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Original Research Article

Antifungal Efficiency of Biological Control Agents against Phytopathogenic Fungi

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ABSTRACT

In the present study, six different biological control agents were used against phytopathogenic fungi to determine the antifungal activities. The aim of this study was to assess the *in vitro* activity of *Trichoderma harzianum, T. harzianum-T. viride, Aureobasidium pullulans, Lactobacillus acidophilus* and *Bacillus subtilis*. These bio control agents were used against phytopathogenic fungi such as *Fusarium culmorum, F. oxysporum, Sclerotinia sclerotiorum, Rhizoctonia solani* and *Macrophomina phaseolina*, respectively. *In vitro* antagonism screening was carried out to test six antagonists against phytopathogenic fungi. Assessment of phytopathogenic fungi mycelial growth was done in 7 days at 25ºC after its placement on the culture medium. The all antagonists were shown high inhibition on fungi mycelial growth. The most effective antagonist against fungi was *T. harzianum* and efficiency rate were continued with *Bacillus subtilis*, *Aureobasidium pullulans* and *Lactobacillus acidophilus*, respectively. Subsequently, in the second phase of *in vitro*, Captan, Maneb and Thiram were used to determine efficiency on fungi's culture and survivability of the biocontrol agents. Fungicide dose was selected in label for each one and was used half, recommended and double concentrations. The most resistant plant pathogens against fungicides were *M. phaseolina* and *F. oxysporum*, on the other hand, most sensitive were *R. solani* and *S. sclerotiorum* when compare to all.

Keywords: Biological control, Phytopathogenic fungi, Efficiency, Inhibition, Antagonism

INTRODUCTION

Plant pathogens, are major treats for plants that causing economic losses on the products (Siddiqui *et al.* 2002). Chemical management is one of the ways to ensure food and products quantitatively and qualitatively and also most preferred one. Furthermore, synthetic fungicides are extensively used in agriculture to control or prevent plant diseases (Dayan *et al.* 2009). Thus, the pesticide resistant problem has been increasing day by day (Dekker 1976, Brent and Hollomon 1998). But, still uses in pest management. Cause applicants have only a few options to synthetic. However, soil borne pathogen management is much more difficult when compared to above ground pathogens (Yangui *et al.* 2008). Fungicides have limited effect on soil borne plant pathogens for some reasons, **1)** limited contact efficiency in soil, **2)** can't reach target organism properly, **3)** fungicides compose more quickly in the soil and finally **4)** developing resistant problem (Papavizas and Lumsden 1980). For mentioned reasons, many research and study have done about biological agents against soil borne pathogens to achieve more reliably management (Dayan *et al.* 2009). Biological management is one of the alternatives to using synthetic fungicides. There are too many commercial biological agent products on the market and some of them are; *Trichoderma harzianum*, *T. viride*, *Aureobasidium pullulans*, *Lactobacillus acidophilus* and *Bacillus subtilis*. On the other hand, most common soil borne pathogens are; *F. oxysporum*, *F culmorum*, *R. solani*, *S. sclerotiorum* and *M. phaseolina* (Agrios 1988). This study was an attempt to examine antagonists-soil borne pathogen interaction and determine the antagonists' efficiency rate and survivability within fungicide usage areas.

MATERIALS AND METHODS

Fungi isolate

The fungi cultures were isolated from infected plants in Bursa. Fungi were identified and storage suitable condition in potato dextrose agar (PDA) media. The fungi isolates were; *Fusarium culmorum* (Sacc.), *Fusarium oxysporum* (Schlecht. emend. Snyder & Hansen), *Macrophomina phaseolina* (Goid.), *Rhizoctonia solani* (J.G. Kühn) and *Sclerotinia sclerotiorum* (de Bary), respectively.

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Biological Control Agents

All bio control agents were selected from inside commercial bio fungicide products pool. *Aureobasidium pullulans* (G. Arnaud), *Bacillus subtilis* (Cohn), *Lactobacillus acidophilus* (Hansen & Mocquot), *Trichoderma harzianum* (Rifai) and *Trichoderma viride* (Pers.) were chosen. Antagonists were selected to determine the efficiency of the products and their mycelial inhibition rates. Antagonists used in experiments were given in Table 1.

Table 1. The antagonists and commercial products.

Antagonists	Commercial Product
Aureobasidium pullulans	Botector
Bacillus subtilis QST 713	Serenade
Bacillus subtilis Y 1336	Biobac WP
Lactobacillus acidophilus	ISR-2000
Trichoderma harzianum	T-22 WP
T. harzianum-T. viride	Root guard

Fungicides

Captan, Maneb and Thiram were selected to determine and examine the effect of the fungicides on soil borne pathogens. Moreover, this study planned to evaluate fungicide-antagonist interactions. For these purposes, fungicides were selected in widely uses ones and were given in Table 2 with their commercial products.

Table 2. The fungicide and commercial products.

Fungicide	Commercial Product
Captan	Koruma, Koruma Captan 50 WP
Maneb	Syngenta, Dithane M-22, 80 WP
Thiram	Bayer, Pomarsol Forte, 80 WP

Fungi and Antagonist Cultures

In *in vitro* experiment, both pathogenic and biological control agents were cultured in PDA media for 7 days at 25^{o}C. After an incubation period 5 mm disc (plate) was obtained via cork borer from each media culture. Biological control agents' (antagonists) discs were placed center of the petri dishes. Plant pathogenic fungi's discs were placed around it (Hjelm *et al.* 2004). Afterwards, every petri dish was placed in an incubator for the incubation period for 3-6 days at 25^{o}C. On a daily basis, all petri dishes were observed. After the incubation period, all phytopathogenic fungi's radials and mycelial growth were measured. Inhibition zones (mm) were determined by measuring the distance from mycelia to the center of the disc (Adesina *et al.* 2007, Kavroulakis *et al.* 2010, Cuesta *et al.* 2012). Antagonist free plates were used as a control group. Antagonist-pathogen interactions were observed on petri dishes.

Fungicide Application Concentrations

Fungicides concentrations were determined regarding by their label (recommended) doses. The concentrations were used in experiments, has shown in Table 3 below.

Table 3. Fungicide and application concentrations.

	Concentrations (g/100 L)		
Fungicide	**Half**	**Label (Recommended)**	**Double Dose**
Captan	200	400	800
Maneb	200	400	800
Thiram	300	600	1200

Adjusting Fungicide Doses

In *in vitro* experiments three fungicides were used to determine the efficiency not only soil borne pathogens but also on the antagonists. For this purpose all fungicides' half, recommended and double of recommended

concentrations were used. For Captan and Maneb, 0.2 g, 0.4 g and 0.8 g, on the other hand, for Thiram, 0.3, 0.6 and 1.2 g were scaled. To mix granule of fungicides to media, ten unit 100 ml PDA were autoclaved. After autoclaved PDA solutions were cooled to 35^{o}C. Scaled doses of fungicides were added to PDA media and shake properly to mix well and prevent sedimentation (Delen and Tezcan 1986). This step was repeated nine times and last unit PDA (no fungicide inside) was used for control.

Statistical Analysis

One way ANOVA was performed to incidence data, and mean values were separated by using LSD test ($P \leq 0.05$). At tables, values in columns followed by the same letter are not significantly different according to LSD test ($P \leq 0.05$). Experiments were conducted three times.

RESULTS

Antagonistic Activity

Almost all the antagonists were shown inhibition zone (mm) on soil borne pathogens. Soil borne pathogens mycelial growth prevention was highly variable (Figure 1). Surprisingly, *T. harzianum* and *T. viride* combination was not effective against long odds (Table 4). Moreover, combination (*T. harzianum-T. viride*) has also shown no effect on *R. solani*, *F. culmorum* and *S. sclerotiorum*.

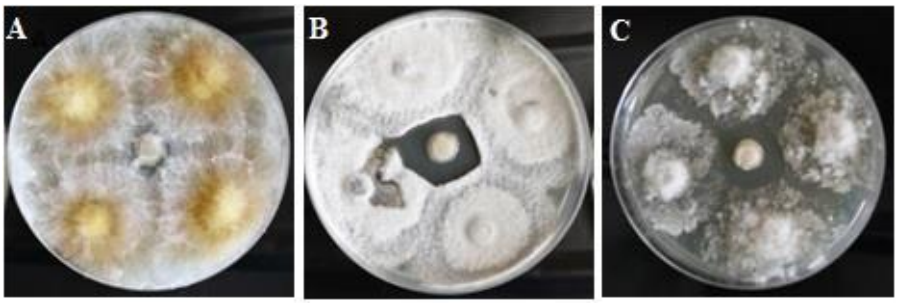

Figure 1. *L. acidophilus* inhibition zone (mm) against soil borne pathogens **A)** *R. solani*, **B)** *S. sclerotiorum*, **C)** *M. phaseolina*.

On the other hand, *Trichoderma harzianum* alone application was the most dominant (Figure 2) and significantly inhibited all the soil borne pathogens mycelial growth when compared with control (Table 4).

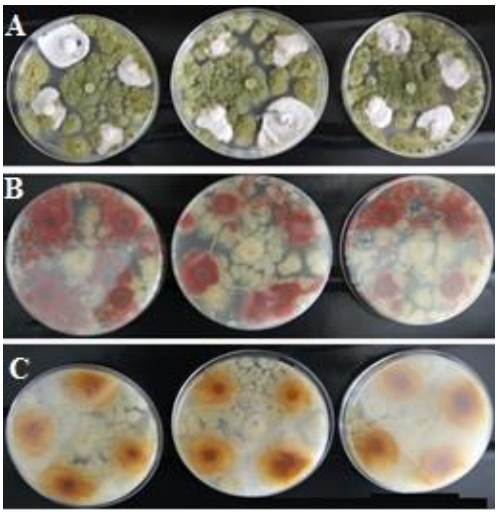

Figure 2. *Trichoderma harzianum* mycelial growth inhibition efficiency on soil borne pathogens **A)** *M. phaseolina*, **B)** *F. oxysporum*, **C)** *F. culmorum*

A. pullulans, *B. subtilis* QST 713, *B. subtilis* Y1336, and *L. acidophilus* has shown minor inhibition on soil borne pathogens. Within pathogenic fungi, the most resistant one was *R. solani* afterwards *F. culmorum*, respectively. Otherwise, *F. oxysporum* and *M. phaseolina* were most sensitive one. *T. harzianum* was point highest inhibition zone (mm) on *M. phaseolina* (15.66) and *F. oxysporum* (15.00), respectively (Table 4).

Table 4. The Effect of biological control agents against soil borne pathogens.

	Antagonist-Pathogen Interaction Inhibition Zone Mean (mm)				
Antagonist	***F. culmorum***	***F. oxysporum***	***M. phaseolina***	***R. solani***	***S. sclerotiorum***
Control	0 c	0 e	0 d	0 c	0 c
A. pullulans	0,25 c	4,41 cd	4,66 b	3,33 a	3,66 b
B. subtilis QST 713	1,33 b	4,66 c	3,03 c	1,58 b	3,50 b
B. subtilis Y1336	0 c	4,83 c	3,33 c	0 c	2,75 b
L. acidophilus	1,33 b	3,83 d	3,66 c	0 c	6,91 a
T. harzianum-T. viride	0 c	6,66 b	3,66 c	0 c	0 c
T. harzianum	10,50 a	15,00 a	15,66 a	4,00 a	9,00 a

Values in columns followed by the same letter are not significantly different according to LSD test (P≤0. 05).

Fungicide Activity

All concentrations of fungicide were shown highly effect on *R. solani* and *S. sclerotiorum* (Table 5). Captan, Maneb and Thiram were inhibited mycelial growth of *R. solani* and *S. sclerotiorum* totally, in all used concentrations. However, to inhibit *M. phaseolina*, *F. culmorum* and *F. oxysporum* mycelial growth was needed higher concentrations of the fungicide. Captan was shown highly effect on *F. oxysporum* and totally prevents mycelial growth (Figure 3).

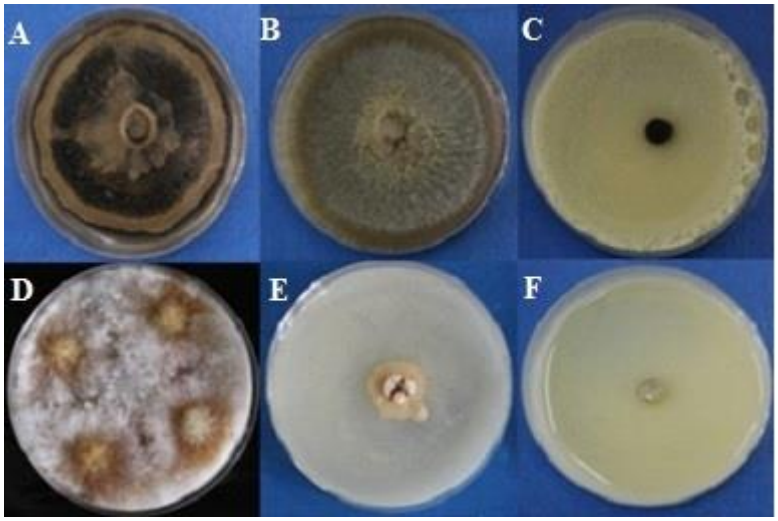

Figure 3. Effect of the Captan and Maneb on fungi mycelial growth; **A)** *M. phaseolina*; control, **B)** *M. phaseolina*; Maneb 400 g/100 L, **C)** *M. phaseolina*; Maneb 800 g/100 L, **D)** *F. oxysporum*; control, **E)** *F. oxysporum*; Captan 400 g/100 L, **F)** *F. oxysporum*; Captan 800 g/100 L.

On the other hand, only highest concentrations of Captan could stop mycelial growth of *F. culmorum*. Inhibition was not observed on none of the Maneb's concentrations against *F. culmorum*. Besides, only the highest concentration of Maneb was effective on *M. phaseolina* (Table 5). Similarly, slightly inhibition was detected on *F. oxysporum* at Maneb applications. The label dose of Maneb was shown minor inactivation on *F. oxysporum* as statistically.

Table 5. The effect of the fungicides concentrations on fungi mycelial growth.

Mycelial Growth (mm)					
	Soil borne Plant Pathogens				
Application (g/100 L)	***R. solani***	***S. sclerotiorum***	***M. phaseolina***	***F. culmorum***	***F. oxysporum***
Control	54,25	52,75	55 a	52 a	43,5 a
Captan					
200	0	0	53,75 a	13,25 b	0 g
400	0	0	36,75 b	8,5 c	0 g
800	0	0	22 c	0 d	0 g
Maneb					
200	0	0	55 a	51,75 a	36 c
400	0	0	53 a	52,25 a	32,5 b
800	0	0	0 d	50,25 a	14,25 d
Thiram					
300	0	0	0 d	0 d	11,25 e
600	0	0	0 d	0 d	7,25 f
1200	0	0	0 d	0 d	6,25 f

Values in columns followed by the same letter are not significantly different according to LSD test (P≤0. 05).

Fungicide-Antagonist Interaction

Antagonist and fungicide interaction was determined. The purpose of this part was determined antagonist survivability within fungicide. But only most successful antagonist (*T. harzianum*) was selected. Others antagonists' inhibition efficiency was not enough for proper biological control and also shown inadequate efficiency on laboratory tests. Hereby, *T. harzianum*, development was tested in PDA media that fungicide in it. The results were respectable when compared with control group (Table 6). When examined Captan-*T. harzianum* interaction antagonist mycelial development was not observed, and Captan inhibited the mycelial growth of *T. harzianum* totally with only half (½) of the label dose. Correlatively, Thiram was shown inhibition on *T. harzianum* mycelial growth. Furthermore, using Thiram was delayed the sporulation and also spore germination. However, Maneb has shown minimal inhibition effect with its label dose and it was a promising result for usage of the antagonist together with pesticide (Table 6).

Table 6. The effect of the fungicides on *T. harzianum* development.

Mycelial Growth (mm)	
	Antagonist
Application (g/100 L)	***T. harzianum***
Control	50 a
Captan	
200	0 f
400	0 f
800	0 f
Maneb	
200	50 a
400	47,75 b
800	47,5 b
Thiram	
300	19,5 c
600	16,25 d
1200	11,75 e

Values in columns followed by the same letter are not significantly different according to LSD test (P≤0. 05).

DISCUSSION

Trichoderma, *Aureobasidium* and *Bacillus* have shown good antagonistic effect in laboratory conditions (Pane *et al.* 2012). However, *Trichoderma* was certainly most effective biological control agents (Bailey *et al.* 2008) when compared others and also has a broad host range (Harman *et al.* 2004) than others. And mostly was studied antagonist in all time (Harman 2000, Howell 2003, Harman *et al.* 2004). *Trichoderma* inhibition efficiency has been successfully on both *Fusarium* and *Sclerotinia* (Gonzalez-Cardenas *et al.* 2005, Avila-Miranda et al 2006). The effect of the *Trichoderma* could come from its multi effect mechanism. It has competition, antibiosis, tropism to the host and most importantly mycoparasitism (Brozova 2004, Ibarra-Medina *et al.* 2010). On the other hand, *A. pullulans*, *B. subtilis* and *L. acidophilus* primarily have competition mechanism. The competition mechanism has a limits, it needs deficiency of nutrient, host, iron, spot etc. However, in laboratory condition there is no deficient of nutrient, minerals or other essential needs and no competition for them in media culture. Furthermore, the competition was decreasing or not working properly in it and finally, their antagonism shown no or minor effect on pathogens. On the other side, the most effective fungicides among used one was Thiram. Captan and Maneb also has shown good inhibition rate on soil borne pathogens mycelial growth. Yet, especially Captan inhibits *T. harzianum* development totally and their combination could not be used together. However, Thiram and Maneb could be combined with *T. harzianum* for efficient control methods. In conclusion, *T. harzianum*, Thiram or Maneb combination could be used in conventional agriculture system to prevent pesticide resistant and get more efficient management. Further studies will be about using antagonist and fungicide in combination with Maneb or Thiram to achieve more effective results to prevent disease development and will continue on pot and field trials.

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