

RESEARCH PAPER

Biological control of date palm diseases with native antagonistic fungi of Oued Righ region (Algerian Sahara)

Lutte biologique contre les maladies fongiques du palmier dattier avec des champignons antagonistes de la région d'Oued Righ (sahara algérien)

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Abstract

The date palm (*Phoenix dactylifera* L.) is one of the principal species in the south of Algeria. However, this speculation is confronted to some problems including plant health. Several fungal and bacterial antagonists were used to control plant diseases. The present study was undertaken to investigate the interaction of antagonistic fungi (*Trichoderma harzianum*, *Pythium* sp. and *Aspergillus niger*) and pathogenic fungi (*Fusarium* sp. and *Alternaria* sp.) of date palms. The confrontation test of two groups of microorganisms with the antagonists on nutrient agar revealed the inhibition of mycelia growth of the two fungi. The *Fusarium* sp. was inhibited with 68% by *T. harzianum*, 63% by *Pythium* sp. and 46% by *A. Niger*. As for the *Alternaria* sp., inhibition rates of 63% and 54% were exercised by *T. harzianum* and *Pythium* sp., respectively. Starting from the sixth day of the cultivation, the antagonistic fungi invaded the colonies of the pathogenic fungi and began to sporulate marking a very important myco-parasitic power.

Keywords: Date palm, biological control, *Fusarium* sp., *Alternaria* sp., antagonistic fungi, Algeria.

Résumé

Le palmier dattier (*Phoenix dactylifera* L.) est l'une des principales espèces cultivées dans le sud algérien. Cette spéculation a été confrontée à quelques problèmes phytosanitaires. Plusieurs champignons et bactéries antagonistes sont utilisés en lutte biologique. La présente étude a été menée afin d'étudier l'interaction entre des champignons antagonistes (*Trichoderma harzianum*, *Pythium* sp. et *Aspergillus niger*) et des champignons phytopathogènes (*Fusarium* sp. et *Alternaria* sp.) du palmier dattier. Le test de confrontation des deux groupes de microorganismes dans un milieu de culture artificiel (PDA) a révélé un effet inhibiteur très marqué. Le *Fusarium* sp. a été inhibé de 68% par *T. harzianum*, 63% par *Pythium* sp. et 46% par *A. Niger*. Quant à l'*Alternaria* sp., des taux d'inhibition de 63% et 54% ont été exercés par *T. harzianum* et *Pythium* sp., respectivement. A partir du sixième jour de la mise en culture, les champignons antagonistes envahissent les colonies des champignons phytopathogènes et commencent à sporuler et marquer un pouvoir myco-parasitaire très important.

Mots-clés: Palmier dattier, lutte biologique, *Fusarium* sp., *Alternaria* sp., champignon antagoniste, Algérie.

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1. INTRODUCTION

The date palm is one of the well adapted species to the arid, semi-arid and hot areas. It has the ultimate fruit of the desert where it plays at the same time an economic role thanks to the production of the dates which constitutes a base of human and animal consumption, and an ecological role since it confers on the oasis. Its structure makes protection against the wind and the projection of the desert while creating under its cover a microclimate favorable to the development of many additional cultures that condition the saving of the oasis and the stability of the populations which live there.

Date palms can be subject to many diseases or complexes of diseases, among which some are serious, can result an inescapable death. Many review works were published on the pathology of date palm; by many authors. Thus, laboratory studies were conducted into the pathology laboratory of the station of INRAA, 13 fungi were found on different parts of the dead palm trees. The following fungal types were identified: *Diplodia phoenicum*, *Theilaviopsis paradoxa*, *Phytophthora* sp., *Helminthosporium* sp., *Stemphylium botryosum*, *Alternaria* sp., *Aspergillus niger*, *Aspergillus* sp., *Cladosporium* sp. and *Fusarium* sp. (Lakhdari et al., 2013).

In order to look for other alternatives methods by using biological control against aggressive pathogenic fungi of date palm, in this work we studied the interaction between three antagonistic fungi isolated from potato culture of Oued Righ region in the experimental station of Sidi Mehdi (INRAA) against two pathogenic fungi which cause diseases on the date palm of the southeastern Algeria.

This work therefore consists by studying *in vitro* the interaction between these antagonistic and pathogenic fungi of palm trees by direct contact.

2. MATERIAL AND METHODS

2.1. Fungal agents

Infected vascular tissues from stem, leaflet and root parts of date palm cultivar showing symptoms were collected separately from farmer's field in the regions of Megarine and Beldet Omar which are situated in the southeastern of Algeria (Oued Righ) (Fig. 01).

The analytical techniques used were those usually practiced in a laboratory of plant pathology. Fragments of reached parts from 5 to 10 mm presenting of the typical symptoms are cut out then planted in a suitable culture medium after disinfection, rinsing with sterile distilled water, and then drying (Fig. 02). Incubation takes place at a temperature between 24-26 °C. Once well differentiated colonies, they will be then re-inoculated in the same medium of sowing (for obtaining the purified cultures).

The identification of fungal flora is not only carried out by the color and the shape of the colony but also from an examination of mycelia and conidia on the nutrient medium. We used the following identification keys: Barnett (1972) and Ellis (1971).

2.2. Antagonistic agents

For the isolation of the antagonist *Trichoderma harzianum*, we collected the leaves of the potato which have morbid symptoms were cut into small pieces and then subjected to disinfection with sodium hypochlorite (5%) for 3 min, then rinsed with sterile distilled



Figure 01: Infected palm trees

water. This operation aimed to eliminate saprophytic flora. After these, fragments were dried on a paper sterile filtered and aseptically placed on an agar medium (PDA) into Petri dishes with 3 fragments per dish. Incubation took place at 20 °C under a photoperiod of 12 hours (Fig. 03).

The other antagonistic fungi were isolated from soil (*Pythium sp.*) and palm trees (*Aspergillus niger*).

2.3. Method of mycelia growth measurement

The direct confrontation between the antagonistic fungi and the pathogens agent was studied by the method of Rapilly (1968). This method consists of putting on the same Petri, two agar pellets (5 mm diameter) one strain of the antagonist and other of pathogen are positioned along a diametrical axis 3 cm away (Figs. 04, 05). We have tested every antagonist on every pathogen (*T. harzianum* vs *Fusarium* sp., *T. harzianum* vs *Alternaria* sp.; *Pythium* sp. Vs *Fusarium* sp., *Pythium* sp. vs *Alternaria* sp. and *A. niger* vs *Fusarium* sp.) with three repetitions in every test; incubation is performed at 25°C for six days in the dark.

The evolution of mycelia growth was performed every 24 hours by measuring the diameter of the colony of the pathogen and the antagonist. The valuation of inhibition by the antagonist is estimated by calculating the percentage of inhibition of mycelia growth by the following formula:

$$I \% = [1 - C_n / C_o] \times 100$$

Cn: average diameter of colonies of pathogen in the presence of the antagonist.

Co: average diameter of colonies of control.

2.4. Statistical analyses

For the *in vitro* (on Petri plates) assays, the data were collected as mean colony diameter (average of two perpendicular diameters) values in each replication. These analyses were calculated by statistical software (STAT BOX 6.0.4., GRIMMERSOFT). The device is held in total uni-factorial randomization by Newman and Keuls test at 5% and 1% ($P \leq 0.05$ and $P \leq 0.01$). The antagonistic treatments (fungi species and untreated control) and *Fusarium sp.*, *Alternaria sp.* were the two fixed factors.



Figure 02: Sowing fungi

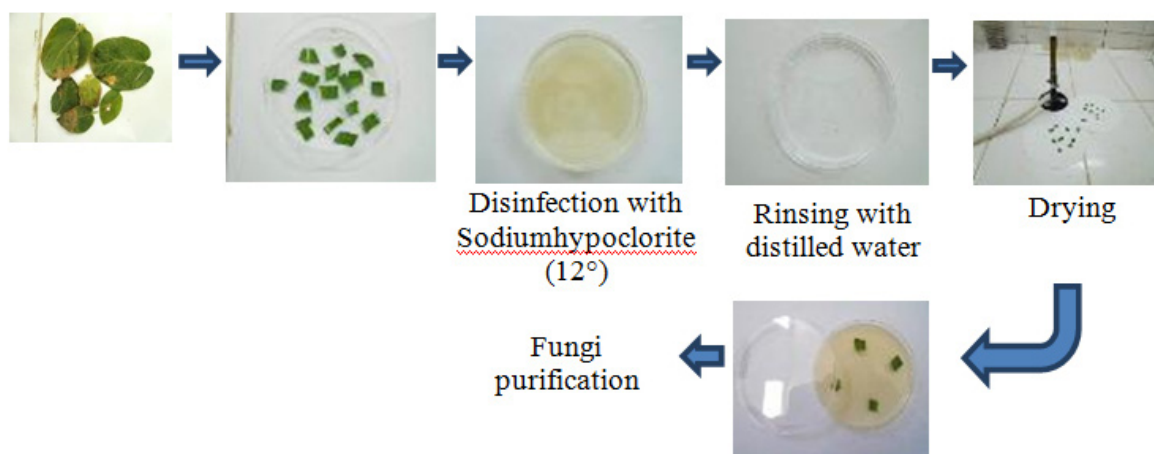


Figure 03: Sowing and purification of indigenous fungi

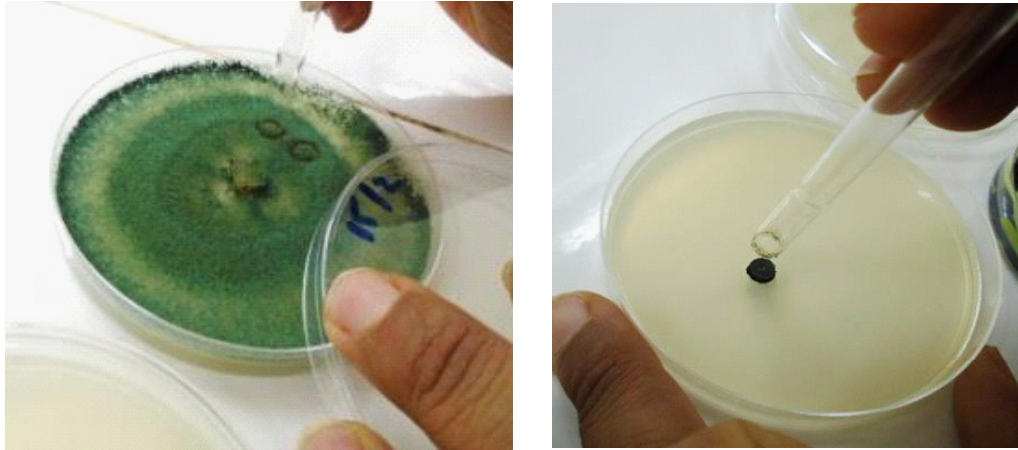


Figure 04: Antagonism method

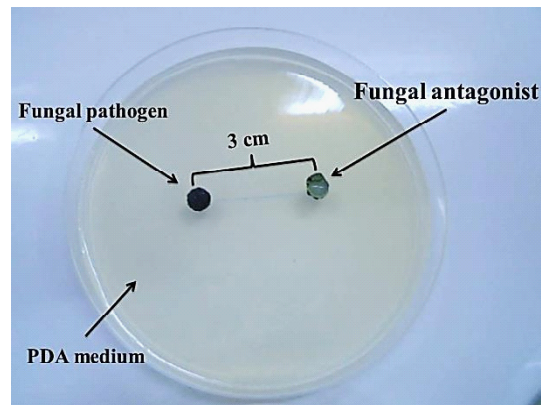
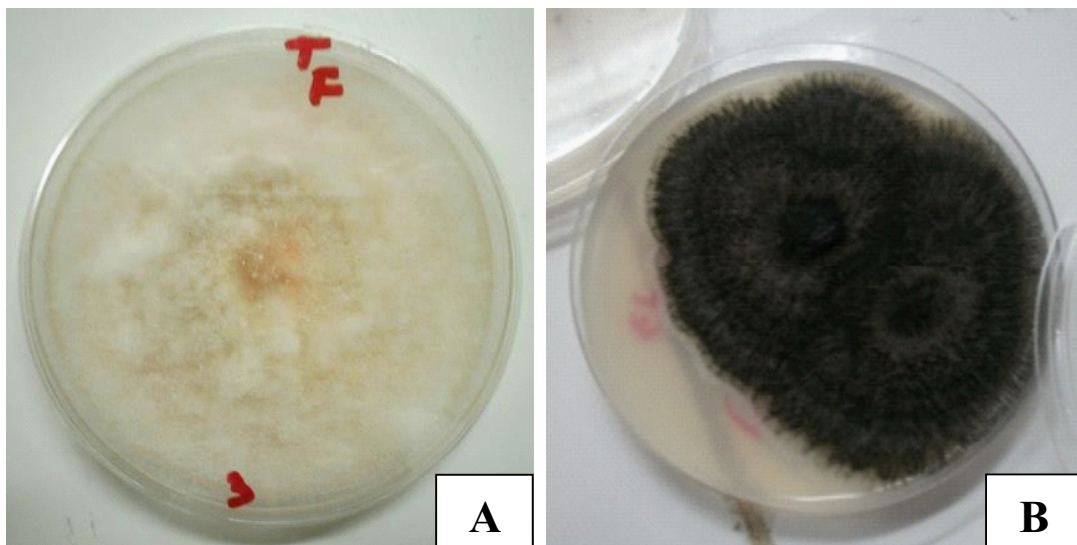


Figure 05: Measurement of mycelia growth

Figure 06: Fungal pathogens (A: *Fusarium* sp.; B: *Alternaria* sp.)

3. Results

3.1. Fungal antagonists and pathogens

Among the isolated fungi from the palm trees, we have observed that the most important fungi were *Fusarium sp.* and *Alternaria sp.* (Fig. 06) with an abnormal symptoms on the date palms; and according to the antagonism effects of several fungi, we were isolated from the soil of the experimental station of Sidi Mehdi (INRAA) some fungi which contain an antagonist characters like *T. harzianum* and *Pythium sp.*, with an addition one (*A. niger*) which were isolated from palm trees (Fig. 07).

3.2. Direct confrontation between *Fusarium sp.* and *Trichoderma harzianum*

Direct confrontation of *T. harzianum* and *Fusarium sp.* showed a faster mycelia growth of *T. harzianum* than of *Fusarium sp.* (Fig. 08). After three days of incubation, the Petri was completely filled by the antagonist, while the *Fusarium sp.* only occupied a surface of 12,33 mm diameter; which corresponded to an inhibition of mycelia growth greater than 68% (Tab. 01). The control of *Fusarium sp.* occupied an area of approximately 39 mm in diameter until the fifth day (Fig. 08).

Table 1: Direct confrontation between *Fusarium sp.* and *Trichoderma harzianum*

Days	Pathogen <i>Fusarium sp.</i>	Antagonist <i>T. harzianum</i>	Control <i>Fusarium sp.</i>	Inhibition percentage
1	4,5 mm	8 mm	5 mm	10 %
2	9,66mm	26,33 mm	15,16 mm	36 %
3	12,33 mm	39 mm	24,16 mm	50 %
4	12,33 mm	39 mm	34,16 mm	63 %
5	12,33 mm	39 mm	39 mm	68 %
6	12,33 mm	39 mm	39 mm	68 %

Table 2: Direct confrontation between *Alternaria sp.* and *Trichoderma harzianum*

Days	Pathogen <i>Alternaria sp.</i>	Antagonist <i>T. harzianum</i>	Control <i>Alternaria sp.</i>	Inhibition percentage
1	-	-	-	-
2	3,16 mm	3,83 mm	3,5 mm	10 %
3	5,75 mm	7 mm	10,25 mm	44 %
4	7,66 mm	10,66 mm	15,5 mm	51 %
5	8,66 mm	14,83 mm	18,16 mm	53 %
6	9 mm	18,33 mm	24,16 mm	63 %

3.3. Direct confrontation between *Alternaria sp.* and *T. harzianum*

Direct confrontation of *Trichoderma harzianum* and *Alternaria sp.* showed a faster mycelia growth of *T. harzianum* than that of *Alternaria sp.* (Fig. 09). After six days of incubation, the Petri was filled by the antagonist, with a remarkable growth rate. Whereas, *Alternaria sp.* occupied a surface area of 9 mm in diameter; with more or less aerial mycelium and a low growth rate compared to the antagonist. This correspond an inhibition of mycelia growth by 63% (Tab. 02).

3.4. Direct confrontation between *Fusarium sp.* and *Pythium sp.*

The antagonism test between *Pythium sp.* and *Fusarium sp.* showed a faster mycelia growth of *T. viride* than that of *Alternaria sp.* (Fig. 10). Four days after incubation, the Petri was completely filled by the antagonist, with an amazing growth rate. Whereas, *Fusarium sp.* occupied a surface area of 2,25 mm in diameter (Fig. 10). This antagonist correspond an inhibition of mycelia growth by 63% (Tab. 3).

3.5. Direct confrontation between *Alternaria sp.* and *Pythium sp.*

The results presented in the table below, the direct confrontation of *Pythium sp.* and *Alternaria sp.* showed a faster mycelia growth of *Pythium sp.* than of *Alternaria sp.* (Fig. 11). After four days of incubation, the Petri was completely filled by the antagonist, while the *Alternaria sp.* only occupied a surface of 9 mm diameter; which reached to an inhibition of mycelia growth greater than 54% (Tab. 4). The control of *Alternaria sp.* occupied an area of approximately 28,5 mm in diameter until the sixth day (Fig. 11).

Table 3: Direct confrontation between *Fusarium sp.* and *Pythium sp.*

Days	Pathogen <i>Fusarium sp.</i>	Antagonist <i>Pythium sp.</i>	Control <i>Fusarium sp.</i>	Inhibition percentage
1	-	-	-	-
2	1 mm	13 mm	3 mm	67%
3	2,25 mm	32,25 mm	4,75 mm	53%
4	2,25 mm	40,75 mm	6,62 mm	66%
5	3,45 mm	43,5 mm	8,5 mm	59%
6	3,75 mm	45 mm	10,25 mm	63%

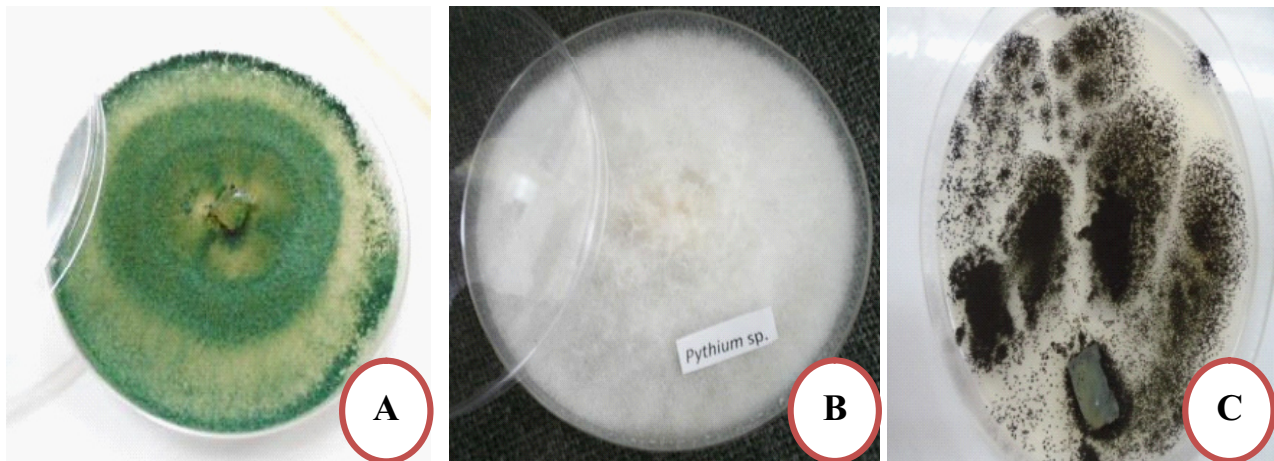


Figure 07: Fungal antagonists (A: *T. harzianum*; B: *Pythium* sp.; C: *A. niger*)

Figure 08: Direct confrontation between *Fusarium* sp. and *Trichoderma harzianum* after six days of incubation

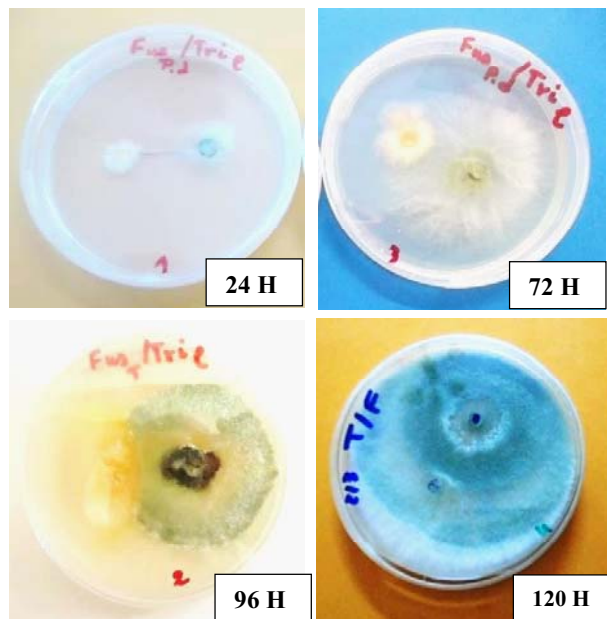


Figure 09: Direct confrontation between *Alternaria* sp. and *Trichoderma harzianum* after six days of incubation

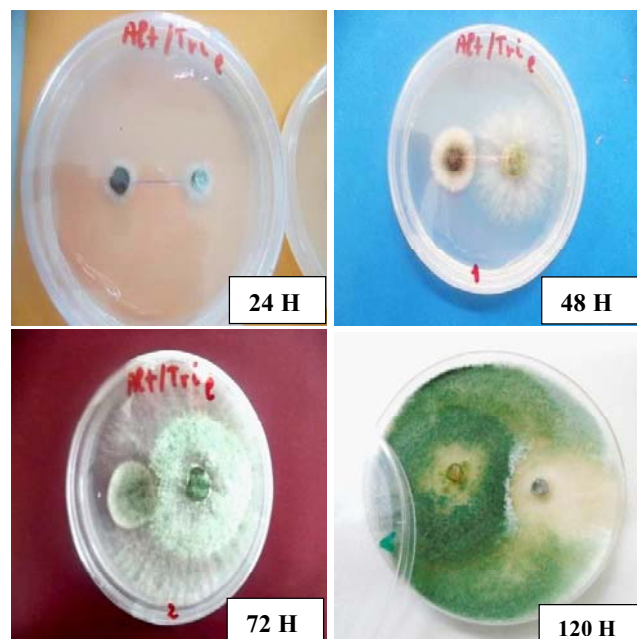


Table 4: Direct confrontation between *Alternaria sp.* and *Pythium sp.*

Days	Pathogen <i>Alternaria sp.</i>	Antagonist <i>Pythium sp.</i>	Control <i>Alternaria sp.</i>	Inhibition percentage
1	-	-	-	-
2	3,83 mm	18,33 mm	5,66 mm	32%
3	8,66 mm	32 mm	12 mm	28%
4	9 mm	38 mm	17,83 mm	50%
5	12,16 mm	47,16 mm	23,5 mm	48%
6	13 mm	47,5 mm	28,5 mm	54%

Table 5: Direct confrontation between *Fusarium sp.* and *Aspergillus niger*

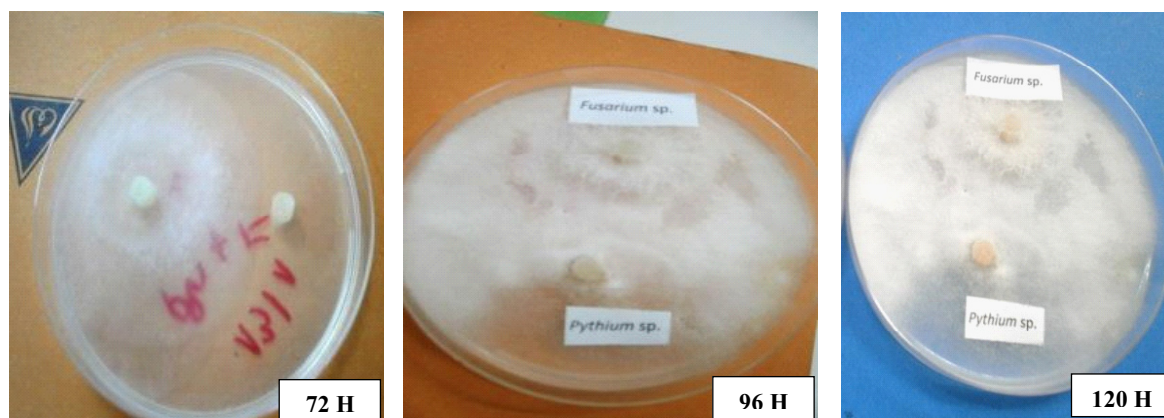
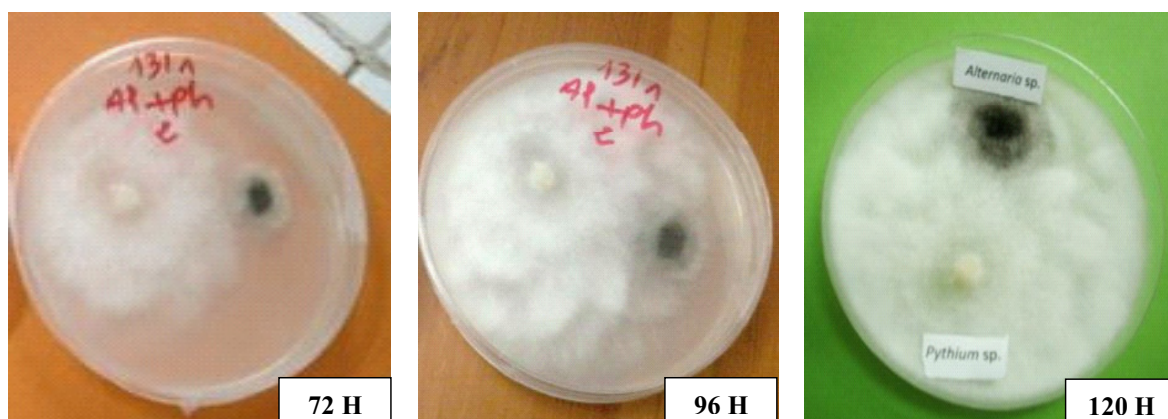
Days	Pathogen <i>Fusarium sp.</i>	Antagonist <i>A. niger</i>	Control <i>Fusarium sp.</i>	Inhibition percentage
1	-	-	-	-
2	1,66 mm	2 mm	3 mm	45%
3	2,25 mm	3,16 mm	4,75 mm	53%
4	3,33 mm	3,66 mm	6,62 mm	50%
5	5,5 mm	29,66 mm	8,5 mm	35%
6	5,5 mm	45,16 mm	10,25 mm	46%

3.6. Direct confrontation between *Fusarium sp.* and *Aspergillus niger*

The data of the table below showed us a great inhibition between the antagonist *Aspergillus niger* and the pathogen *Fusarium sp.* (Fig. 12). After six days of incubation, the Petri was completely filled by the antagonist, while the *Fusarium sp.* occupied a surface of 5,5 mm diameter; which corresponds to an inhibition of mycelia growth greater than 46% (Tab. 5).

3.7. Statistical analyses

The statistical analyses showed that all treatments tested on the pathogenic fungal of palm trees had a significant effect. These results confirm that there is an inhibition caused by the antagonistic fungi against the two pathogenic fungi.

**Figure 10:** Direct confrontation between *Fusarium sp.* and *Pythium sp.* after six days of incubation**Figure 11:** Direct confrontation between *Alternaria sp.* and *Pythium sp.* after six days of incubation

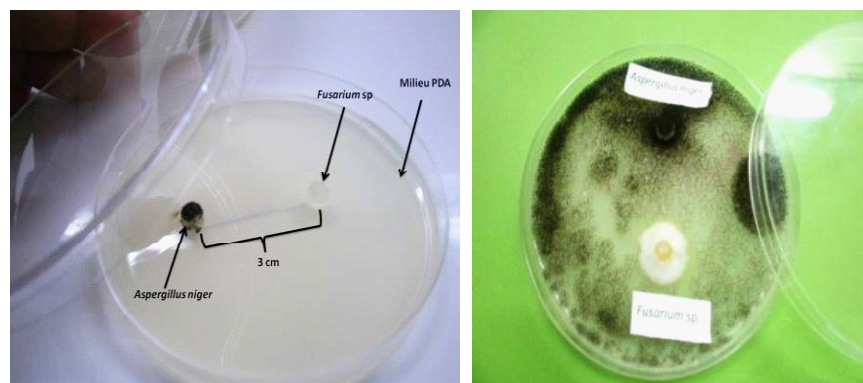


Figure 12: Direct confrontation between *Fusarium sp.* and *A. niger* after six days of incubation

4. Discussion

Whipps and McQuilen (1993) reported that *A. niger*, *A. terreus*, *P. citrinum*, *T. harzianum* and species of *Bacillus* control soil-borne diseases (In Alwathnani, 2012). According to the last author, biological control of *Fusarium oxysporum f. sp. lycopersici* by *A. niger* revealed a rate of 35,6% and by *T. harzianum* was about 44,4%.

The *Trichoderma* have been known a long time ago by their antagonistic activities against many fungi such as: *Fusarium solani var coeruleum*, *Fusarium roseum var sambucinum* and *Fusarium roseum var graminearum* (Daami-Remadi, 2001); *Alternaria alternata* (Gveroska & Ziberoski, 2011); *Fusarium oxysporum f. sp. Lentis* (Essalmani & Lahlou, 2004).

Also Sundaramoorthy and Balabaskar (2013) have found that the efficacy of *Trichoderma spp.* against wilt of tomato caused by *Fusarium oxysporum f. sp. Lycopersici* note an important rate of 57%. Therefore, the antagonist *T. harzianum* is chosen to be the most promising bio-control agent for *F. oxysporum f. sp. lycopersici*.

The same author said that the mycelia growth inhibition of *F. oxysporum* (60%) at the 5th day in the confrontation plate assay was negatively correlated with mycelia growth of *F. oxysporum* in the presence of *T. harzianum*.

Allowing to Jat & Agalave (2013), in an investigation *Trichoderma harzianum* hampered the growth of *Alternaria alternata* by 48.33% but Hussainet al., (2009) found that *Trichoderma harzianum* reduced the growth of *Alternaria alternata* by 67.7%.

Chanchal (2014), in antagonistic tests, found that *T. harzianum* inhibited 70% growth of *Fusarium moniliforme* and 55% of *Fusarium sacchari*.

The results of Bouziane (2011), showed that the inhibition of mycelia growth in the direct confrontation between *T. harzianum* and *Alternaria sp.* is about 34%.

Alkatatnyet al. (2007) found that the fungal antagonist *Pythium oligandrum* inhibited the fungal pathogen *F. oxysporum* by 100%.

Bouneghou (2011) noted that the mycelium growth of *Alternaria alternata* was inhibited by *Pythium sp.* with an inhibition percentage of 50.3%, unlike, *Fusarium roseum* which was inhibited less than the first pathogen with 40%.

Conclusion

The present evaluation gave clear indication that the isolates of *Trichoderma harzianum* (isolated from leaves of potato), *Pythium sp.* (isolated from soil) and *Aspergillus niger* (isolated from palm trees) are strong and virulent antagonists.

Indeed, the direct confrontation tests between the pathogen (*Fusarium sp.* and *Alternaria sp.*) and antagonists (*T. harzianum*, *Pythium sp.* and *A. niger*) on a medium culture (PDA) have shown an inhibition of the pathogen mycelia growth tested. the Confrontation of the fungal pathogen with the antagonists revealed the inhibition of mycelia growth of the three fungi: *T. harzianum* with (*Fusarium sp.* = 68% and *Alternaria sp.* = 63%); *Pythium sp.* with (*Fusarium sp.* = 63% and *Alternaria sp.* = 54%) and *A. niger* with *Fusarium sp.* = 46%).

These results allowed us to believe that these three fungal antagonists have ability to remove *Fusarium sp.* and *Alternaria sp.* from date palm and can be tested *in vivo* and have practical interest in the biological control against diseases of this culture.

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