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Antagonistic Potential of Native *Trichoderma* species against Tomato Fungal Pathogens in Yemen

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Abstract:
The present study was conducted to determine the antagonistic potential of native *Trichoderma* species against tomato fungal pathogens in Yemen. A total of 200 rhizosphere soil samples of different crops (sorghum, corn and potato) were collected from different provinces in Yemen (Ibb, Sana'a, Taiz, Amran, Thamar and Al-Hodaida) and screened for the presence of *Trichoderma* species isolates and their antifungal activity against four pathogenic fungi (*A. solani*, *F. oxysporum*, *P. ultimum* and *R. solani*) using dual culture technique. Thirteen *Trichoderma* species were identified among a total of 96 *Trichoderma* isolates. *T. harzianum* (33.33%) was the most predominant species occurring in the present soil samples. *Trichoderma* species showed the ability to grow in the moisture content of the soil ranging from 12.28% to 23.49% and pH values ranging from 7.16 to 7.88. Results showed that antagonistic potential of *Trichoderma* isolates varied significantly which inhibited the growth of the pathogen isolates at varying degrees. Among the isolates of *Trichoderma*, only 16 isolates showed strong antagonistic activity and inhibited four pathogenic fungi by more than 50%. These potential isolates of *Trichoderma* may be further exploited as biocontrol agent against soilborne pathogenic fungi.
Keywords: *Trichoderma*, biological control, tomato, Yemen.

INTRODUCTION

Tomato (*Lycopersicum esculentum* Mill) is one of the most important vegetable crops in Yemen (El Gouri *et al.,* 1996). In the year 2015, tomato is grown in an area of 9,582 ha with a production of fruit about 148,669 tons (ASYB, 2015). *Fusarium* wilt caused by *F. oxysporum* (Akrami and Yousefi, 2015), damping-off, stem rot and root canker caused by *Rhizoctonia solani* and *Pythium* spp. (Agrios, 1997; Gravel *et al.,* 2006) and early blight caused by *Alternaria solani* (Tewari and Vishunavat, 2012) is the major fungal disease posing a threat in tomato production (Akrami and Yousefi, 2015).

Nowadays fungicides have been used in agriculture to protect crops against the damaging losses caused by plant diseases (Leadbeater, 2015), however intensified use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans, pollution of the environment, can lead to the development of resistant strains of the pathogen with repeated use and altered the biological balance in the soil by over-killing the non-targeted microorganisms (Muthukumar *et al.,* 2008; Vinale *et al.,* 2008; Houssien *et al.,* 2010). The soil is a rich compendium of diverse organisms which have multifaceted roles in the ecological dynamics (Islam, 2018; Siyar *et al.,* 2019). The growing concern about the hazards involved relating to human health and environmental contamination has led to a demand for the development of alternatives to control plant diseases (Alsohiby *et al.,* 2016; Carmona-Hernandez *et al.,* 2019).

Biological control of plant diseases is a viable alternative method to manage plant diseases (Cook, 1993l; Iqbal and Ashraf, 2017; Hashmi *et al.,* 2018). *Trichoderma* strains have long been recognized as biological agents for the control of plant disease, for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, uptake and use of nutrients (Ranasingh *et al.,* 2006). *Trichoderma* species possess several control mechanisms to combat against phytopathogenic organisms. These biocontrol mechanisms include competition with plant pathogens, mycoparasitism, antibiosis, production of lytic enzymes and secretion of secondary metabolites (Vinale *et al.,* 2008). Many previous studies have proved the potential activity of *Trichoderma* species as biological agents antagonistic to large number of soilborne plant pathogens including *F. oxysporum*, *R. solani*, *P. aphanidermatium*, *F. culmorum*, *Gaeumannomyces graminis* var. tritici, *Sclerotium rolfsii*, *Phytophthora cactorum*, *Botrytis cinerea* and *Alternaria* species (Pan *et al.,* 2001; Jash and Pan, 2004; Harman, 2006).

This study aimed to isolate, identify native *Trichoderma* species and evaluate their antagonistic activity against four fungal pathogens on tomato.

MATERIALS AND METHODS

Collection of soil samples

A total of 200 soil samples were collected from different provinces in Yemen (Ibb, Sana'a, Taiz, Amran, Thamar, and Al-Hodaida), during 2013-2015. Soil samples were collected from rhizosphere soils of sorghum, corn, potato and tomato crops. The rhizosphere soil samples were collected by manually uprooting plants and shaking-off the adhering soil into sterile polythene bags and transferred to the laboratory (Sule and Oyeyiola, 2012).

Estimation of moisture content and pH of the soil samples

Soil moisture content (MC) was measured on the oven dry basis. The moisture content was calculated using the following formula provided by American Wood Preservation Association's Standards (AWPA, 1986).

$$\mathrm{MC}(\%) = \mathrm{W} - \mathrm{w}/\mathrm{W} \times 100$$

Where:

MC is moisture content

W is the initial weight

w is the constant weight after oven drying.

The pH of all soil samples was determined by mixing soil samples with distilled water at ratio 1:1 (w/v), mixed well and allowed to stand for 30 minutes. The pH of soil suspension was recorded using a pH meter (Intana, 2003).

Isolation and identification of *Trichoderma* species

Trichoderma species were isolated in *Trichoderma* selective media (Elad *et al.,* 1981) using dilution plate technique (Johnson *et al.,* 1959) incubated at 28 ± 1 °C for 1 week. Three replicates were prepared for each sample. The isolates were maintained on PDA slants at 4°C for further use (Kavitha and Nelson, 2013). *Trichoderma* isolates were identified according to the taxonomic key of (Rifai, 1969; Bissett, 1991; Samuels, 2004) by Assiut University, Mycological Center (AUMC), Egypt.

Antifungal activity of *Trichoderma* isolates against pathogenic fungi by dual culture method

Ninety-six *Trichoderma* isolates were tested for antifungal activity using dual culture method against four plant pathogenic fungi namely, *Fusarium oxysporum* (AUMC 208) and *Rhizoctonia solani* (AUMC 6594) collected from Microbiological Resources Center, Faculty of Agriculture, Assiut University, Assiut, Egypt. *Alternaria solani* (EMCC 756) and *Pythium ultimum* (DSM 62987) were collected from Mycological Center, Faculty of Science, Al-Azhar University, Cairo, Egypt.

Plates of PDA were inoculated with 0.5 cm disc obtained from the five-day-old culture of the plant pathogen fungi 10 mm from the edge of the plate. After two days, 0.5 cm disc of the *Trichoderma* isolates (5 days) was placed on the opposite side from the pathogen fungi disc. *Pythium ultimum* and the *Trichoderma* isolates were inoculated at the same time (Siameto *et al.,* 2011).

In the control plate, 0.5 cm disc of pathogenic fungi was placed on the edge of the plate 10 mm from the periphery. Inoculated plates were incubated at 28 ± 1 ^{o}C for 5-7 days. The experiment was carried out thrice to ensure repeatability. After incubation, the radial growth of pathogen was recorded in control and in the presence of antagonists and percentage inhibition calculated in relation to control according to the formula of Hajieghrari et al. (2008).

$$L = \{(C - T)/C \times 100$$

Where:

L= inhibition of radial mycelial growth.

C= radial growth measurement of the pathogen in control.

T= radial growth measurement of the pathogen in the presence of antagonists.

The degree of antagonism between each *Trichoderma* and test pathogen in dual culture was scored on a scale of 1-5 as proposed by Bell et al. (1982).

1. Antagonist completely overgrew the pathogen and covered the entire PDA medium surface.
2. Antagonist overgrew at least two third of the PDA medium surface.
3. Antagonist and the pathogen each colonized one half of the PDA medium surface (more than one third and less than two third) and neither organism appeared to dominate each other.
4. The pathogen colonized at least two third of the medium surface and appeared to withstand encroachment.

5. The pathogen completely overgrew the antagonist and occupied the entire medium surface.

RESULTS AND DISCUSSION

In the present study, the moisture content of 200 soil samples ranged from 2.04 to 25.44%. *Trichoderma* spp. showed the ability to grow in moisture content ranged from 12.28% to 23.49%. Similar results were obtained by Khang et al. (2013), who found that *Trichoderma* spp. lived in various humid conditions ranging from 18.85 - 51.65%. High moisture content might be helpful to increase the population of *Trichoderma*. Populations of *Trichoderma* spp. have been reported to be greater in moist soils or even in soils with excessive moisture as compared to dry soils (Danielson and Devey, 1973).

The pH of all soil samples in this investigation ranged from 6.13 to 8.48. *Trichoderma* spp. showed the ability to grow in pH values ranged from 7.16 to 7.88. *Trichoderma* spp. showed the ability to grow in weak alkaline soils. Lo et al. (1996) found that *Trichoderma* spp. had equal ability to colonize roots in both alkaline and acidic soils. Intana (2003) also reported that 165 isolates of *Trichoderma* spp. from 148 soil samples could grow in differing pH of soil 5.4 - 7.2. Whereas Gherbawy et al. (2004) found very low *Trichoderma* biodiversity in agricultural soils of the Nile valley in Egypt, which contained only *T. harzianum* and the anamorph of *H. orientalis.* The alkalinity of the soils (pH 7.3-7.4) may influence the low degree of diversity. This ability of *Trichoderma* species to live in a wide range of pH soils can be explained by the fact that they can adjust to surrounding environmental conditions by regulating metabolism, growth, and sporulation (Eziashi, 2007).

It was possible to obtain 96 isolates of *Trichoderma* during this investigation, from which 49 isolates from corn, 45 isolates from sorghum and 2 isolates from potato. The highest number of *Trichoderma* were obtained from Ibb (83 isolates) followed by Sana'a (8), Taiz (3) and Thamar provinces (2) (Figure 1). This is due to the Ibb is known as "the fertile province", one of the wettest areas of Yemen, moderate climate and temperatures are warm (Alsanoy and Abdulwadood, 2014).

It was worth mentioning that not even a single *Trichoderma* isolate was obtained from tomato soil (Table 1). Similar results with Jiang et al. (2016), found that the composition and proportion of *Trichoderma* species and the dominant species were distinct between different regions and cropping types. In contrast, Körmöczi et al. (2013) found that the number of isolated strains was the highest in the case of the tomato rhizosphere sample derived from Szeged-Szikszós, Hungary (15 isolates from 2 species). Kale et al. (2018) isolated 16 *Trichoderma* isolates from rhizosphere soil of tomato crop of eight (8) districts of Marathwada region, India. Ranga et al. (2017) isolated five *Trichoderma* isolates from soils collected from groundnut rhizosphere region, two from redgram and two from tomato. This result may be attributed to differences in region, topography climate, season, soil type or plant type (Garbeva *et al.,* 2004; Jiang *et al.,* 2016).

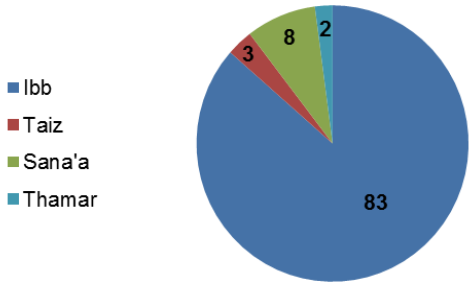

Fig. 1. The occurrence (%age) of *Trichoderma* isolates in different provinces in Yemen.

Table 1. Number of *Trichoderma* isolates obtained from 200 soil samples collected from different localities in Yemen during (2013-2015).

Crop	No. of soil samples analyzed	No. of *Trichoderma* isolates (%)
Corn	70	49 (51.04)
Sorghum	65	45 (46.88)
Potato	45	2 (2.08)
Tomato	20	0
Total	200	96

A total of 96 *Trichoderma* isolates belonging to 13 species were isolated and identified. *T. harzianum* (33.33 %) was the most predominant species occurring in tested soil samples during this investigation followed by *Trichoderma* sp. anamorph of *H. vinosa T. citrinoviride* (8.33 %) each, *T. longibrachiatum*, *T. reesei*, *T. koningii*, *T. inhamatum* each of them representing (7.29%), *T. atroviride* (6.25%), *T. viride* (5.20%), *T. pseudokoningii* (4.16%), *T. aureoviride*, *T. parceramosum*, (2.08%) each, and *Trichoderma* sp. anamorph of *H. semiorbis* (1.04%) (Table 2). This result agrees with other researchers in different parts of the world (Naeimi *et al.,* 2011; Rahman *et al.,* 2011; Sun et al., 2012; Körmöczi *et al.,* 2013; Digambar, 2017; Redda *et al.,* 2018) which listed *T. harzianum* as a predominant species. In contrast, Reddy et al. (2014) found that *T. viride* was the most predominant species occurring in the soil samples followed by *T. harzianum*, *T. virens, T. pseudokoningii*, *T. reesei*, *T. koningii*, and *T. atroviride. T. harzianum* has a worldwide distribution and is often the dominant species in other environments, such as Egypt (Gherbawy *et al.,* 2004) Asia (Kubicek *et al.,* 2003) and Europe (Błaszczyk *et al.,* 2011).

Table 2. *Trichoderma* species isolated from different rhizosphere soil samples.

Trichoderma species	No. of isolates	%age of isolates
T. harzianum	32	33.33
Trichoderma sp. anamorph of *H. vinosa*	8	8.33
T. citrinoviride	8	8.33
T. longibrachiatum	7	7.29
T. reesei	7	7.29
T. koningii	7	7.29
T. inhamatum	7	7.29
T. atroviride	6	6.25
T. viride	5	5.20
T. pseudokoningii	4	4.16
T. aureoviride	2	2.08
T. parceramosum	2	2.08
Trichoderma sp. anamorph of *H. semiorbis*	1	1.04
Total isolates	96	

Results of this study showed that the majority of *Trichoderma* isolates grew faster than plant pathogenic fungi. The interaction of *Trichoderma* isolates and plant pathogenic fungi showed significant differences in growth inhibition of the pathogen isolates. Sixteen (16) out of the 96 isolates tested were able to inhibit the mycelial growth of four plant pathogens each by more than 50%. Six and twenty-six isolates showed significant inhibition of mycelial growth (above 70.0%) over the control against *F. oxysporum* and *P. ultimum*, respectively. Also,

11 and 21 of *Trichoderma* isolates showed significant inhibition of mycelial growth (above 60.0%) of *A. solani* and *R. solani*, respectively. Similar results were obtained by Rai (2017), showed that all 20 isolates of *Trichoderma* inhibited mycelial growth of fungal phytopathogens (*F. oxysporum, A. alternate*, *Colletotrichum gleosporoides,* and *R. solani*) by more than 50%.

In this investigation, all *Trichoderma* isolates showed antagonistic towards fungal pathogens. Most of them showed a degree of antagonists up to two-third of the medium surface and were placed in class-2 according to Bell's scale against *F. oxysporum*, *P. ultimum*, *A. solani,* and *R. solani* by 73.96%, 69.80%, 68.75%, and 39.58%, respectively (Table 3). The degree of effectiveness varies according to the nature, quality and quantity of antibiotics (Kubicek *et al.,* 2001; Singh, 2006; Woo *et al.,* 2006). Antagonist potential of *Trichoderma* species against different fungal phytopathogens has been reported by several researchers (El-Katatny *et al.,* 2001; Marco *et al.,* 2003; Sanjay *et al.,* 2008; Indira and Kamala, 2011; Bastakoti *et al.,* 2017). Inhibition growth of the pathogens which may be due to fungistatic effect (Cook and Baker, 1983), secretion of antibiotics or other inhibitory substances such as viridin, gliovirin, geodin, terricin, terric acid harzianic acid, alamethicins, tricholin, peptaibols, 6-penthyl-α-pyrone and dermadin, etc. (Howel, 1998; Mondal *et al.,* 2000; Vey *et al.,* 2001; Landreau *et al.,* 2002; Yan *et al.,* 2006).

Table 3. Antagonistic effect of Trichoderma spp. against plant pathogenic fungi using Bell et al. scale

Bell's scale	*F. oxysporum*		*A. solani*		*P. ultimum*		*R. solani*	
	No. of isolates	Percent (%)	No. of isolates	Percent (%)	No. of isolates	Percent (%)	No. of isolates	Percent (%)
1	3	3.13	28	29.17	18	18.75	34	35.42
2	71	73.96	66	68.75	67	69.80	38	39.58
3	22	22.92	2	2.08	7	7.29	12	12.5
4	-	-	-	-	-	-	12	12.5
5	-	-	-	-	-	-	-	-

CONCLUSION

Ninety-six isolates of *Trichoderma* collected from a different province in Yemen and 13 *Trichoderma* species were recovered from rhizosphere soil of corn, sorghum, and potato. Most of *Trichoderma* isolates were active against four tested pathogenic fungi *in vitro* with variable antagonistic activity.

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CONFLICT OF INTEREST

All the authors have declared that no conflict of interest exists.

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