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Full Length Research Paper

In vitro antagonistic activity evaluation of some selected fungi isolated from burned soils in Mila region (East of Algeria)

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The present study was initiated to (i) determine burned forest-inhabiting fungi in Zouagha, TerriBeinène, Mila and (ii) study the antagonistic activities of *Trichoderma* sp against *Fusarium* sp, *Penicillium* sp, *Rhizoctonia* sp, and *Alternaria* sp. Eighteen fungal strains representing six genera were isolated from soil samples obtained from the burned forest of Zouagha in the Mila region: *Trichoderma* sp, *Fusarium* sp, *Penicillium* sp, *Rhizoctonia* sp, *Alternaria* sp, and *Rhizopus sp.* The direct antagonistic activity assays of *Trichoderma sp* on Potato Dextrose Agar medium (PDA) against the four fungi: *Fusarium* sp, *Penicillium* sp, *Rhizoctonia* sp, and *Alternaria* sp revealed that the fungus *Trichoderma* sp reduced the mycelium growth of *Fusarium* sp, *Penicillium* sp, *Rhizoctonia* sp and *Alternaria* sp to 23.13, 33.13, 33.75, and 38.31%, respectively, compared to the control after six days at room temperature. The results illustrated an inhibitory action of the antagonist *Trichoderma sp* characterized by slowing the mycelial growth of fungal strains. Strains of *Fusarium* sp, *Penicillium* sp, *Rhizoctonia* sp and *Alternaria* sp showed differences in the sensitivity to the antagonist. Because *Trichoderma* occurred more frequently in burned soils and were more antagonistic to phytopathogenic fungi in culture than isolates from unburned soils, the judicious use of fire may increase the abundance of *Trichoderma* isolates and their inhibitory action may be used for the control of fungal plant diseases.

Key words: Fungi, burned soil, Zouagha, Antagonism, *Trichoderma* sp.

INTRODUCTION

Forest ecosystems of cork and oak often present a balance of extreme complexity and their burning generate a cascade of degradations, which spread over many years and sometimes prove to be irreversible. Certainly, the trees which are weakened after a wildfire, present the ideal conditions for massive colonization by various species of fungi. Some of these are phytopathogenic fungi (Belhoucine and Bouhraoua, 2013). Some microorganisms are considered to be more sensitive to heat than others, for example fungi compared to bacteria

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Figure 1. The canton of BeniAfak (forest Zouagha, Mila) [source: Google Earth].

(Gema et al., 2011).The forest fire is one of the most widespread disruption and potentially most destructive affecting fungi that inhabit the soil (Lygis et al., 2010). The genus *Trichoderma* includes a set of saprophytic imperfect fungi which are commonly found in soil, dead wood, plant debris and aerial parts of the plants. They are easily recognized in the culture through the generally greenish color of their spores and their typical phialides (bowling-like).

The antagonistic properties of *Trichoderma* have long been recognized since the first publication in 1887. However, the comprehensive study of the phenomenon of antagonism and its application as a means to fight crop pests started between the two world wars (Johanne, 2002). *Trichoderma* has a set of potentially usable mechanisms for attacking, but remain complex. It can use one or more modes of action simultaneously to control a pathogen. The deployment of modes of action also varies according to the partners involved and the physical and chemical conditions of the medium (temperature, humidity, etc). *Trichoderma* is effective when it is allowed to install before the pathogenic fungi. Therefore its action is preventive (Johanne, 2002). In this study, the effects of selected *Trichoderma* sp isolated from burned soils on the growth and development of *Fusarium* sp, *Penicillium* sp, *Rhizoctonia* sp and *Alternaria* sp were evaluated.

MATERIALS AND METHODS

Study site and sample collection

The work focuses on the study of antagonistic fungi isolated from burned forests in Mila region. Soil, bark and leaves samples were collected from different sites in the forest Zouagha which is bound from the north by Djijal, the west by OuledRabeh, the south by BeniHaroune and the east by Grarem-Gouga. The study area is located on the topographical map of SidiMarouane scale 1/50.000, sheet N°50, between 804.8 to 805 altitude E and 366 à 366.2 longitude N. Z. Zouagha forest is spread over an area of 353, 50 ha and is entirely limited and divided into five regions: El Bahloul, Bouzourane, DjbelDaya, Arres and BeniAfek (Figure 1).

Sampling was conducted on February 19, 2014 at 11: 30 am. Three random sites of burned forest of Zouagha, BeniAfek region in Terri Beinène, Mila were selected. Samples were taken from tree bark and 1 kg of soil (after the removal of 3 cm of the upper layer of soil) of each site and as well as leaves from site 2 to evaluate the presence of fungi. After that, they were stored at 4°C in cooler, then transported to the laboratory until use.

Isolation and purification of fungi

Isolation of fungal strains was carried out according to the suspension dilution method (Davet and Rouxel, 1997). One gram of the soil sample, from each site, was aseptically added to 9 ml sterile saline water. The suspension was vortexed and diluted up to 10^{-6}. The bark and leaves samples were washed first in bleach for 5 min, then with ethanol for 5 min to remove microorganisms from the surface. Purification of strains was done on PDA agar (pH =5.1). Plates were incubated at 25°C for 6 days.

Identification of fungi

Strain's identification was conducted following the conventional dichotomous identification scheme. The macromorphology identification was done on the basis of colonies' properties of the isolation media. The micromorphology of the isolates were determined by direct light microscopic examination at 10X and 40X (optical microscope EXACTA+OBTEC) according to the determination keys of Botton et al. (1990).

Antagonism test

Direct confrontation method also called opposite cultures technique was used to determine antagonism activity. In a Petri dish containing 15 ml of PDA medium, two agar pellets (8 mm in diameter) of antagonist and pathogen were placed 4 cm from each other. Petri dishes containing pathogenic fungi were used as control. The plates were incubated at a temperature of 25°C and continuous light as an activation factor of certain enzymes. The

Table 1. Source of isolates and their frequencies.

Fungal isolate	Site	Leaf	Bark	Soil	Frequency %
*Trichoderma sp*1		-	-	+	
*Trichoderma sp*2		-	-	+	
Fusarium sp1	**1**	-	-	+	33.33
*Trichoderma sp*3		-	-	+	
*Trichoderma sp*4		-	-	+	
*Trichoderma sp*5		-	-	+	
*Trichoderma sp*6		-	-	+	
*Fusarium sp*2		-	-	+	
Alternaria sp	**2**	+	-	-	27.77
*Trichoderma sp*7		-	-	+	
*Rhizoctonia sp*1		-	-	+	
*Rhizoctonia sp*2		-	+	-	
Rhizopus sp		-	-	+	
*Trichoderma sp*8		-	-	+	
*Fusarium sp*3	**3**	-	-	+	38.88
Penicillium sp		-	-	+	
*Trichoderma sp*9		-	-	+	
*Trichoderma sp*10		-	-	+	

*(+) Presence of fungi, *(-) Absent of fungi.

development of mycelia growth was monitored every 24 h by measuring diameters of mycelial colony in millimeter. The percentage of mycelial growth inhibition was determined using the following formula (Hmouni et al., 1996):

$$I\ (\%) = (1 - Cn/Co) \times 100$$

I(%): is the percentage of mycelia growth inhibition
Cn: is the average diameter of the colonies in the presence of the antagonist
Co: average diameter of the control colonies.

RESULTS

Isolation and identification of fungi

Fungi were isolated from almost all analyzed samples and were identified. Eighteen fungal strains belonging to six genera: *Alternaria* sp, *Fusarium* sp, *Penicillium* sp, *Trichoderma* sp, *Rhizoctonia* sp and *Rhizopus* sp were isolated. Results are illustrated in the Table 1. The isolation results demonstrate that the highest frequency of the fungal isolate which is 38.88% is from site number 3. Five different genera including *Rhizoctonia* sp2, *Rhizopus* sp, *Trichoderma* sp 8, 9, 10, *Penicillium sp,* and *Fusarium* sp3 were isolated. The site number 1 had 33.33% , including *Trichoderma sp*1, 2, 3, 4, 5, and *Fusarium sp*1 and finally the site number 2 had 27.77%; *Trichoderma* sp6, 7, *Fusarium* sp2*, Alternaria* sp, and *Rhizoctonia* sp1.

The percentages of fungal isolates varied; 16.66% for *Fusarium* sp, 5.55% for *Alternaria sp,* 55.55% for *Trichoderma* sp, 5.55% for *Rhizopus* sp, 11.11% for *Rhizoctonia* sp and 5.55% for *Penicillium* sp (Tables 2 and 3).

Antagonism

The results of antagonism test of *Trichoderma* against four fungal isolates (*Alternaria* sp, *Fusarium* sp, *Penicillium* sp, and *Rhizoctonia* sp) show a medium reduction of mycelial growth of colonies of different fungal isolates compared to control (Figure 2 and Table 4). The tested colonies of *Trichoderma* sp inhibited the germination of *Fusarium* sp conidia by 23.13%, followed by *Penicillium* sp by 33.13%, *Rhizoctonia* sp *by* 33.75% and *Alternaria* sp by 38.31% (Figure 3).

DISCUSSION

In the study, the fungus flora of the burned forest soil of Zouagha, Mila region was determined. Among the genera obtained *Trichoderma* sp, *Fusarium* sp, *Penicillium* sp, *Rhizoctonia* sp, *Alternaria* sp, and *Rhizopus* sp.

Lucarotti (1981) obtained higher frequencies of *Trichoderma, Penicillium, Mucor*Mich ex Fr. and

Table 2. Samples analysis of unburned soil from Zouagha forest.

Parameter / Sample	Content expressed as % by weight of dry materials				
	Carbonates NF P(94-48)	Sulfates	MO	CO_2	pH
Site 01	40.08	Traces	15,56	1.70	6.08
Site 02	40.27	Traces	6.14	1.30	6.27
Site 03	41.41	Traces	5.30	0.80	6.41

Table 3. Samples analysis of soil obtained from Zouagha forest.

Parameter / Sample	Content expressed as % by weight of dry materials				
	Carbonates NF P(94-48)	Sulfates	MO	CO_2	pH
Site 01	4.88	Traces	10.0	2.15	7.0
Site 02	6.32	Traces	4.0	2.78	7.13
Site 03	6.32	Traces	4.0	2.78	7.80

Figure 2. Representative images of controls of five fungal genera after six days.

Table 4. The effect of *Trichoderma sp* on *Fusarium sp*, *Penicillium sp*, *Rhizoctonia sp* and *Alternaria sp.*

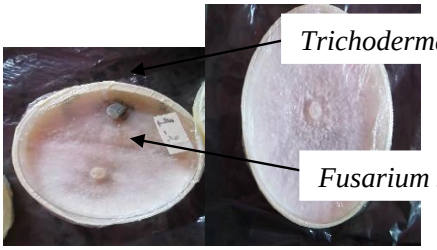
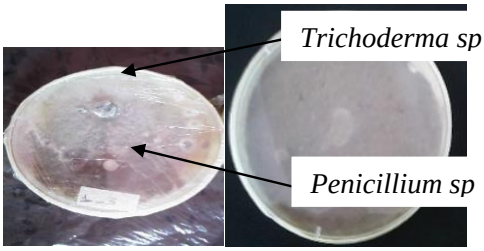
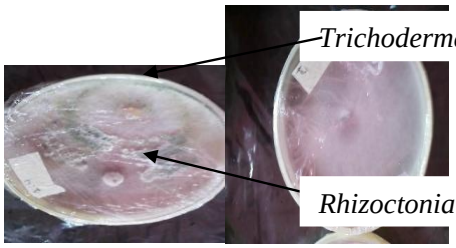
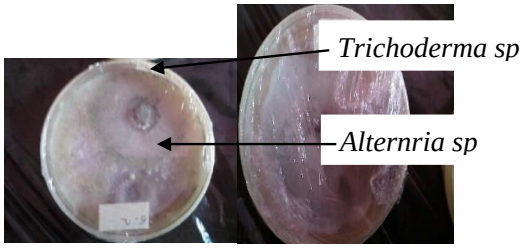
Antagonist – Pathogen	Antagonistic activity	
Trichoderma sp + Fusarium sp		T2
Trichoderma sp + Penicillium sp		T3

Table 4. Contd.

Trichoderma sp + Rhizoctonia sp		T4
Trichoderma sp + Alternria sp		T5

T2 :*Fusarium sp*, **T3 :***Penicillium sp*, **T4 :** *Rhizoctonia sp* and **T5:** *Alternaria sp.*

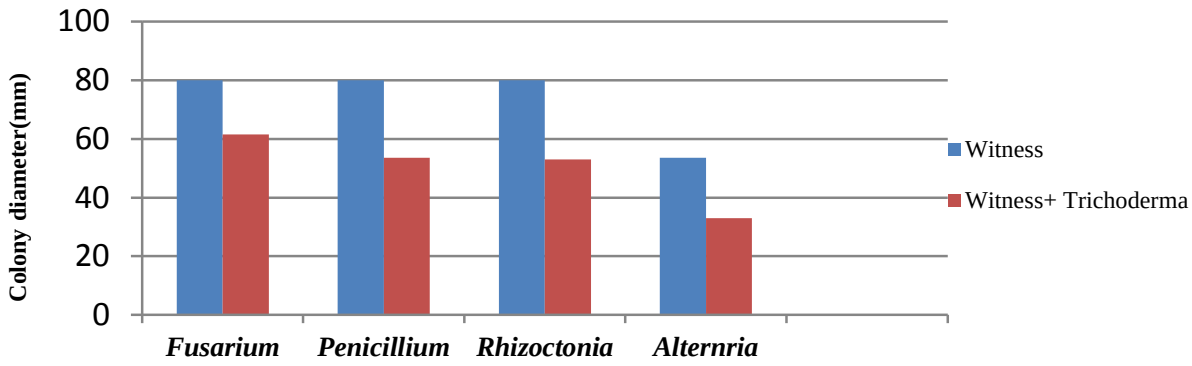


Figure 3. Colony diameter of *Fusarium sp*, *Penicillium sp*, *Rhizoctonia sp* and *Alternaria sp* in the presence of *Trichoderma sp* after 6 days.

Mortierella Coemans at in soil of burned forest in Canada. It can be postulated that these species do not show much sensitivity to ecological extremes and are more resistant to negative conditions. Also, Reaves et al. (1990) reported that they obtained *Trichoderma citrinoviride* Bissett most frequently in burned forest soil. Chwalinski (1989) determined that the variety of species following a fire was renewed within a year but the fungal density was not renewed completely in this period.

Many researchers reported that the soil humidity, soil pH (RamaRao, 1970), salt amount (Hasenekoglu and Sulun, 1990), and organic matter content (Behera and Mukerji, 1985) influence the activity of soil microorganisms. The fact that the amount of organic matter is very high in all soils proves that the rapidly spread fire, did not do much harm underground and the fire was only on the surface (Hasenekoglu and Sulun, 1990).

In addition, 20% of organic matter is nitrogen, and thus these soils are considered to be very rich in nitrogen. This may have a positive effect on microorganism activity in the soil (Table 2). The fact that the soil has a low rate of salt and lime (Ca^{+2}) could exclude their negative effect on the activity of soil microorganisms.

Suciatmih (2006) found that a significant positive correlation existed between the fungal population and the total organic carbon content. Waid (1960), listed temperature, humidity, CO_2, oxygen concentration, size of the soil pores, longevity of fungal mycelium, interaction between soil fauna and soil fungi and soil reaction as

factors that may influence growth and production of the mycelium of fungi in the soil.

According to Suciatmih (2006), the forest fire leads to a reduction and possibly an elimination of soil fungi. Chet (1984) reported studies on mode of action of *Trichoderma* used for biological control against *Rhizoctonia solani* in the case of cotton and strawberry cultivation, the results highlighted the importance of mycoparasitism phenomenon in the effectiveness of *Trichoderma*.

In the case of direct confrontation between *Alternria alternata* and *Trichoderma harzianum. A. alternria* has a very short development time but *T. harzianum* grows faster and surrounds the pathogen on the second day. *T. harzianum* develops without obstacles, and it has opportunities to stop the development of *A. alternate*. It grows over the colony of the pathogen at the same time (Biljana and Jugosslave, 2011; Gveroska and Ziberosk, 2011).

Rajendiran et al. (2010) demonstrated the inhibitory effect of *Trichoderma viride* against *Fusarium* sp., *Penicillium* sp. And *Aspergillus* sp. Growth inhibition of these fungi is due to its rapid growth nature, extracellular secretion of harmful compounds such as antibiotics, enzymes that can degrade cell wall such as gluconases, endochitinases, chitinases and mycoparasitism. Harman et al. (2004) described the mycoparasitic action of *Trichoderma* sp against pathogens. It coils around the hyphae of pathogen and produced peptaible which facilitate the entry of hyphae of *Trichoderma* sp into the lumen of parasitic mold.

Conflict of Interests

The authors have not declared any conflict of interests.

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