



Biocontrol Capacity of the Soil Fungus *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *albedinis*, a Causal Agent of Fusarium Wilt (Bayoud) Disease of Date Palm (*Phoenix dactylifera* L.)

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ABSTRACT

Background: This study has the objective to study antifungal effect of the antagonistic fungus of soil named *Trichoderma harzianum* on mycelial growth of twenty isolates of *Fusarium oxysporum* f. sp. *albedinis* (Foa), agent of the bayoud disease on the date palm, originated from south western region of Algeria.

Methods: Twenty Foa isolates obtained from isolation of the spines carrying the typical symptoms of Bayoud disease were used. The strain of *T. harzianum* was isolated from rhizosphere soils of the date palm trees. The *in vitro* test affected by two tests of confrontation, direct which is dual confrontation and indirect confrontation by volatiles substances, on mycelial growth.

Result: A statistical analysis showed a significant difference ($P < 0.05$), of Foa isolates reaction against the antagonistic fungus *T. harzianum* in the test realized. The results of *in vitro* test showed a significant effect ($P < 0.05$), on mycelial growth in both tests used. The inhibition rate (IR) of mycelial growth of Foa by *T. harzianum* varies between 28.42 and 59.12%, with 12 Foa isolates showed IR, more than 50% in the direct test and between 3.3 and 56% with just three (3) isolates have IR more than 50% in the indirect test by volatile substances. Based on these encouraging results, we can apply of this antagonistic fungus to protect our groves contaminated by Bayoud disease and also contain this susceptible commercial variety.

Key words: Bayoud disease, Biocontrol, Confrontation, Date palm, *Trichoderma harzianum*.

INTRODUCTION

In the oasis system of southern Algeria, the date palm represents the key to the agroecological equilibrium of the desert and ensures the socio-economic stability of the local Saharan populations (Dubost, 1992). The phoeniculture is an essential element in the agro-economic development of oases (Bouguedoura *et al.* 2015). The proposal of new management method using biotechnological procedures like plant breeding, phylogenic selection, micropropagation *etc.*, has become more than necessary for the future of this field (Sedra, 2003). But all these methods have limits against bayoud disease on date palm culture (Djerbi, 2003). Bayoud disease, is a vascular wilt caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa), is considered as the most destructive fungal disease in Moroccan and Algerian palm groves cultivated with important economical varieties like Bouskri, Majhoul, Boufeggous, but all are susceptibles to this plant pathogen (Sedra 2005a, b; Djerbi, 2003). Since its first appearance, which goes back more than a century, in the Moroccan palm groves where it has been recorded the disappearance of more than 12 million palm trees (Djerbi, 2003). Its progression continues to advance from the west to the palm groves of central and eastern of Algeria (Djerbi, 2003).

In the absence of an updated estimate of the last years, the losses reported since 2003 (Djerbi, 2003), estimated at more than 3 million palms are in a clear evolution, especially

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since several unaffected regions in the past are reconsidered as contaminated by Bayoud (Essarioui and Sedra, 2017). Bayoud represents not only the risk to eliminate the best commercial varieties, but also to the phenomenon of desertification and the immigration of oasis habitants to large urban cities (Bouguedoura *et al.* 2015).

Due to the biological and pathogenic characteristics of *Fusarium oxysporum* f. sp. *albedinis* and its host date palm, any attempt at chemical control remains ineffective for old palm groves where their rooting is deeper and harmful to the environment and human health (Saaidi, 1990). On the other hand, the proposed prophylactic measures don't seem able to stop its progression, especially since the epidemiological data are favoured by a progressive spread of this disease, but these measures are important to protect the Southern Algerian of healthy date palms (Benzohra *et al.*, 2017).

Genetic control by resistant varieties is therefore another promising way of reducing the damage caused by this constraint, but unfortunately all resistant cultivars haven't high quality in date fruits (Benzohra *et al.*, 2021a; Sedra, 2005b).

In this latest decade, a bayoud has eliminated thousands of individuals "Khalts" (natural hybrids) and important populations of better quality date, were widely cultivated as Mejhoul, Boufeggous and Jihel in Morocco, have disappeared (Sedra, 2005b). The selection of productive date palms, with good quality date palms and resistance to Bayoud requires a rigorous methodology and a broad time to select these cultivars (Benzohra *et al.*, 2021b; Sedra, 1993; Sedra, 1994a, b; Sedra and Besri, 1994).

To control and limit this constraint, the biological control through the use of antagonistic (El-Hassni *et al.*, 2007; Negi *et al.*, 2019; Kuchlan *et al.*, 2017) and mycorrhizal microorganisms remains the best way to combat this threat in palm groves contaminated by Bayoud (Jaiti *et al.* 2006; 2008; Dihazi *et al.*, 2012).

The objective of this work is to study the *in vitro* antagonistic effect of *Trichoderma harzianum* on the mycelial growth of twenty isolates of *Fusarium oxysporum* f. sp. *albedinis* (Foa).

MATERIALS AND METHODS

Isolates of *Fusarium oxysporum* f. sp. *albedinis*

Twenty (20) isolates of *Fusarium oxysporum* f. sp. *albedinis* (Foa) used in this study of different origins provided by CRSTRA (Center for Scientific and Technical Research on Arid Regions, Biskra). The citation of isolates and its origins was presented in Table 1. All isolates were conserved in PDA medium (For 1000 ml : 200 g potato juice; 15 g glucose; 20 g agar-agar; pH =5.5), with temperature of conservation at 04°C and incubation at 23°C (Sedra, 2005a).

Strain of antagonist *Trichoderma harzianum*

The strain of the antagonist *Trichoderma harzianum* (Th-Lpv-Most-2018), was provided by the Laboratory of Plant Protection (LPV) of the University of Mostaganem (Table 2). The strain was used in the confrontation tests.

Dual confrontation

We performed the method of Howell (2003) to evaluate the inhibitory action of *T. harzianum* on mycelial growth of Foa. We have in Petri dishes containing 15 ml of diametrically opposed PDA medium; two explants (5 mm

in diameter) from the Foa and the antagonist (Fig 1), with four repetitions were performed. The control consisted by colonies of Foa in petri dishes without the antagonist. The mycelial growth of Foa isolates was assessed by measuring the radial diameter of mycelial growth of Foa for 6 days of incubation.

Indirect confrontation

This technique consists in depositing at the center of each Petri dish containing the PDA culture medium, an explant of 5 mm in diameter one from colony of the antagonist and another from Foa (Aydi *et al.*, 2013; Belaidi *et al.*, 2021). The lower part containing the antagonist *Trichoderma harzianum* is sealed with another containing the pathogen, by a band of parafilm so as to avoid any contamination and any loss of volatile substance (Aydi *et al.* 2018). Four repetitions are made with an untreated control (Aydi *et al.*, 2014).

Evaluation of mycelial growth

For the estimation of mycelial growth, the technique used is that indicated by Hibar *et al.* (2007b). This method consists in measuring the daily linear mycelial growth of the colonies until the seventh day, according to the following formula:

$$L = (D-d) / 2$$

Where

L: Mycelial growth (mm).

D: Colony diameter (mm).

d: Explant diameter (5 mm).

The mycelial growth averages are calculated by the following formula:

$$V(\text{mm/j}) = \sum (L_n - L_{n-1}) / n$$

Where

V: Mycelial growth per day (mm/j).

L_n, L_{n-1} : Mycelial growth during the day n.

The inhibition rate (%), is calculated as follows:

$$IR (\%) = (L_t - L) \times 100 / L_t$$

Where

IR: Pourcentage of inhibition rate (%).

L_t : Daily mycelial growth of control.

L: Daily mycelial growth by *T. harzianum* effect.

Multiplication of foa

The multiplication of the inoculum was carried out in petri dishes containing PDA medium (Potato Dextrose Agar), autoclaved for 25 min at a pressure of 1 bar and a temperature of 121°C, to which we have added 10 ml of a liquid culture of *F. oxysporum* f. sp. *albedinis*. These dishes are then incubated at 22±3°C for two weeks (Hibar *et al.* 2007).

Multiplication of the antagonist

The multiplication of *T. harzianum* was carried out in the same way as for *F. oxysporum* f. sp. *albedinis*, but with an incubation period of one week. This is explained by the fact that *T. harzianum* has a faster of mycelial growth than *F. oxysporum* f. sp. *albedinis* (Hibar *et al.* 2005).

Table 1: Foa isolates with its origin.

Foa isolates	Provinces	Oases
S1	Saoura	Ouakda, Bechar
S2	Saoura	Bechar
S3	Saoura	Beni Abbes
S4	Saoura	Tabelbella
S5	Saoura	Igli
S6	Gourrara	Charouine
S7	Gourrara	Ouled Said
S8	Gourrara	Tinerkoug
S9	Gourrara	Telmine
S10	Gourrara	Charouine
S11	Touat	Tililane
S12	Touat	Ouled Ahmed Timi
S13	Touat	Reggane
S14	Touat	Inzeghemir
S15	Touat	Adrar
S16	Touat	Adrar
S17	Mizab	Atteuf
S18	Mizab	Atteuf
S19	Mizab	Ghardaïa
S20	Mizab	Metlili

S: Strain of *Fusarium oxysporum* f. sp. *albedinis*.

Statistical analysis

The device used is a univariate total randomization and nonparametric comparison of K samples. The significance study is performed with the 5% Kruskal-Wallis test ($P < 0.05$). The analysis was done using the XLStat 2009.1.02 software, (AddinSoft, USA). The studied factor is the inhibition of mycelial growth; the observation is the Foa strains and the experimental units are the linear or radial of mycelial growth.

RESULTS AND DISCUSSION

Dual confrontation

Statistical analysis revealed a highly significant effect ($P < 0.05$), a remarkable difference in mycelial growth of Foa isolates with *Trichoderma harzianum* (TH), compared to the control (Table 2), with formation of zone inhibition (Z), (Fig 1). We found that the mycelial growth in the control is always greater than that obtained with the direct (dual) confrontations (Foa + TH), (Table 3).

The averages of mycelial growth of Foa isolates were consistently lower (between 2.04 and 2.53 mm / day), than the control (between 3.15 and 5.42 mm / day), (Table 3). This reduction is followed by a complete stop of the growth.

The inhibition rate (%) of mycelial growth (RI), of Foa under the direct effect of *T. harzianum* varies between 28.42 and

Table 2: Informations of antagonistic fungi strains' species used in 'in vivo' biocontrol test against bayoud disease.

Strain name	Antagonistic fungus strain information		
	Origin of sampling	Date of sampling	Antagonism type
<i>Trichoderma harzianum</i> (T-22)	Mostaganem	2018	Antibiosis

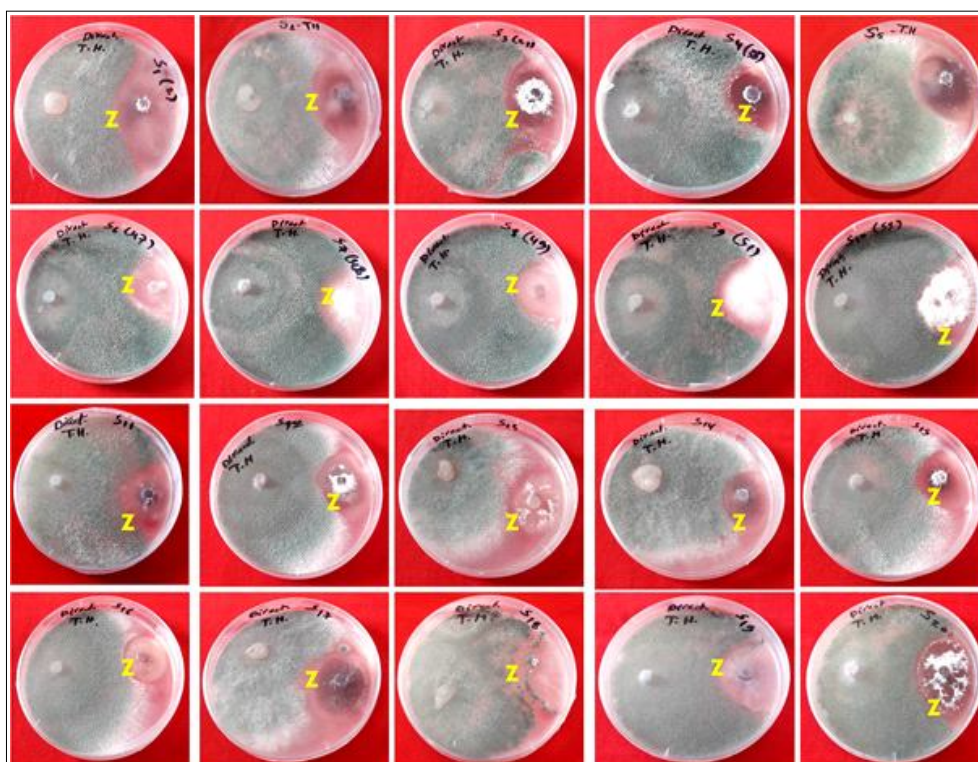


Fig 1: Colonies of twenty Foa isolates in dual confrontation with *T. harzianum*. Z: Zone of inhibition.

59.12% (Fig 2), according to the isolate. 12 isolates (S1, S3, S9, S10, S11, S12, S15, S16, S17, S18, S19, S20), showed a very high inhibition rate that exceeded 50%. Also, we observed that all four isolates of Ghardaia region showed IR more than 50% (S17, S18, S19 and S20), (Table 3; Fig 2).

Indirect confrontation

Also, a significant effect ($P < 0.05$) was observed by appearance of a difference on mycelial growth of Foa isolates in the effect of volatile substances indirectly confronted against *Trichoderma harzianum* compared to the control (Table 4).

We observed that the average of the mycelial growth of Foa isolates by the effect of *T. harzianum* (Table 3), is always lower (between 2.04 and 2.53 mm/day), than the control (between 2.13 and 4.88 mm/day).

The inhibition rate (IR%) of the mycelial growth of Foa under the effect of *T. harzianum* varies between 3.3 and 52.9% (Fig 2).

In comparison between the two tests of confrontation effects, we observed that the dual confrontation presented IR values very important with global average very close to 50% (49.80%), than IR values of indirect confrontation with global average under 50% (35.9%).

The potential of microorganisms as biological control agents against plant diseases has been identified for several

Table 3: Averages of mycelial growth of foa by direct effect of *T. harzianum* in dual confrontation.

Foa isolates	Mycelial growth (mm/day) ($\bar{x} \pm \sigma$)	
	Control (mm/day)	Foa + TH (mm/day)
S1	4.92 ^a ±0.08	2.44 ^b ±0.11
S2	4.22 ^a ±0.31	2.50 ^b ±0.00
S3	4.71 ^a ±0.00	2.24 ^b ±0.13
S4	4.79 ^a ±0.35	2.53 ^b ±0.23
S5	4.65 ^a ±0.26	2.44 ^b ±0.11
S6	4.88 ^a ±0.07	2.51 ^b ±0.03
S7	4.78 ^a ±0.14	2.46 ^b ±0.04
S8	4.71 ^a ±0.20	2.44 ^b ±0.11
S9	4.89 ^a ±0.13	2.33 ^b ±0.11
S10	5.42 ^a ±0.00	2.44 ^b ±0.11
S11	5.35 ^a ±0.14	2.30 ^b ±0.10
S12	5.21 ^a ±0.08	2.35 ^b ±0.10
S13	2.85 ^a ±0.00	2.04 ^b ±0.19
S14	3.15 ^a ±0.14	2.06 ^b ±0.17
S15	5.00 ^a ±0.00	2.40 ^b ±0.11
S16	4.33 ^a ±0.10	2.17 ^b ±0.30
S17	5.26 ^a ±0.12	2.15 ^b ±0.27
S18	5.00 ^a ±0.00	2.06 ^b ±0.17
S19	5.21 ^a ±0.08	2.40 ^b ±0.11
S20	5.35 ^a ±0.14	2.35 ^b ±0.10
LSD 5%		126.83*
C.V.		12.8%

*Significant effect at $P \leq 0.05$; σ : Standard deviation; \bar{x} : Average of mycelial growth; a and b: Homogenate groups; Foa: *Fusarium oxysporum* f. sp. *albedinis*; TH: *Trichoderma harzianum*; LSD: Least significative difference; C.V.: Coefficient of variation.

years (Howell, 2003). The antagonism action of microorganisms has been studied for many plant diseases (Wells *et al.* 1972; Schirmbock *et al.* 1994; Elad and Kapat, 1999; Yedidia *et al.* 1999; Yedidia *et al.* 2001; Harman, 2000; Sharon *et al.* 2001; Ozbay and Newman, 2004; Hibar *et al.* 2007; El-Hassni *et al.* 2007; Kuçuk *et al.* 2007; Dawidziuk *et al.* 2016; Benzohra *et al.* 2020).

The direct and indirect confrontation between the fungal antagonist *Trichoderma harzianum* and the pathogen *Fusarium oxysporum* f. sp. *albedinis* (Foa) showed significant reductions in mycelial growth compared with the control in all the isolates used. This reduction was represented by the inhibition rate (IR%) of mycelial growth. In dual confrontation, this rate varies between 28.42 and 59.12% and the indirect confrontation showed an inhibition rate varies between 3.3 and 56%, by the effect of *T. harzianum*.

Hibar *et al.* (2007) reported that *T. harzianum* was able to show a growth inhibition rate of 70 to 76% of *Fusarium oxysporum* f. sp. *radici-lycopersici*, tomato vascular wilt agent (*Lycopersicon esculentum* L.). While Boughedid and Filali (2015) reported an inhibition rate of mycelial growth by *T. harzianum* varies between 50 and 57% on *Fusarium graminearum*, Fusarium wilt agent of barley (*Hordium vulgare* L.). Concerning a bayoud disease, Sidaoui *et al.* (2018) reported that *Trichoderma longibrachiatum* had a 63% inhibition rate on mycelial growth. These last two results are very closer to those obtained in our tests.

This inhibition of mycelial growth is known in the bibliography as "antibiosis". Antibiosis is one of antagonism mode actions carried out by antagonistic fungi by the secretion of secondary volatile substances such as glioviridines and gliotoxins, substances that act as antibiotics, can inhibit the development of several phytopathogenic fungi (Howell, 2003).

In direct confrontation, there is another mode of antagonism called "mycoparasitism". An example of this mode of action is on the *T. atroviride* strains, which showed a mycoparasitism action against *Fusarium oxysporum* f. sp. *ciceris* (Foc), a vascular wilt agent in chickpea (*Cicer arietinum* L.), by its ability to invade Foc colonies and sporulate above (Mach *et al.* 1999). A significantly positive correlation exists between the efficacy of *Trichoderma* strains in reducing disease index and growth inhibition in indirect confrontation under the effect of volatile antifungal substances (Howell, 2003; Kala *et al.* 2016; Fitrianiingsih *et al.* 2019). This finding suggests that the antagonistic activation of our Foa isolates may be due in part to these volatile antifungal substances (Mach *et al.* 1999). Many researches showed the biological control activity of *T. atroviride*, that was due in part to the production of endochitinases (Kullnig *et al.* 2000), to the production of antibiotics (antifungals), including aromatic antibiotics such as pyrones (Keszeler *et al.* 2000), peptides (Oh *et al.* 2000) and induction of resistance mechanisms in plants (Brunner *et al.* 2005).

T. atroviride has shown a good efficacy in the biological control of *Rhizoctonia solani* on potato in the field (Kullnig *et al.* 2000) and also good protection against *Fusarium*

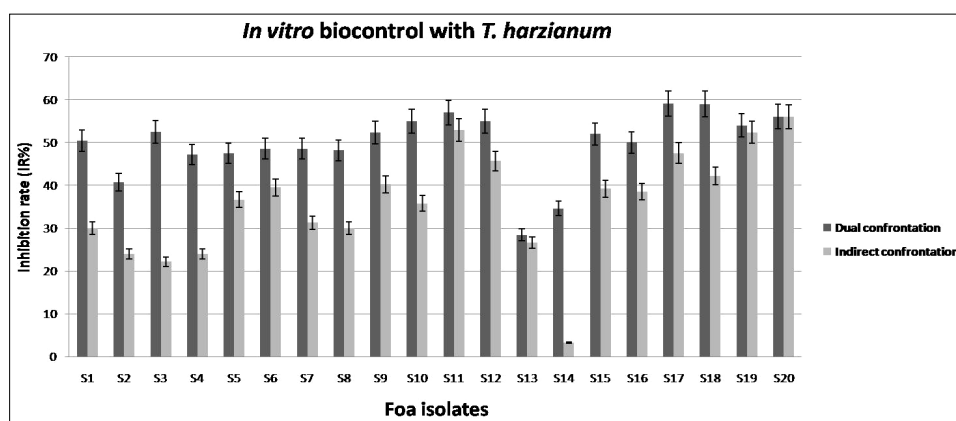


Fig 2: Inhibition rates (IR) of mycelial growth of Foa isolates by direct and indirect effects of *T. harzianum*.

Table 4: Averages of mycelial growth of foa isolates by indirect effect of *T. harzianum* in indirect confrontation.

Foa isolates	Mycelial growth (mm/day) ($\bar{x} \pm \sigma$)	
	Control (mm/day)	Foa + TH (mm/day)
S1	3.47 ^a ±0.44	2.44 ^b ±0.11
S2	3.29 ^a ±0.47	2.50 ^b ±0.00
S3	2.88 ^a ±0.53	2.24 ^b ±0.13
S4	3.33 ^a ±0.56	2.53 ^b ±0.23
S5	3.85 ^a ±0.32	2.44 ^b ±0.11
S6	4.15 ^a ±0.16	2.51 ^b ±0.03
S7	3.58 ^a ±0.52	2.46 ^b ±0.04
S8	3.49 ^a ±0.45	2.44 ^b ±0.11
S9	3.90 ^a ±0.17	2.33 ^b ±0.11
S10	3.80 ^a ±0.36	2.44 ^b ±0.11
S11	4.88 ^a ±0.09	2.30 ^b ±0.10
S12	4.33 ^a ±0.61	2.35 ^b ±0.10
S13	2.78 ^a ±1.30	2.04 ^b ±0.19
S14	2.13 ^a ±0.82	2.06 ^b ±0.17
S15	3.95 ^a ±0.12	2.40 ^b ±0.11
S16	3.53 ^a ±0.37	2.17 ^b ±0.30
S17	4.10 ^a ±0.36	2.15 ^b ±0.27
S18	3.53 ^a ±0.37	2.04 ^b ±0.19
S19	4.33 ^a ±0.61	2.06 ^b ±0.17
S20	4.88 ^a ±0.09	2.15 ^b ±0.27
LSD 5%		110.2*
C.V.		15.2%

*Significant effect at $P \leq 0.05$; σ : Standard deviation; \bar{x} : Average of mycelial growth; a and b: Homogeneous groups; Foa: *Fusarium oxysporum* f. sp. *albedinis*; TH: *Trichoderma harzianum*; LSD: Least significant difference; C.V.: Coefficient of variation.

graminearum, when applied by treatment wheat seed (Roberti *et al.* 2000). Recently, the biological control capabilities of this species have been shown against *Cryphonectria parasitica* (Murril) Barr., the agent of chestnut canker in USA (Dodd *et al.* 2003).

According to Hervas *et al.* (1997), the contribution of *T. harzianum* (marketed biofungicide) to soil significantly

reduces the incidence of Fusarium wilt of chickpea, caused by *Fusarium oxysporum* f. sp. *ciceris*. Dubey *et al.* (2007) reported a significant reduction in glasshouses by seed application of *T. harzianum* isolates against Pythium root rot.

Wang *et al.* (2003) reported that the other species of the antagonistic fungus, *Trichoderma viride*, influences the development and survival of *A. rabiei*, agent of ascochyta blight on chickpea. Some authors have noted that the absence of Bayoud disease in the palm groves of some Moroccan regions such as Marrakesh, seems linked to the phenomenon of the presence of antagonistic microorganisms in the soil (Sedra, 1993; Sedra and Rouxel, 1989). This remark was observed also in Algeria in the oases of Mizab region (Benzohra *et al.* 2017).

All these results confirmed the importance of application of this fungal species as a means of biological control and are promising for the future in the program of integrated control of the Bayoud date palm.

CONCLUSION

This study investigated the antagonistic capacity of *Trichoderma harzianum* on mycelial growth of twenty isolates of *Fusarium oxysporum* f. sp. *albedinis* (Foa), agent responsible for Bayoud disease on date palm. This *in vitro* test, affected by two antagonism methods was carried out by direct (or dual) and indirect confrontation using 20 Foa isolates.

The results of *in vitro* test indicate that there is an antagonistic effect made by *T. harzianum* on the development of *Fusarium oxysporum* f. sp. *albedinis*. Significant differences ($P < 0.05$) were observed in both tests performed, direct and indirect confrontation.

These results in the two tests carried out, showed appreciable results of antagonistic capacities in *Trichoderma harzianum* on isolates of *Fusarium oxysporum* f. sp. *albedinis*, which can inhibit the development of Bayoud in the soil when these antagonists enter Foa, compete in space and nutrients, in antibiosis by the secretion of antibiotics and in mycoparasitism by invasion of filamentous mycelia of Foa by the secretion of lytic enzymes.

In perspectives, this biological control approach study showed the important effect of the antagonistic fungi on Foa. These results obtained *in vitro*, must be confirmed by *in situ* soil tests, in palm groves contaminated with Bayoud with currently no resistant cultivars exist yet (Djerbi, 2003; Sedra, 2003; Sedra, 2005b).

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Conflict of interest: None.

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