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TRICHODERMA HARZIANUM AS A BIOCONTROL AGENT AGAINST *BIPOLARIS* SP. AND *CHRYSOSPORIUM* SP. ON GRAPE FRUITS

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Abstract: The present investigation aimed is to isolate and identify the mycetes accompanying the grape (*Vitis vinifera*) fruits and to evaluate *in vitro* the ability of *Ttrichoderma harzianum* to control the isolated mycetes. Some infected grape fruits by mycetes were brought from Oum-Elbouaghi market. The results of isolation allowed the identification of *Bipolaris* sp. and *Chrysosporium* sp. One isolate of *T. harzianum* / *Hypocrea lixii* was utilized in this study. The results of direct confrontation (*in vitro*) of *T. harzianum* against *Bipolaris* sp. and *Chrysosporium* sp**.** on PDA medium indicated the inhibition of mycelium growth in variable degrees; it was equal in the fourth days of the experiment to 40% and 36.36% for *Bipolaris* sp. and *Chrysosporium* sp**.** respectively. However, it did not show any growth of the tested fungus when re-planting a disk from the interaction hyphal area between *T. harzianum* and *Bipolaris* sp. or *Chrysosporium* sp. from dual cultures, while *T. harzianum* grew alone in the plates. The microscopic observations of mycelia of dual culture in slide methods showed that the mycelia of *T. harzianum* induced degradation the spores and analyzed the mycelia of *Chrysosporium* sp., overgrowing the mycelim of *Bipolaris* sp. and coiled around of them and degrading spores and mycelium.

Key words: *Bipolaris sp., Vitis vinifera, confrontation, slide methods*

INTRODUCTION

Species of *Bipolaris*, *Drechslera*, *Exserohilum* and *Curvularia* constitute a group of taxonomically related and ecologically similar deuteromycetes (mitosporic fungi) that are important plant pathogens or common saprophytes throughout the world. The first three of these genera were segregated from *Helminthosporium* in several revisions from 1930 to 1974, and some species of *Bipolaris* and *Curvularia* share the same teleomorph (Pratt, 2006). The genus *Bipolaris* includes a number of significant plant pathogens with worldwide distribution. These species are commonly associated with leaf spots, leaf blights, melting outs, root rots, foot rots and other disease symptoms mainly in high value field crops in the family Poaceae, including rice, maize, wheat and sorghum and on various other host plants (Ellis, 1971; Sivanesan, 1987; Berbee et al., 1999). In addition to a host association with Poaceae, species of *Bipolaris* are known to occur on at least 60 other genera in Anacardiaceae, Araceae, Euphorbiaceae, Fabaceae, Malvaceae, Rutaceae and Zingiberaceaeas either saprobes or pathogens (Ellis, 1971; Sivanesan, 1987; Manamgoda et al., 2011). Chrysosporium is a genus groups nearly twenty species. *Chrysosporium fastidum*, *C. pruinosum* and *C. xerophilum* have an industrial interest. These later were isolated from dry plums, ground, textiles and other cellulosic feed stocks (Botton et al., 1990).

Due to hazardous effect of chemical fungicides, search for safer alternative to control the pathogen is better choice. This led to trials on the use of bioagents to control the pathogen.

Biological control using potential microorganisms having strong antifungal activity is coming up as an alternative strategy for disease management, which is also ecology-conscious and environment friendly (Kamala & Indira, 2012). Several biocontrol strategies have been proposed for controlling the plant pathogens, but practical applications are still limited (Hibar et al., 2007). *Trichoderma* species are common soil-inhabiting fungi that have been developed as effective biocontrol agents against various phytopathogenic microorganisms (Bel Haj Khethr et al., 2008).

The objective of this investigation was to isolate and to identify the mycetes accompanying the grape (*V. vinifera*) fruits, and to evaluate the *in vitro* potential of *Trichoderma harzianum* to control that isolated mycetes.

MATERRIALS AND METHODS

1. Fungal strains

Bipolaris sp. and *Chrysosporium* sp. were isolated from infected grape fruits, which were brought from Oum-Elbouaghi market (Figure 1) and identified based on the microscopic observations of their reproductive and colony characteristics in laboratory of microbiology, University of Oum-Elbouaghi (Algeria) (Robert et al., 1981; Botton et al., 1990; Rémi, 1997). A local strain of *Trichoderma harzianum*/*Hypocrea lixii* was identified in the same laboratory and verified in Walloon Center of Biology Industrial, University of Liege, Belgium.

Figure.1. Infected grape fruits with fungi rot (1); The same infected fruits after incubation for one week in laboratory (2)

2. *In vitro*. Evaluation of the antagonistic capability of *T. harzianum* against *Bipolaris* sp. and *Chrysosporium* sp. on PDA medium (direct confrontation)

To study the direct confrontation between *T. harzianum* and *Bipolaris* sp. or *Chrysosporium* sp., two plugs of mycelium (8 mm diameter) were cut with a sterilized cork borer from the margins of actively stock cultures, growing on PDA medium of *T. harzianum* and the other of *Bipolaris* sp. or *Chrysosporium* sp. Two mycelial blocks one from Trichoderma and other from *Bipolaris* sp. or *Chrysosporium* sp were placed in a same time on PDA medium (Potato Dextrose Agar) plate in opposite directions. One plug of *Bipolaris* sp. or *Chrysosporium* sp. were maintained as controls (alone cultures). Each replicate has three plates. Both the dual and control cultures were incubated at 25°C for four days, and measurement of colony diameters (in millimeters) was taken every day. The percentage of inhibition growth (I) was calculated by using the formula: [I (%) = (C-T/C) x 100]. where: I = Percentage inhibition of pathogen growth by antagonists; C = Radial growth in control; T = Radial growth in the treatment (Berber et al., 2009; Hamitou & Dehimat, 2015).

3. Evaluation of dual culture using slide methods

For each pathogen (*Bipolaris* or *Chrysosporium*) - *T. harzianum* interaction, a clean slide was placed in 9 cm diameter plates and sterilized. Following that, a small amount of PDA medium was spread over the slide to make a thin PDA film on the slide. The 5 mm discs of one week old of each pathogen and *T. harzianum* isolates were placed on the opposite sides of the slide 3 cm apart on the PDA surface. Then 5ml of distilled water was added to the plate to prevent drying and then incubated at 25°C for a week. At the end of incubation period, region of contact between *T. harzianum* - Pathogen hyphae was stained with lacto phenol and cotton blue and examined under a light microscope (Al-Saeedi & Moqdad, 2014).

RESULTS AND DISSCUSSION

1. *In vitro*. Evaluation of the antagonistic capability of *T. harzianum* against *Bipolaris* sp. and *Chrysosporium* sp. on PDA medium (direct confrontation)

The results of dual culture indicated that the *T. harzianum* inhibited the growth of *Bipolaris* sp. and *Chrysosporium* sp. at varying degrees. The maximum growth inhibition was recorded in the second day of experiment on the *Bipolaris* sp. with 44.44%. The highest inhibition on the *Chrysosporium* sp. was recorded in the fourth day of the experiment with 36.36 % (Table1 and Figure 2). Besides, the results presented in the Figures 3.3 and 3.6 showed no mycelia growth of *Bipolaris* sp. or *Chrysosporium* sp. when re-planting the disks from the interaction hyphal area between *T. harzianum* and *Bipolaris* sp. or *Chrysosporium* sp. from dual cultures, in the new plates containing PDA, while *T. harzianum* grew alone in the re-planting plates.

Table 1. *In vitro* effect of *Trichoderma harzianum* on the mycelia growth of *Bipolaris* sp. and *Chrysosporium* sp. on PDA medium

		Radial growth rate (mm) after:			
	Fungus species	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Dual culture	*Trichoderma harzianum*	18	60	100	130
	Bipolaris sp.	6	10	20	24
Alone culture	*Bipolaris* sp.	10	18	28	40
% inhibition of mycelia growth	*Bipolaris* sp.	40	44.44	28.57	40
Dual culture	*Trichoderma harzianum*	18	60	100	130
	Chrysosporium sp.	4	16	24	28
Alone culture	*Chrysosporium* sp.	4	20	32	44
% inhibition of mycelia growth	*Chrysosporium* sp.	0	20	25	36.36

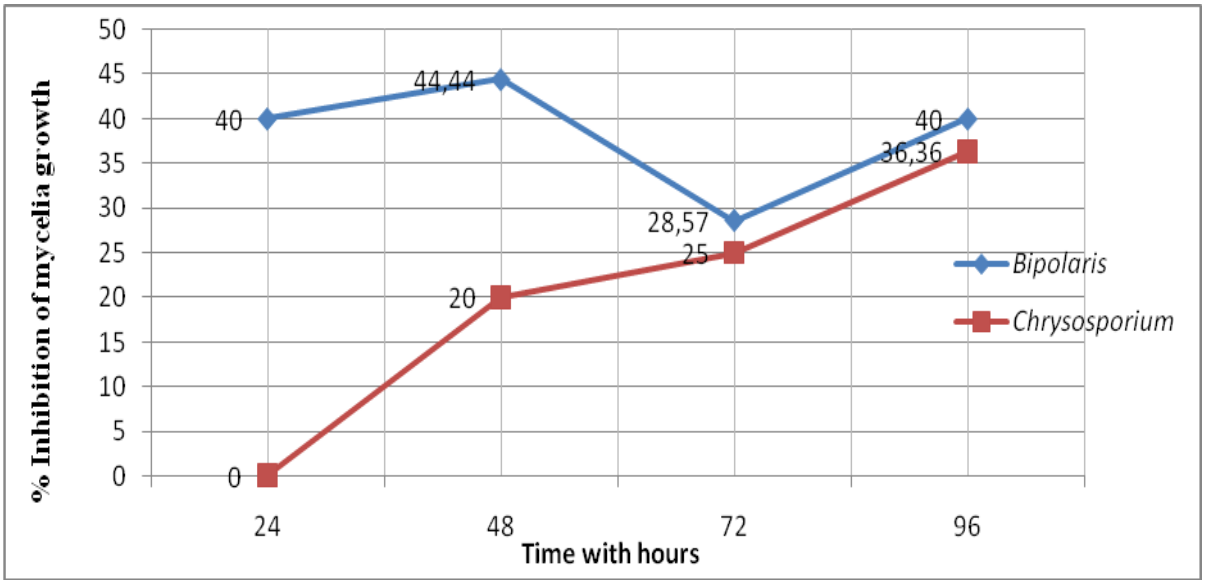

Figure 2. Effect of *Trichoderma harzianum* in mycelia growth of *Bipolaris* sp. and *Chrysosporium* sp.

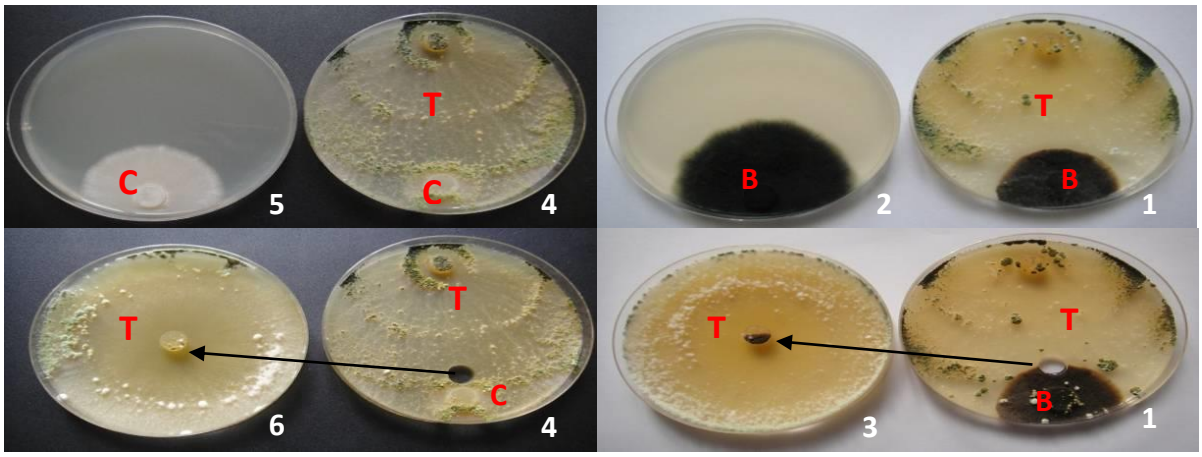

Figure 3. *In vitro* effect of *Trichoderma harzianum* against *Bipolaris* sp. and *Chrysosporium* sp. dual cultures (1) and (4), controls (2) and (5), re-planting plates (3) and (6)
B = *Bipolaris*, C = *Chrysosporium*, T = *Trichoderma*

2. Evaluation of dual culture using slide methods.

The microscopic observations of mycelia of dual culture in slide methods showed that the mycelia of *T. harzianum* overgrowing the mycelia of *Bipolaris* sp. and coiled around of them (Figure 4.5). Degrading the mycelium and spores of *Bipolaris* sp. (Figure 4.2) and *Chrysosporium* sp. (Figure 4.4) compared with controls (Figures 4.1 and 4.3).

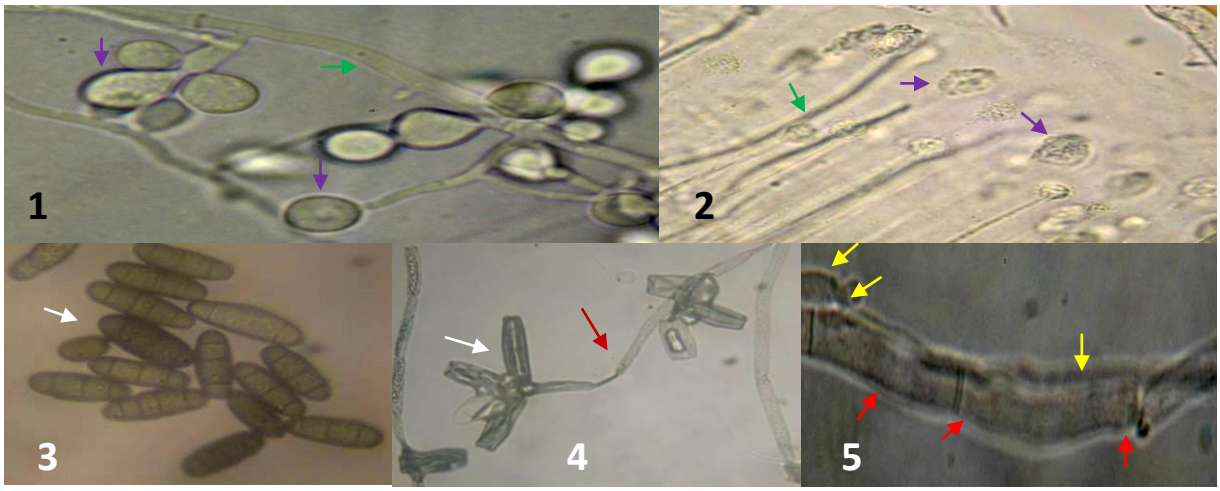

Figure 4. Microscopic observations from the slide methods showed the effect of *Trichoderma harzianm* against *Bipolaris* sp. and *Chrysosporium* sp. Decomposition (lyses) phenomenon (2) and (4); Mycoparasitism phenomenon (*Trichoderma* hyphal coiling around of *Bipolaris* hyphal) (5); Controls (1 and 3).
Bipolaris (hyphe = red arrows, spores = white arrows); *Chrysosporium* (hyphe= green arrows, spores=purple arrows); *Trichoderma* (hyphe = yellow arrows)

Similar results were confirmed by many studies, where found that *T. harzianum* can inhibit the growth of *Bipolaris* sp., *Fusarium oxysporium*, *Fusarium* sp. and *R. solani* with a different ratios, and inhibit there spore's formation, with recording a different degrees of parasitism (Camporota, 1985; Azza & Allam, 2004; Hibar et al., 2005; Berber et al., 2009; Suraiya et al., 2014). The observation is similar to the findings of Manimegalai & Bahkali (2011), Agarwal et al., (2011) and Seema & Devaki (2012) where found that *T. harzianum* checked the growth of the brown spot fungus *Bipolaris oryzae* pathogen in the dual culture with 71.2% and inhibited the mycelia growth of *R. solani*, *A. flavus* and *A. fumigates* with 74.53%, 77.77% and 67% respectively in the dual culture compared with controls. Besides, *T. harzianum* strains produced lytic enzymes as 1, 3-β-glucanase and chitinase which were inhibited the growth of *G. graminis* var. *tritici*, *F. culmorum* and *F. moniliforme* on PDA medium (Cigdem & Merih, 2004). Also, *T. harzianum* can produce nonanoic acid into a liquid culture medium, which has a strong effect on mycelial growth and spore germination of the cacao pathogen (*Crinipellis perniciosa* and *Moniliophthora roreri*) (Anejaa et al., 2005). Many compounds have been isolated from a *T. harzianum* strain such as azaphilone, butenolides, hydroxy-Lactones, harzianolide and its derivatives T39 butenolid, deydro-harzianolide. That compound containing a highly activity towards several plant pathogenic fungi. The pyrone 6-pentyl-2H-pyran-2- is a metabolite commonly purified in the culture filtrate of *T. harzianum* and is responsible for the coconut aroma released by axenically developed colonies and shown both *in vivo* and *in vitro* antifungal activities towards several plant pathogenic fungi and a strong relationship has been found between the biosynthesis of this metabolite and the biocontrol ability of the producing microbe (Almassi et al., 1991; Claydon et al., 1991; Vinale et al., 2014). Yacoub (1999) found that the *Trichoderma sp.* reduced the lesion development and number of conidiophores of *Botrytis sp.* in foliar discs of strawberry test, compared with the non - treated (control).

CONCLUSIONS

From the results obtained *in vitro*, we have established that the *Trichoderma harzianum* can be classified as BCA (biological control agent) for grape *Bipolaris* leaf spot disease and *Chrysosporium* soft rot.

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