a	1161 a	160 a
3rd cut	761 b	67 b	1051 b	135 b
4th cut	561 d	55 d	851 c	87 c
LSD value	0.97	0.81	1.4	1.16

Table 8. Stem and Crown Disease severity index of Egyptian clover (Thiophanate Methyl over control).

Treatments	Chemical	Control
Week 1	2.1 o	6.1 p
Week 2	3.8 n	10.4 o
Week 3	5.6 m	15.1 n
Week 4	7.4 l	19.4 m
Week 5	9.2 k	24.1 l
Week 6	11.2 j	28.6 k
Week 7	11.7 i	33.3 j
Week 8	12.6 h	37.5 i
Week 9	16.3 g	42.4 h
Week 10	18.3 f	46.6 g
Week 11	20.4 e	51.3 f
Week 12	20.6 de	55.6 e
Week 13	20.9 d	60.4 d
Week 14	25.3 c	64.5 c
Week 15	27.2 b	69.2 b
Week 16	28.7 a	73.5 a

Table 9. Green and dry fodder weight of Egyptian clover (Thiophanate Methyl over control).

Treatment	Control		Chemical	
	Fresh weight in gram	Dry weight in gram	Fresh weight in gram	Dry weight in gram
1st cut	610 c	63 c	991 c	102 c
2nd cut	964 a	95 a	1271 a	172 a
3rd cut	761 b	67 b	1131 b	161 b
4th cut	561 d	55 d	930 d	92 d
LSD value	0.97	0.81	1.4	1.12

DISCUSSION

Several studies have been conducted so far, controlling the Stem and crown rot disease on many crops caused by *Sclerotinia* spp. Pathologists have tested a variety of chemicals and alternative control methods such as biological control and cultural control. However, we could not find any reliable information regarding the control of Stem and crown rot in Egyptian clover, especially in Pakistan. The literature described that *S. sclerotiorum* causing basal stalk rot in beans was controlled by seed treatment with Topsin-M (Thiophanate Methyl) and rhizolex-T fungicides (El-Wakil and Ghonim, 2000; Helmy *et al.*, 2001; Mueller *et al.*, 2002; Shaat and El-Argawy, 2011). Use of fungicides and biocontrol agents (especially *Trichoderma* species) significantly inhibited the disease Sclerotinia stem rot of soybean caused by *S. sclerotiorum* (Sumida *et al.*, 2015), fluazinam inhibited 100% of the *S. sclerotiorum* followed by thiophanate-methyl (Costa and da Silva Costa, 2004), fluazinam and procymidone inhibited the *S. sclerotiorum* in common bean (Vieira *et al.*, 2012; Reis *et al.*, 2010), different fungicides have a different mechanism of action to inhibit the pathogen like procymidone retard the spore germination of *S. sclerotiorum* (Picinini and Goulart, 2002). Application time of fungicide is a critical factor that reduces the disease incidence and increases the yield (Mueller *et al.*, 2004). Thiophanate methyl, Fluazinam, and procymidone proved effective against the white mold of common beans caused by *S. sclerotiorum* (Lehner *et al.*, 2015). Fluazinam is a fungicide that could control the diseases caused by Sclerotinia species (Lemay *et al.*, 2002; Matheron and Porchas, 2004; Vieira *et al.*, 2012; Mahoney *et al.*, 2014). Thiophanate methyl, boscalid, and fluazinam are effective fungicides against *S. sclerotiorum* cause white mold in dry beans (McCreary *et al.*, 2016).

Mahoney *et al.* (2014) and Ramasubramaniam *et al.* (2008) also reported Thiophanate methyl effective fungicides controlling *S. sclerotiorum*. Similarly, we also found promising results in the disease's chemical control, as the other scientists reported. Thiophanate Methyl (0.5 cm) with 90.7% inhibition was found more effective among seven tested fungicides (Thiophanate Methyl, Tebuconazole, Tubeconazole+Emdachloprid, Chlorothalonil+cymoxanil, Azoxystrobin, Pyraclostrobin, Pyraclostrobin, and Mancozeb+Metalaxyl) at three concentrations under in vitro trials. Thiophanate Methyl's application significantly reduced the disease severity compared to control plants. Disease severity in fungicide-treated trays was 28.7% as compared to untreated/control 73.5%. Green and dry fodder weight significantly increased by the application of Thiophanate Methyl. Green fodder weight of fungicide-treated trays was 931 grams with 92 grams of dry weight compared to untreated/control 561 grams with 55 grams of dry weight in the 4th cut. For biological management trials, we tested three Trichoderma species, including *T. harzianum*, *T. longibrachiatum,* and *T. asperellum,* against *S. trifoliorum*; among these three, the best response was achieved by *T. harzianum* with 80.61% inhibition in comparison to control under *in vitro* conditions. Moreover, the culture filtrate of *T. harzianum* was proved highly effective at concentrations 1/10 with 2.4 average No. of Sclerotia and 66% inhibition compared to control. Under field conditions**,** *T. harzianum* and its culture filtrates at 1/10 dilution reduced the disease severity to 51.7% compared to untreated/control 73.5%. Green and dry fodder weight significantly increased by the application of *T. harzianum*. Green fodder weight of *T. harzianum* treated trays was 851 grams with 87 grams of dry weight than untreated/control 561 grams with 55 grams of dry weight in the 4th cut. Similarly, many plant scientists proved that Trichoderma species had more significance among different fungal antagonists due to their wide range against fungal plant pathogens, including *Fusarium, Pythium, Sclerotinia* species (Sarma *et al.*,

2014; Steindorff *et al.*, 2014; Woo *et al.*, 2014). *T. harzianum* had 56.3% inhibition efficiency in dual culture test against *S. sclerotiorum* cause stem rot in soybeans, and its culture filtrates inhibit the 51.2% (Muthukumar *et al.*, 2011; Zhang *et al.*, 2016). Tancic, 2013 reported that the *Trichoderma* species has the potential to antagonize the *S. sclerotiorum*. Seed treatment with *Trichoderma* species gave a significant increase in seed germination of soybean and plant height (Singh *et al.*, 2008; Joshi *et al.*, 2010; Mukhtar *et al.*, 2012). *T. hazianum* and *T. viridae* proved effective biocontrol agents for managing the *S. sclerotiorum* by antagonizing the mycelium or production of antibiotics. (Chet and Baker, 1981; Papavizas, 1985; Shaat and El-Argawy, 2011).

Conclusion

Sclerotinia trifoliorum causing Stem and crown rot disease (46% to 55% incidence) on *T. alexandrinum* L. in Pakistan. For management of this disease fungal antagonist and chemicals were evaluated under *in vitro* and field conditions. Among antagonistic fungi *T. harzianum* and Thiophanate Methyl's application among chemicals showed promising inhibition of disease.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.