POWERFUL STRAINS OF *PSEUDOMONAS* SPP. FLUORESCENT TESTED ON FLAX UTILISED IN THE BIOLOGICAL CONTROL OF BAYOUD

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(Manuscript received 7 June 2007)

Abstract

Fusarium wilt of date palm caused by the fungus *F oxysporum* f.sp. *albedinis* (Kill and Mayor) Malençon known under the name of "bayoud", is generally considered one of the most dangerous and menacing disease of date palm in the north Africa. In Algeria, several famous commercial varieties and clones of very high fruit quality were extinct and others can disappear because of their susceptibility towards the bayoud disease. In order to preserve these plants, one strategy to control Fusarium wilt is the use of antagonistic root-colonizing *Pseudomonas* spp. It has been demonstrated that different strains of these bacteria suppress disease by different mechanisms. Application of these biocontrol strains in interaction with the flax, who is often used as an indicating plant in the place of date palm, which requires one longer period to have the appearance of symptoms of bayoud. The ability to suppress Fusarium wilt of flax was tested *in vivo* in presence and absence of indigenous microflora (disinfected soil and original soil) in presence of F.o.ln (=*F. oxysporum* f.sp. *lini*). The strains used of different origins showed a beneficial effect in the suppression of disease. Antagonistic activities of DN20 strain isolated from the date palm and the strain of reference D2 strains in presence of *Fusarium oxysporum* f.sp. *lini* is very brought closer under the conditions of our experiments. The effect of antagonism in the original soil (not disinfected) for a going rate of inhibition from 94.45% to 100% more outstanding remainder, whereas in the disinfected soil, it is of about an 18.43% to 42.12%. A good antagonistic activity was recorded for DN20 strain under the two conditions of our experiments; on the other hand the antagonistic activities noted for the D2 strain are variable in the two types of tests. This indicates the beneficial effect of DN20 strain and the necessity of their application on his plant origin (the date palm).

Key words: Fusarium wilt, date palm, bayoud, flax, *F oxysporum* f.sp. *albedinis*, *F. oxysporum* f.sp. *lini*, *Pseudomonas* spp., biological control.

INTRODUCTION

The "bayoud" which is a disease of date palm, it is endemic in the North of Africa and was initially announced in 1870 in Morocco. In Algeria, the "bayoud" prevails in several palm plantations of Algerian south in particular in the areas of Gourara (Timimoun), Touat (Adrar) and Mzab (Ghardaia) (Tantaoui, 1996) where it destroyed more than three million palm trees (Fernandez and *al.*, 1997). The causal

agent of fusarium wilt of date palm is *F oxysporum* f.sp. *albedinis* (Kill and Mayor) Malençon. The first symptom of this disease manifest by the appearance of a gray colouring ashed on the level of a palm of the average crown, which takes a leaded aspect. Thereafter, the leaflets and the spines of this palm desiccate unilateral way gradually upwards and are folded up towards the rachis (attacks hemiplegics) while taking a blank colour from where the Arab name of bayoud (Louvet, 1972; Djerbi, 1988). Drying continues other with dimensions of the palm while progressing this from top to bottom time. The desiccated palm takes the aspect of "wet feather" and becomes hanging along feather-grass (Bounaga–Rivielle, 1985 ; Djerbi, 1988). The disease then extends to the others rachis, which show brownish scratchs. The central bouquet formed by the young palms remains green. At an advanced stage of the disease, the totality of the final bud is desiccated thus resulting in the death of the tree (Louvet, 1974).

The fight against this disease, it is not always easy. The systemic nature and the epidemic and infectious character, limit the possibilities of fight and prevention against this disease. The use of systemic fungicides remains the means more used to fight against the latter in spite of the risk of appearance of resistant strains. The biological control using the potentialities of the antagonism of *Pseudomonas* spp. fluorescent with respect to the populations of *F oxysporum to* fact the object of several research tasks (Kloepper and *al.*, 1980; Alabouvette, 1990; Duijff and *al.*, 1991; Lemanceau and Alabouvette, 1991; Lemanceau and *al.*, 1992; Lemanceau and Alabouvette, 1993 ; Alabouvette and *al.*, 1993; Benchabane and *al.*, 2000). This work completed under experimental or natural conditions showed the effectiveness of fluorescent *Pseudomonas* spp. in the presence of special forms of *Fusarium oxysporum* (Scher and Baker, 1982; Van Peer and *al.*, 1990). *Pseudomonas* spp. fluorescent belong to the group of PGPR (= "Plant Growth Promoting Rhizobacteria") which are known for their intervention in the improvement of the growth of the plants like in the biocontrol of soil-born pathogens agents. They stimulate the growth of plants by the improvement of their mineral food and by the synthesis of substances of growth (direct action). Moreover, they intervene in the biocontrol of soil-born pathogens agents by their nutrient competition and the phenomenon of antibiosis (indirect action).

The objective of this study is based on the effectiveness of some strains of *Pseudomonas* spp. fluorescent for the suppression of special form of *F oxysporum*: *F O.* f.sp. *lini* in interaction with the flax, who is often used as an indicating plant in the place of date palm, which requires one longer period to have the appearance of the symptoms of bayoud. The test is carried out in presence and absence of indigenous microflora (disinfected soil and original soil).

MATERIALS AND METHODS

Biological material

The fluorescent strains of *Pseudomonas* spp. were isolated from the environment rhizospheric of tomato, date palm and a naked soil. These strains assembled a significant antagonistic capacity *in vitro*. The pathogenic agent is *F O.* f.sp. *lini* comes from the collection of laboratory of biology of soil in Dijon–France. The plant host is the flax (*Linum usitatissinum* var. opaline). The soil used disinfected and not disinfected (natural) is taken on the level of a parcel under greenhouse of ITCMI (Staoueli - Algeria). The D2 strain was used as a reference strain (Table 1).

Table 1. Strains selected for this study

Strains	Plant host	Origin
D2	Flax	Laboratory of Biology of soil (INRA- Dijon France).
F.o.ln (=*F. oxysporum* f.sp. *lini*)	Flax	Laboratory of Biology of soil (INRA Dijon, France).
DN20	Date palm (Algeria).	Laboratory of Biology of soil Blida
S5	Naked soil	Laboratory of biology of soil Blida (Algeria).
TRS24	Tomato	Laboratory of biology of the soil Blida (Algeria).

Experimental device: The test *in situ* of antagonism was carried out under greenhouse of glass according to an experimental device in complete random blocks with four repetitions. Each block contains the six treatments:

- T1: witness seedlings (witness negative) inoculated with distilled water sterilised
- T2: Seedlings inoculated with DN20 strain and the pathogen.
- T3: Seedlings inoculated with the S5 strain and the pathogen.
- T4: Seedlings inoculated with TRS24 strain and the pathogen.
- T5: Seedlings inoculated with the D2 strain and the pathogen.
- T6: witness seedlings (witness positive) inoculated with the pathogen.

Each treatment contains three seedlings is on the whole (3 ×6) 18 seedlings per block. Thus, each

Treatment is illustrated in the device by (3 ×4) 12 seedlings is on the whole (12 ×6) 72 seedlings.

Soil preparation: The soil comes from a parcel under greenhouse of ITCMI (Staoueli-Algeria). It was used in a natural state with its indigenous flora and after its

disinfection at 120°C during two hours twice of continuation separated by 24 hours. The disinfected soil or not was distributed in 100g/ pot.

Bacterial inoculations: The seeds of flax are disinfected during 20 minutes with bleach titrating 6°, then they are rinsed with sterile distilled water, then they are dried and sown at a reason of two seeds per pot. The sown pots are deposited under a greenhouse of glass and are sprinkled daily with sterile water. After three weeks, the seedlings of flax are bacterised on the level of the snare by bacterial suspensions having a concentration of 5 X 10^5 CFU/g of soil. These suspensions were prepared from bacterial creams of 24 hours old cultivated in the medium KB (King et *al.*, 1954) then transferred in 100 ml from plug MgSO4, $7H_2O$.

Fungus inoculations: The suspensions of conidia were obtained starting from fungus of 10 days old cultivated on PDA medium. The concentration of the suspensions of conidia prepared in the plug $MgSO_4$, $7H_2O$ is adjusted to 10^7 conidia/ml. The inoculation of F.o.In. was carried out 24 hours after the contribution of the bacteria laugh at a reason of 5 ml per pot.

Estimation of the level of infection of fusarium wilt of flax: The level of infection was carried out using a scale given by Fuchs and Defago (1991). This scale comprises five degrees of notation.

- 0 = No symptom
- 1 = partial Yellowing
- 2 = wilting of the top in the form of arc Shepherd's Crook
- 3 = wilting of half of the plant
- 4 = wilting of the whole plant
- 5 = Died of the plant.

Daily observations on the health state and the degree of infection of seedlings were carried out for 12 weeks. The index of disease and its severity were then estimated according to the method used by Fuchs and Defago (1991). The index of disease expressed as a percentage was calculated according to the following report:

I = [A number of sick seedlings/a total number of seedlings] X 100

The severity of disease was evaluated according to the equation given below which takes into account the degrees of notation of the scale of Fuchs and Defago (1991):

$S = \sum (E.a / N.T) \times 100$

where

S = the severity of the expressed as a percentage disease

E = the varying degree of notation from 0 to 6 for the flax.

A = the number of sick seedlings of the degree of notation 6.

N = the number of sick seedlings.

T = the value of the highest degree of notation which is 6.

In the case of significant difference, the comparison of the treatments was carried out according to the smallest significant amplitude of test of Newman and Keuls to the threshold of risk of error α = 5% (Dagnelie, 1975).

RESULTS

Antagonistic activity in the disinfected soil

Index of infection: The number of sick seedlings is less significant compared to the positive witness. The enumeration of the sick seedlings is carried out two weeks after the fungus inoculation for the sick witness as well as the treatments associated with the bacterial strains S5, TRS24 and D2. For the treatment [DN20 +F.o.ln.], counting began three weeks after the fungus inoculation, to reach its maximum at the end of the test (Table II). After twelve weeks of inoculation, the sick witness showed an index of infection of about 83.33%. For the bacterised treatments, the highest index of infection (75%), was noted for the bacterial strain S5 and the lowest rate of infection (58.33%) is obtained in interaction with the bacterial strain D2 (Table II). The comparison of the indices of infections showed a significant difference. The test of Newman and Keuls to the threshold α = 5%, made possible to classify the treatments studied in three homogeneous groups. The sick witness and treatment [S5 + F.o.ln.] are classified in group A, and the other treatments in the group B, the group C contains the healthy witness (Table II).

Table II: Evolution of the index of the fusarium wilt (%) on the seedlings of the flax according to time

Treatments	Week After The Inoculation Numbers											
	1	2	3	4	5	6	7	8	9	10	11	12
Sick witness (T+)	0	25.0	33.3	58.3	83.3	83.3	83.3	83.3	83.3	83.3	83.3	83.33 (A)*
DN20 + F.o.ln.	0	0.0	8.3	16.7	16.7	33.3	58.3	58.3	66.7	66.7	66.7	66.66 (B)*
S5 + F.o.ln.	0	16.7	41.7	58.3	75.0	75.0	75.0	75.0	75.0	75.0	75.0	75.00 (A)*
TRS24+ F.o.ln.	0	8.3	33.3	33.3	33.3	33.3	58.3	66.7	66.7	66.7	66.7	66.66 (B)*
D2 + F.o.ln.	0	0.0	8.3	16.7	16.7	25.0	33.3	58.3	58.3	58.3	58.3	58.33 (B)*
Healthy witness (T-)	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	00.00 ©*

*: Values followed by the same letter are not significantly different according to the test from Newman and Keuls to the threshold α = 5%.

-Severity of the fusarium wilt: The symptoms of this disease appeared two weeks after the inoculation for the sick witness and the treatments bacterised with S5 and TRS24 strains, after three weeks for the treatment associated with DN20 strain and after four weeks for the bacterised seedlings with the D2 strain (Table III). On the majority of the seedlings, we have observed at the beginning of the appearance of symptoms, of partial yellowing, which spread thereafter. Total wilting and the death of seedlings were noted with the sick witness. Twelve weeks after the fungus inoculation, the sick witness showed a rate of severity of fusarium wilt of about 63.33%. In the case of the interactions with the bacterial strains, the rates of severity of the disease are weak compared to the sick witness. The highest rate (51.66%) was noted with the S5 strain and the lowest rate (36,66%) was recorded with D2 and DN20 strains (Table III). The comparison of the rates of severity of the vascular wilt shows a significant difference. According to the test of Newman and Keuls to the threshold α = 5%, the treatments are classified into four homogeneous groups. The sick witness is affiliated with group A. The treatments associated with TRS24 and S5 strains are classified in the group B, whereas the group C presents the treatments associated with the D2 and DN20 strains. The healthy witness is classified in the group D (Table III; fig 1).

Table III : Evolution of the severity of the fusarium wilt (%) on the seedlings of the flax according to time.

Treatments	Week After The Inoculation Numbers											
	1	2	3	4	5	6	7	8	9	10	11	12
Sick witness (T+)	0	5.0	8.3	16.7	33.3	40.0	50.0	50.0	53.3	58.3	61.7	63.33 (A)*
DN20 +F.o.ln.	0	0.0	1.7	3.3	3.3	6.7	11.7	15.0	23.3	36.7	36.7	36.66 (C)*
S5 + F.o.ln	0	3.3	11.7	15.0	30.0	30.0	30.0	41.7	43.3	45.0	48.3	51.66 (B)*
TRS24 + F.o.ln	0	1.7	8.3	8.3	8.3	10.7	25.0	28.3	28.3	40.0	43.3	45.00 (B)*
D2 + F.o.ln	0	0.0	0.0	3.3	3.3	8.3	11.7	16.7	23.3	35.0	36.7	36.66 (C)*
Healthy witness (T-)	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	00.00 (D)*

*: Values followed by the same letter are not significantly different according to the test from Newman and Keuls to the threshold α= 5%. F.o.ln. *Fusarium oxysporum* f sp. *lini*

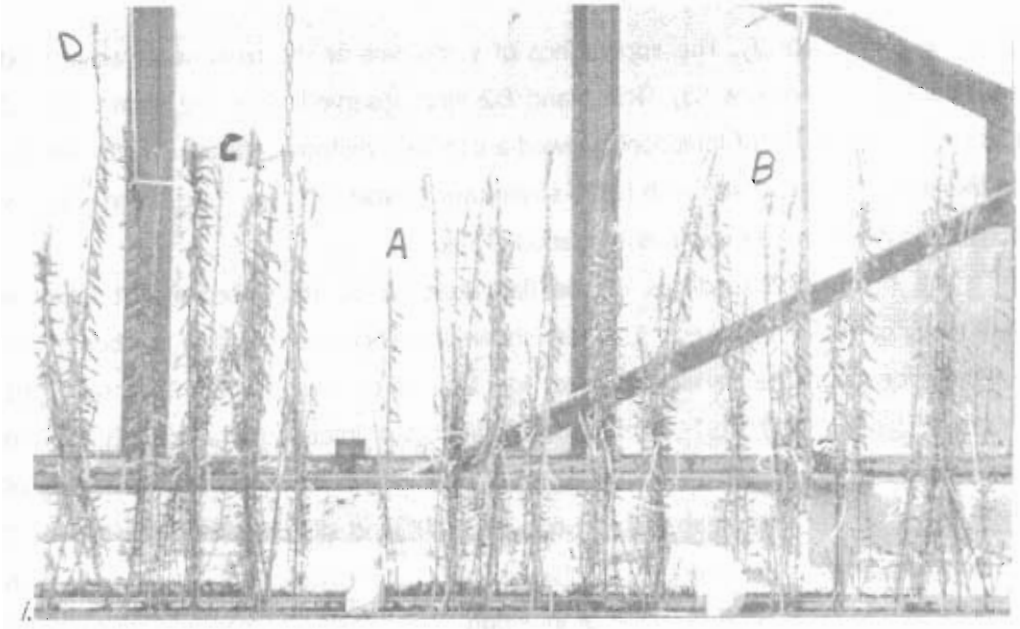

Figure 1. Symptoms of wilting of the flax. A: wilting of the top.

B: Generalised wilting. **C**: Died of the seedlings. **D**: Healthy seedling.

The rates of inhibition of the severity of vascular wilt are variable according to bacterial strains tested. According to the test of Newman and Keuls to the threshold α = 5%, the bacterised treatments are classified in three homogeneous groups of which first is consisted the treatments associated with the bacterial strains DN20 and D2, which showed the highest rate of inhibition (42.12%). The second group comprises the treatment [TRS24 + F.o.ln.] who showed a rate of 28.95%. The third group is consisted the treatment [S5 + F.o.ln.], the rate of inhibition is about 18.43% (Table IV).

Table IV: Rate of inhibition of the severity of the fusarium wilt on the seedlings of the flax.

Treatments	Severite Of The Disease (%)	Rate Of Inhibition Compared To The Temoin (+)
Dn20 + F.O.Ln	36.66	42.12(A)*
S5 + F.O.Ln	51.66	18.43 (C)*
Trs24 + F.O.Ln	45	28.95(B)*
D2 + F.O.Ln	36.66	42.12(A)*

*: Values followed by the same letter are not significantly different according to the test from Newman and Keuls to the threshold α= 5% F.o. ln. *Fusarium oxysporum* f sp. *lini*

--Antagonistic activities in the original soil

-Index of infection: We noticed that the fusarium wilt of flax began dice for the first week for the sick witness where the index of infection is about 8.33%. The number of sick seedlings increased considerably to reach its maximum at the end of the test is

twelve weeks (Table V). The appearance of symptoms at the treatments associated with the bacterial strains S5, TRS24 and D2 was observed after the sixth week of inoculation. The index of infection followed a constant rhythm until the twelfth weeks. On the seedlings bacterised with DN20 strain, any typical symptom of disease was not observed during all the experimental period (Table V).

The number of seedlings of the flax attacked by the vascular wilt is more significant for the sick witness. This last showed an index of infection of about 75% largely exceeding the rates recorded for the other treatments. The seedlings bacterised with S5 and TRS24 strains showed indices of infection of about 8.33%, and the treatment associated with the with D2 strain of reference showed an index of about 16.66%. For the treatment [F.o.ln.+ DN20] no sick seedling was observed (Table V). A significant difference is given between the treatments tested energy in absence of disease at a rate of 75% of infection. The test of Newman and Keuls to the threshold α= 5%, make it possible to classify these treatments in four homogeneous groups or group A is represented by the sick witness. The group B is consisted the treatment associated with the D2 strain. The group C include understand the treatments bacterised with the TRS24 and S5 strains. The healthy witness and the treatment related to DN20bstrain are affiliated with the group D (Table V).

Table V Evolution of the index of the fusarium wilt (%) on the seedlings of the flax according to time.

Treatments	Week After The Inoculation Numbers											
	1	2	3	4	5	6	7	8	9	10	11	12
Sick witness (T+)	8.33	25	41.7	41.66	50	75	75	75	75	75	75	75.00 (A)*
DN20 +F.o.ln.	0	0	0	0	0	0	0	0	0	0	0	00.00 (D)*
S5 + F.o.ln.	0	0	0	0	0	8.33	8.33	8.33	8.33	8.33	8.33	08.33 (C)*
TRS24 + F.o.ln.	0	0	0	0	0	8.33	8.33	8.33	8.33	8.33	8.33	08.33 (C)*
D2 + F.o.ln.	0	0	0	0	0	16.66	16.66	16.66	16.66	16.66	16.66	16.66 (B)*
Healthy witness (T-)	0	0	0	0	0	0	0	0	0	0	0	00.00(D)*

*: Values followed by the same letter are not significantly different according to the test from Newman and Keuls to the threshold α= 5%. F.o.ln. *Fusarium oxysporum* f sp. *Lini*

-Severity of the fusarium wilt: For the sick witness, the symptoms are noted dice the third week after the inoculation of pathogen [F.o.ln.] with an index of severity of about 1.66%. For the treatments bacterised by the S5, TRS24 and D2 strains, the severity of disease began dice the sixth week and remains constant until

the end of the test. No symptom of vascular wilt was observed on the seedlings of the flax bacterised with DN20 strain during all the probation period (Table VI). The severity results in the appearance of yellowing partial followed by unilateral wilting at the bacterised plants. The severity recorded in these last presents a definitely significant difference compared to the sick witness for whom the severity generated the mortality of some seedlings (Table VI).

On the basis of rate of severity, the test of Newman and Keuls with the threshold α = 5% gather the treatments tested in three homogeneous groups. The sick witness is classified in group A for a rate of 60%. The group B is consisted the treatments associated with the bacterial S5, TRS24 and D2 strains, which show, respectively, of rates of about 1.66%, 1.66% and 3.33%. The healthy witness and the treatment [F.o.ln. + DN20] are affiliated with the group C (Table VI)

Table VI Evolution of the severity of the fusarium wilt (%) on the seedlings of the flax according to time.

Treatments	Week After The Inoculation Numbers											
	1	2	3	4	5	6	7	8	9	10	11	12
Sick Witness (T+)	1.7	5.0	11.7	11.7	30.0	36.7	41.7	41.7	55.0	56.0	60.0	60.00(A)*
Dn20 + F.O.Ln	0	0	0	0	0	0	0	0	0	0	0	00.00(C)*
S5 + F.O.Ln	0	0	0	0	0	1.7	1.7	1.7	1.7	1.7	1.7	01.66(B)*
Trs24 + F.O.Ln	0	0	0	0	0	1.7	1.7	1.7	1.7	1.7	1.7	01.66(B)*
D2 + F.O.Ln	0	0	0	0	0	3.3	3.3	3.3	3.3	3.3	3.3	03.33(B)*
Healthy Witness (T-)	0	0	0	0	0	0	0	0	0	0	0	00.00 (C)*

*: Values followed by the same letter are not significantly different according to the test from Newman and Keuls to the threshold α= 5% F.o.ln. *Fusarium oxysporum* f. sp. *Lini.*

The treatments bacterised with the S5, DN20, TRS24 and D2 strains show significant rates of inhibition of severity of the disease compared to the sick witness. The test of Newman and Keuls to the threshold α= 5% class these treatments in only one group. The treatment associated with DN20 strain records the highest rate of

inhibition (100%). The seedlings bacterised by S5 and TRS24 strains show a rate of inhibition of abou97.24%. A rate of 94.45% is noted for the treatment [F.o.ln + D2] (Table VII).

Table VII Rate of inhibition of severity of the fusarium wilt (in %) on the seedlings of the flax.

Treatments	Severite Of Disease (%)	Rate Of Inhibition Of Disease Compared To The Sick Temoin (T+)
Dn20 + F.O.Ln.	0	100.00 (A*
S5 + F.O.Ln.	1.66	97.24 (A)*
Trs24 + F.O.Ln.	1.66	97.24 (A)*
D2 + F.O.Ln.	3.33	94.45 (A)*

Values followed by the same letter are not significantly different according to the test from Newman and Keuls to the threshold*: α= 5%. F.o.ln. *Fusarium oxysporum* f sp. *lini*.

A phenomenon of nanism is observed on the whole of the seedlings of the flax cultivated on an original soil (not disinfected). The average size of these seedlings about 17 cm to 42 cm is compared with the seedlings of the flax of healthy witness, which have an average size of about 65 cm (Fig.2).

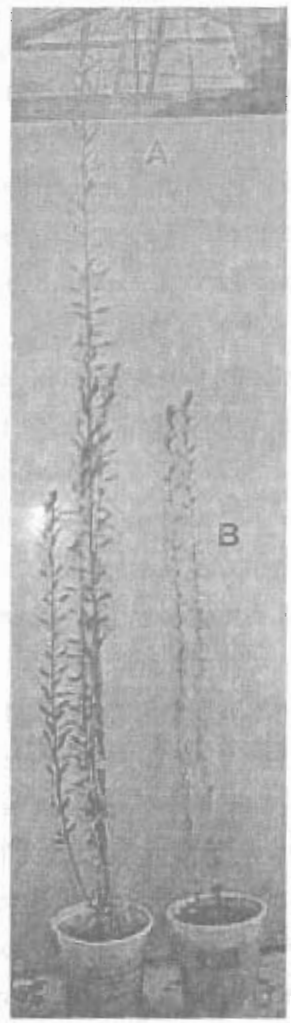

Figure 2 . Nanism observed on the seedlings of the flax led in original soil A: Healthy seedling. B: Sick seedling.

DISCUSSION

Antagonistic activities of DN20 and D2 strains in the presence of *Fusarium oxysporum* f.sp. *lini* is very brought closer under the conditions of our experiments. The effect of antagonism in the original soil (not disinfected) for a going rate of inhibition from 94.45% to 100% more outstanding remainder, whereas in the disinfected soil, it is of about an 18.43% to 42.12%. A good antagonistic activity was recorded for DN20 strain under the two conditions of our experiments; on the other hand the antagonistic activities noted for the D2 strain are variable in the two types of tests.

The test *in vivo* of antagonisms live has screw of *Fusarium oxysporum* f.sp. *lini*, made it possible to note a very apparent variability at the bacterial strains tested according to the experimental conditions. This type of observation at summer brought back in several work which takes into account the influence of the biotic and a-biotic

factors on exteriorization of antagonistic activities (Misaghi and *al.*, 1982 , Weller, 1988 ; Leeman and *al.*, 1991 ; Van Peer and *al.*, 1991 ; Toua, 1996 ; Toua and *al.*,1997).

The effect of antagonism exerted in the rhizosphere of the flax revealed that DN20 strain isolated from the rhizosphere of date palm, presents a significant activity with respect to *Fusarium oxysporum* f.sp. *lini* with an inhibition of exteriorisation of the symptoms in an original soil.

These results show that the antagonistic activity noted in the original soil is higher compared to the disinfected soil, which explains the influence of the telluric microflora on the beneficial effects exerted by the fluorescent strains of *Pseudomonas* spp. So the biological characteristics of the soil are determining (Lemanceau, 1992). In absence of pathogenic micro-organisms, a strain of *Pseudomonas* whose mode of action is microbial antagonism will not modify the growth of the inoculated plant. Thus, the bacterisation of potato tubers cultivated in a soil presenting a reduced noxious microflora with WCS358 strain is not accompanied by a significant increase in the output of culture (Bakker and *al.*, 1986). It is necessary thus that the limiting factor on which the bacterium acts is effective so that the effect of the bacterium is visible. A strain of *Pseudomonas* also remains without effect when the reduction of output of the witness plants is due to a pathogenic micro-organism insensitive with the bacterial antagonistic activity (Weller, 1988). In addition, the mixture of a purified soil resistant of pathogenic micro-organisms to a soil treated beforehand with heat allowed the flora of *Fusarium* sp. introduced with the substrate to limit the re-colonization by the pathogenic agent by the means of a competition will intrageneric to specify the importance of the ratio density of the population of total *Fusarium* oxysporum pathogenic / density of the population of *Fusarium oxysporum* (Couteaudier and *al.*, 1985).

Under the natural conditions, the severity of the vascular wilt varies from a soil to another. Several studies showed to the existence of naturally resistant soils due to the presence at the same time of strains of *Pseudomonas* spp. fluorescent antagonists and of the isolates of nonpathogenic *Fusarium oxysporum* (Lemanceau and *al.*, 1988; Lemanceau and Alabouvette, 1991; Bao and Lazarovits, 2001).

The beneficial effects of the bacterisation result at the same time from the specific activities of the bacteria and their density in the rhizosphere of the plant host. Thus the effectiveness of fluorescent strains of *Pseudomonas* spp. depends on their aptitude to produce certain metabolites (Siderophores, antibiotics, HCN growth hormones and lipopolysaccharides) to reinforce the resistance and to improve the growth of the plant (Lemanceau, 1992).The weak rhizospheric competence of the

introduced bacteria can generate a loss in the beneficial effects waited (Lemanceau, 1992). Indeed Baker and Cook (1974) suggest that the rhizospheric microflora is made up of a whole of micro organisms in equilibrium; if the introduced strain does not colonize the rhizosphère in an aggressive way, its beneficial activity cannot be expressed because microbial equilibrium former to the inoculation is restored quickly. This report was checked in a series of work based on the use of certain strains of *Pseudomonas* spp. fluorescent ready to develop on the root system (Burr and *al* .,1978 ; Kloepper and Schroth, 1978; Kloepper and *al*, 1980; Stutz and *al.*, 1986).

The selection of powerful strains of *Pseudomonas* spp. fluorescent should not be however limited to these only activities which would be little of utility if the bacteria are not ready to be maintained and to colonize the rhizosphere of the plant host (Lemanceau, 1992).

According to Bahme and Schroth (1987); Weller (1988) good rhizospheric competence is conditioned by the characteristics of the bacterium which are related to the aptitude of the strains for:

- To stick to the root
- To move along the root
- To enter in effective competition of way with the microflora resident.

The attachment of the bacteria to the root results from physicochemical and biological interactions between the bacteria and the root of the plant host. This specificity and affinity result from the presence of characters genotypic in the bacterium having a certain compatibility with certain genotypes of the plant host (Benizri and *al.* 2001). The colonization of *Pseudomonas* spp. fluorescent is also influenced by their localization on the level of the root.Thus, the bacteria endophyts or endorhizospheric would be less prone to the competition than those located outside the root. Indeed, the density of the bacterial populations and the intensity of the competition are more reduced inside than has the outside of root (Van Peer and *al.*, 1990). The aptitude of certain strains to colonize the endorhizosphere is correlated for their aptitude to bind it self under the action of agglutinins roots. These strains are also characterized by the nature of their lipopolysacharids and their membrane proteins (Van Peer and *al.*, 1990).

To study roots colonization of fluorescent strains of *Pseudomonas* spp., Lagopodi and *al.* (2001) tested bacterial strains marked and known by their effect of biocontrol, WCS365 strain of *P. fluorescens* and PCL 1391 strain of *P. chlororaphis* were inoculated on tomato seedlings in interaction with *Fusarium oxysporum* f.sp.

lycopersici inoculated directly in the soil or the roots of tomato. These micro-organisms occupy the same ones quote, but *Pseudomonas* spp. fluorescent more quickly colonize the roots and in an intensive way.

In conclusion these results show the effectiveness of DN20 strain in the suppression of the fusarium wilt of the flax under the two conditions. Thus, it is necessary to apply it to the date palm with an aim of realized a biological fight against the bayoud.

REFERENCES

1. Alabouvette, C. 1990. Biological controls of Fusarium wilt pathogens in suppressive soils. Pp: 27-43 in biological controlof soil-born plant pathogens. Ed. D. Hornbey. CAB International. Wallingford.
2. Alabouvette, C., P., Lemanceau and C. Steinberg. 1993. Recent advances in the biological control of fusarium wilts. Pestic. Sci. 37: 365-373.
3. Bahme, J. b. and M. N. Schroth. 1987. Spatial-temporal colonization patterns of a Rhizobacterium under grand organs of potato. Phytopathology 77: 1093-1100.
4. Baker, K. F. and R. J. Cook. 1974. Biological control of plant pathogens. A phytopatol. Soc. ST. PAUL. MN. 433pp.
5. Benchaban, M., R., Bakour D. Toua et A. Boutekrabt. 2000. Mise en évidence de l'effet antagoniste de Pseudomonas fluorescens vis-à-vis de la fusariose vasculaire de la tomate. Bulletin OEPP/EPPO.30 :243-246.
6. Benizri, E., E. Boudouin, C. D. B. Leboeuf et A. Guckert. 2001. Des bactéries pour la santé des plantes. Biofutur 210: 52-55.
7. Burr, T. J., M. N. Schroth and T. Suslow. 1978. Increased potato yields by treatment of seed pieces with specific strain of Pseudomonas fluorescens and P. putida. Phytopathology 68: 1377-1383.
8. Bounaga–Rivielle, N. 1985. Contribution à l'étude de Fusarium oxysporum f.sp. albedinis (Killian et Maire) Gordon, agent de la fusariose de palmier dattier. Thése Doc. USTHB Alger.180pp.
9. Couteaudier, Y. M., M. Letard, C. Alabouvette et J. Louvet. 1985. Lutte biologique contre la fusariose vasculaire de la tomate. Résultats en serre de production. Agronomie 5 : 151-156.
10. Djerbi, M. 1988. Les maladies du palmier dattier. Projet régional de lutte contre le bayoud. Alger. 172pp.
11. Duijff, B. J., J. W. MEIJER, PAHM bakker and B. Schippers. 1991. Suppression of Fusarium wilt of carnation by pseudomonas in soil; mode of action. Pp: 152-156. In: plant growth promoting rhizobacteria. Progress and prospects. Eds. C. Keel, Koller B, et G. Defago. IOBC/WPRS. Bulletin XIV/8.
12. Fernandez, D., M. Ouinten, A. Tantaoui and J. P. Geiger. 1997. Molecular record of micro evolution within the Algerian population of Fusarium oxysporum f. sp. albedinis during its spread to new ases. Eur. J. Plant. Pathol.103: 485-490.
13. Kloepper J. W., J. Leong, M. Teintz and M. N. Schroth. 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 286: 885-886.

14. Kloepper, J. W. and MN Schroth. 1978. Plant growth promoting rhizobacteria radishes. In proc 4th Int Conf Plant Dis 2: 879-882.
15. Kloepper, J. W, J. Leong, M. Teintz and MN Schroth. 1980. Pseudomonas siderophores: a mechanism explaining disease suppressive soils. Current Microbiology 4: 317-320.
16. King, EO, MK Ward and Raney DE 1954. Two simple media for the demonstration of pyocyanin and fluorescin.J Lab Cli Med 44:301-307.
17. Lagopodi, ALAFJRAM, GEM Lamers, PJ. Punt and CAMJJ Vandelhandel. 2001. Confocal laser scaning microscopy analyses of tomato root colonization an infection by fusarium oxysporum f.sp radislycopersici using the green fluorescent protein marker. Submited. Pp 564. in rhizosphere colonization by pseudomonas. Ed. BJJ Lugtenberg. Ann. Rev. Phytopathol. 461-491.
18. Leeman, M., RJ. Scheffer and B. Schippers. 1991. Control of Fusarium wilt of radish by fluorescent Pseudomonas fluorescents WCS374 in greenhouse trial. Pp: 34-38. in: Plant Growth Promoting Rhizobacteria Progress and Prospects. Eds. C. KEEL, B. KOLLER et G. DEFAGO.IOBC/WPRS. Bulletin XIV/8.
19. Lemanceau, 1992. Effets bénéfiques de rhizobacteries sur les plantes : exemples des Pseudomonas spp. Fluorescents. Agronomie 12 :413-437
20. Lemanceau, P. and C. Alabouvette. 1991. Biological control of Fusarium diseases by fluorescent Pseudomonas and non-pathogenic Fusarium. Crop protection 10:279-286.
21. Lemanceau. P., BAKKER PAHM,Kojel de WJ, C. Alabouvette and B. Schippers. 1992. Effet of pseudobactin 358 production by Pseudomonas putida WCS358 on suppression of Fusarium wilt of carnations by non pathogenic Fusarium oxysporum fo47. Appl Environ Microbiol 58: 2978-2982.
22. Lemanceau, P. and C. Alabouvette. 1993. Suppression of Fusarium wilts by fluorescent Pseudomonas mechanisms and applications. Biocontrol Sci. Technol. 3: 219-234.
23. Louvet, J. 1972. La nature de bayoud, fusariose vasculaire du palmier dattier, première semaine internationale sur le bayoud, Alger. 158-161.
24. Louvet, J. 1974. Les recherches sur le bayoud au Maroc. Bull. Agr. Sahara. 1:15-24.
25. Misaghi and al. 1982. Fungistatic activity of water-soluble fluorescent pigments of fluorescent Pseudomonas. Phytopathology 72: 33-36.
26. Scher, FM, and R. Bake. 1982. Mechanism of biological control in a Fusarium – Suppressive soil. Phytopathology 70: 412-417.

27. Stutz, and al. 198. Naturally occuring fluorescent Pseudomonas involved in suppression of black root of tobacco. Phytopathology 76:181-185.
28. Toua, 1996. Essai d'utilisation de Pseudomonas fluorescens dans le biocontrole de Fusarium oxysporum f.sp.lycopersici et de Verticillium dahlia sur la tomate et dans la promotion de la croissance vegetale. Thèse de Magister. I. N. A. El-Harrach. Alger. 138pp.
29. Toua, D., M. Benchabane and R. Bakour. 1997. Effect of some selected Pseudomonas fluorescents strains on suppression Fusarium wilt of tomato. Proceedings 10th Congress of Mediterranean Phytopathological Union. SFP. France pp: 811-814.
30. Van Peer, R, WLM Punte, LA De Weger and B. Schippers. 1990. Characterization of root surface and endorhizosphere pseuomonads in relation to their colonizationof roots. Appl Eviron Microbiol 56:2462-2470.
31. Van Peer and al., 1991. Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of camation by Pseudomonas sp. Strain WCS417. Phytopathology 81: 728-734.
32. Van Peer, R., GJ, Nieman and B. Schippers. 1990. Induced resistance and phytoalexin accumulation in biological control of fusarium wilt of carnation by Pseudomonas sp. strain WCS471r. Phytopathology 81: 728-734.
33. Weller, DM. 1988. Biological control of soilborne plant pathogenes in the rhizosphere with bacteria. Ann Rev Phytopathol 26: 379-407.

اختبار السلالات القوية المضيئة من فطر PSEUDOMONAS المستخدمة على الكتانِ في المكافحة الحيويةِ لمرض البيوض

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ذبول الفيوزاريم الذي يصيب نخيل البلح سببه الفطرِ *F. sp. Oxysporum Albedinis* .

يعتبر مرض البيوض أحد أكثر الأمراض الخطيرة والمُهَدِّدة عموماً لنخيل البلح في شمال أفريقيا. يزرع بالجزائر عدة أصناف تجارية مشهورة لها صفات نوعية عاليةِ جداً انقرضت وأخرى يُمْكِنُ أنْ تختفي بسبب سهولةِ تأثّرهم بمرضِ البيوض.

للحفاظ على هذه الأصناف فأنه توجد إستراتيجية واحدة للسَيْطَرَة على ذبولِ الفيوزاريم وذلك باستعمال المضادات الحيوية لمستعمرات الفطر فى جذور النخيل.

أظهرت السلالات القوية المضيئة من فطر PSEUDOMONAS أنها تَقْمعُ المرض باستخدام الآلياتِ المختلفة.

جربت هذه السلالات فى المكافحة البيولوجية للمرض in vivo وقد أظهرت تأثير مفيد في إخمادِ المرضِ.