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Evaluation of Endophytic Microorganisms (*Trichoderma hazianum* and *Bacillus subtilus*) as Biofungicides against Bayoud Disease (*Fusarium oxysporum* f. sp. *albedinis*) on Date Palm (*Phoenix dactylifera* L.)

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ABSTRACT

Backgraound: Date palm disease named Bayoud, is the major constraint caused by the soil fungus *Fusarium oxysporum* f. sp. *albedinis* (Foa) in Algeria. This work has the objective to *in vivo* biocontrol on bayoud disease incidence on three date palm varieties (Deglet-Nour, Feggous and H-mira), using two antagonistic microorganisms, one fungus (*Trichoderma harzianum*) and one bacterium (*Bacillus subtilus*).

Methods: Foa strains obtained from isolation of the spines carrying the typical symptoms of Bayoud disease. Two strains used *T. harzianum* and *B. subtilus*, are isolated from rhizosphere soils of the date palm. Using the greenhouse screening test, the *in vivo* biocontrol test is carried out on 3-4 month-old date palm seedlings at the rate of 5 ml of inoculum suspension.

Result: A significant ($P<0.01$), difference of date palm seedlings reactions against Foa isolate and antagonistic microorganisms was observed. All three date palm varieties present a high susceptibility against Foa until the total mortality of date palm seedlings. The biocontrol test showed that two antagonists showed different reaction, with the complete resistance of three varieties treated by *T. harzianum*, while, the antagonist *B. subtilus* protect just two varieties (Deglet-Nour and H-mira) and can't protect Feggous variety when showed the susceptibility reaction by presentation of typical symptoms of bayoud disease. We can apply of these antagonistic microorganisms to protect our groves contaminated by Bayoud disease and contain just susceptible commercial cultivars of date palm.

Key words: Antagonist, *Bacillus subtilus*, Bayoud disease, Biocontrol, Date palm, Management, *Trichoderma harzianum*.

INTRODUCTION

The phoeniciculture is an essential element in the agro-economic development of oases (Bouguedoura *et al*., 2015; Vinita and Punia, 2016). 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 Fusarium 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 and Boufeggous in Morocco and Deglet Nour and Feggous in Algeria, but all are susceptible to this parasite (Sedra 2005a,b ; Djerbi, 2003). It's originated from Morocco and its progression continues to advance from the west and central of Algerian palm groves (Benzohra *et al*., 2015; 2017). The losses reported by Djerbi (2003), estimated at more than 3 million palms are in a clear evolution, especially since several unaffected regions in the past are reconsidered as contaminated by Bayoud. This disease 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). The proposal used of prophylactic measures against this disease doesn't seem able to stop its progression,

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especially since the epidemiological data are favored by a progressive spread of this disease, but these measures are important for southern Algeriangroves when the Bayoud is absent (Benzohra *et al*., 2017). Other method was used, a genetic control by resistant varieties is therefore another promising way to reduce this constraint, but unfortunately all resistant cultivars haven't high food quality in date fruits (Sedra, 2005b).

To control and limit this constraint, especially in contaminated groves which contain the susceptible varieties, the biological control through the use of antagonistic microorganisms and mycorrhizal species remains the best way to combat this threat in these palm groves (El-Hassni *et al*., 2007; Jaiti *et al*., 2006; Jaiti *et al*., 2008; Dihazi *et al*., 2012; Benzohra and Megateli, 2017; Belaidi *et al*., 2020).

The objective of this work is to evaluate the capacity of *in vivo* effect in two antagonistic microorganisms *Trichoderma harzianum* and *Bacillus subtilus* on the incidence of bayoud disease by testing of three important commercial date palm varieties, but susceptible to Bayoud diseases (Deglet-Nour, Feggous and H-mira).

MATERIALS AND METHODS

Date palm varieties

Three date palm varieties are used in the *in vivo* biocontrol test. These varieties are: Deglet-Nour, Feggous and H-mira (Fig 1). The Deglet-Nour is cultivated in the South-Central and East palm groves of Algeria and H-mira is cultivated in Saoura oases (South-West groves of Algeria).

Fungal material

The Foa isolate used in the inoculation test of date palm seedlings, was sampled from contaminated groves in Ghardaia province in 2016. The antagonistic microorganisms were obtained from soils of date palm rhizosphere of Bechar region in 2019 (Table 1). These strains of microorganisms used in this study provided by CRSTRA (Center for Scientific and Technical Research on Arid Regions, Biskra, Algeria), from laboratory of Plant Pathology and Protection, of Experimental Station of Biophysical Environment, Naama, Algeria. All isolates were conserved in PDA cultures (For 1000 ml : 200 g potato filtrate; 15 g glucose; 20 g agar-agar; pH=5,5), (Fig 1), with temperature of conservation at 04°C and incubation at 23°C (El-Hassni *et al*., 2007).

Preparation of inoculum

Date palm seedlings (2-3-months-old) were obtained by pre-germination of date palm seeds after their disinfection with Hypochlorite of Sodium at 2%, then rinsed with sterile distilled water three successive times (Sedra, 2005b). The date palm seeds are placed in Petri dishes containing the cotton soaked with sterile distilled water and then stored in the oven at a temperature of 23±2°C (El-Hassni *et al*., 2007). After 20 days of pre-germination, the seeds are transplanted into pots containing sterile peat for two months (Sedra, 1985).

The biocontrol test based on use of mixture 'Foa inoculum + antagonistic', this mixture is considered as treatment and control for plants treated with Foa inoculums alone taking account the same condition as above. Eight seedlings were used per treatment. After injection, seedlings were conserved in small greenhouse at 25±2°C with 16 h photoperiod.

Rating scale

Symptomsrating based on mortality rate of date palm seedlings. This rate mortality based on the calculation of dead plants compared to all the plants inoculated (Sedra, 2005b). The mortality rate (mr) formula is written as follows:

$$mr = \frac{\text{Number of dead plants}}{\text{Total number of inoculated plants}} \times 100$$

Statistical analysis

The experiments schemes were randomized complete blocks. Eight replicates (number of seedlings tested), per treatment were performed and each whole experiment was performed independently. All the collected data were submitted to ANOVA analysis using XLStat software 9 (AddinSoft, USA Ltd.) and the significance of differences among treatments was recorded at $P<0.05$. Multiple comparisons of the means were conducted according to the Newman-Keuls test at $P<0.05$.

RESULTS AND DISCUSSION

The statistical analysis of results showed significant differences between the date palm varieties and also between two antagonistic microorganisms (Table 2). The results showed a high level of effectiveness of the two microorganisms on theincidence of Bayoud disease of date palm compared to the control. The control (direct inoculation with Foa alone), presented a very high mortality rate up to 100% (Table 2), with typical symptoms of bayoud disease by vascular wilt and discoloration (Fig 2). The antagonistic fungus *T. harzianum* showed the total absence of bayoud disease symptoms without mortality rate (*rm*) observed (0%), (Table 2). The second species, the bacterium *B. subtilus* showed an antagonistic behavior less than the fist species (*T. harzianum*), withrm value varied according to the date palm variety. In two varieties (Deglet-Nour and H-mira), *B. subtilus* showed the total absence also of typical symptoms of bayoud (0%), but in the variety Feggous, the *rm* values was 50%. In comparison between these two species, we can say that the fungus *T. harzianum* was more efficiency than the bacterium *B. subtilus*.

Our findings in this study show that the antagonistic microorganisms tested have significant biocontrol activity against Bayoud disease when tested by an *in vivo* biocontrol on bayoud disease incidence. These microorganisms are: one fungus named *Trichoderma harzianum* and one bacterium named *Bacillus subtilus*

Table 1: Microorganisms used in the *in vivo* biocontrol against bayoud disease.

Microorganisms	Microorganisms information		
	Origin of sampling	Date of sampling	Strain type
Fusarium oxysporum f. sp. *albedinis*	Atteuf, Ghardaia	2016	Parasite
Trichoderma harzianum	Mostaganem	2018	Antagonistic
Bacillus subtilus	Bechar	2019	Antagonistic

Table 2: Mortality rates and reaction of date palm varieties in the biocontrol results against bayoud disease.

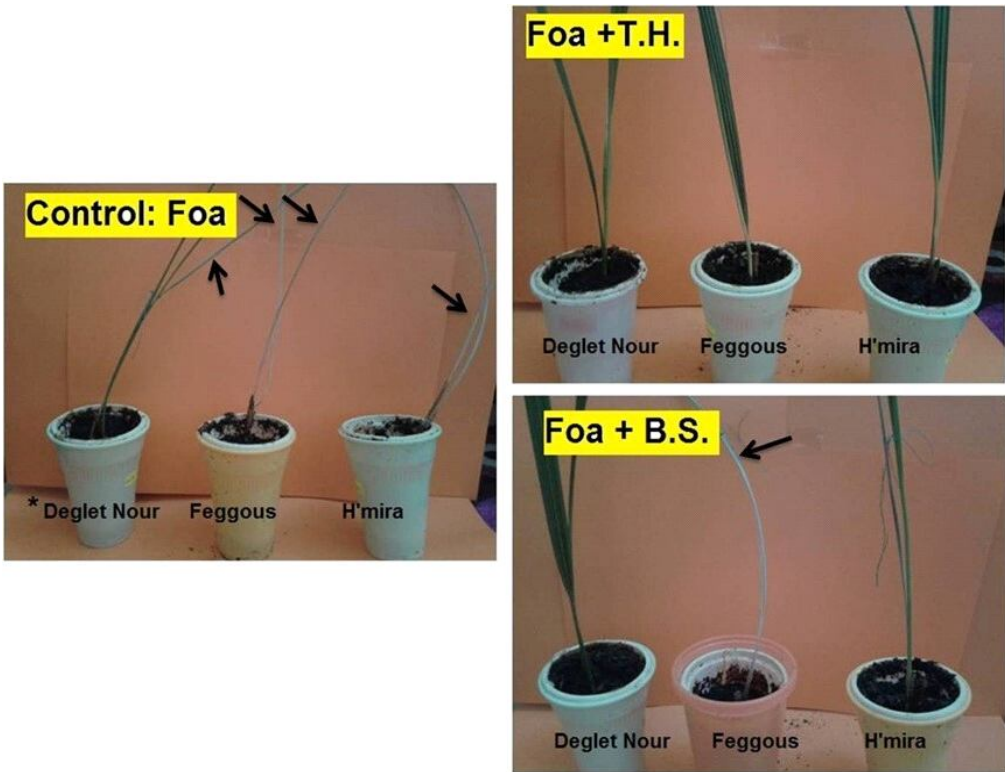
Date palm varieties	Mortality rate (%)		
	Control (Foa isolate)	Foa + *T. harzianum*	Foa + *B. subtilus*
Deglet-Nour	100[a]*	00[c]	00[c]
Feggous	100[a]	00[c]	50[b]
H-mira	100[a]	00[c]	00[c]
Deglet-Nour	S	R	R
Feggous	S	R	S
H-mira	S	R	R

✓*Mean of 08 replicates (date palm seedlings). In the column, values having the same letter are not statistically different (P 0.05) according to the LSD test.

✓Foa: *Fusarium oxysporum* f. sp. *albedinis*; S: Susceptible reaction; R: Resistant reaction.

✓The mortality rate (%) was calculated according to dead date palm seedlings compared with total seedlings tested.

Fig 1: Fungal and plant material used in the *in vivo* biocontrol against bayoud disease.



Foa: *Fusarium oxysporum* f. sp. *albedinis*; antagonistic microorganisms: T.H.: *Trichoderma harzianum*, B.S.: *Bacillus subtilus*; *: Date palm varieties names.

Fig 2: Reactions of three date palm varieties against Bayoud disease in presence of two antagonistic microorganisms.

(Table 2), using screening test based on inoculation by Foa isolate of three important date palm varieties in Algeria (Deglet-Nour, Feggous and H-mira). The mortality rate varied significantly according to the type of antagonist. The absence of mortality rate was observed using *T. harzianum*, while the bacterium *B. subtilus* showed the efficiency two varieties (Deglet-Nour and H-mira), while the Feggous variety showed the mortality arrived at 50%. In comparison between these two antagonistic microorganisms, the fungus *T. harzianum* is more efficient than the bacterium *B. subtilus*.

Many reports from the world has been published about the importance of biological control as the best way to combat Fusarium wilt (Alabouvette, 2006; Sajeena *et al*., 2020; Cheng *et al*., 2019; Abed *et al*., 2016; Ou-Zine *et al*., 2020). This method of control can enhance the plant defense machanisms against Fusarium disease and also other phytopathogenic fungi (El-Hassni *et al*., 2007).

The importance of antagonistic fungi like *Trichoderma harzianum* as biofungicide against fusarium wilt has been reported by Kala *et al*. (2016), against Fusarium wilt of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceris*, by Sahampoor *et al*. (2020), on Fusarium wilt of cucurbits caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* and by Fitrianingsih *et al*. (2019), on Fusarium wilt oftomato (*Lycopersicum esculentum* L.) caused by *Fusarium oxysporum* f. sp. *radicis-lycopersisci*.

The antagonistic bacterium *Bacillus subtilus* was also reported by many authors against Fusarium wilt diseases. Among these fusarium wilt, we can cite: *Fusarium oxysporum* f. sp. *ciceris* of chickpea (*Cicer arietinum* L.), (Zaim *et al*., 2013; 2018; Rouag *et al*., 2019; Suthar *et al*., 2019), *Fusarium oxysporum* f. sp. *radicis-lycopersisci* of tomato (*Lycopersicum esculentum* L.), (Estefania and Ligia, 2018) and *Fusarium oxysporum* f. sp. *abedinis* of date palm (*Phoenix dactylifera* L.), (El-Hassni *et al*., 2007).

In this study, these two antagonistic microorganisms (*T. harzianum* and *B. subtilus*), can enhance the ability to resist bayoud disease by the three date palm varieties used in the *in vivo* biocontrol test (Deglet-Nour, Feggous and H-mira).

CONCLUSION

This work had the objective to *in vivo* biocontrol based on the use of two antagonistic microorganisms, one fungus species*Trichoderma harzianum* and one bacterium *Bacillus subtilus,* against Bayoud disease of date palm caused by the soil fungus *Fusarium oxysporum* f. sp. *albedinis* (Foa), on three date palm varieties (Deglet-Nour, Feggous and H-mira). The results of this study showed significant differences between the two antagonistic microorganisms as well as the control (inoculation with Foa strain inoculum). If we make a comparison between

two antagonistic species, we can say that *T. harzianum* is more efficient than that species *B. subtilus*, *rm*% values equal to 0% for the first, 50 for the second, respectively.

In conclusion, the use of biological agents like antagonistic microorganisms to enhance the resistance in the susceptible varieties against this pathogen represents an ecologically friendly alternative to pesticides repeatedly used to control this kind of plant diseases. This strategy has more significance against soil-borne pathogens such as bayoud disease on date palm in our contaminated palm groves, especially in south-west region of Algeria, for fighting strategies are very restricted or no large used by our farmers.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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