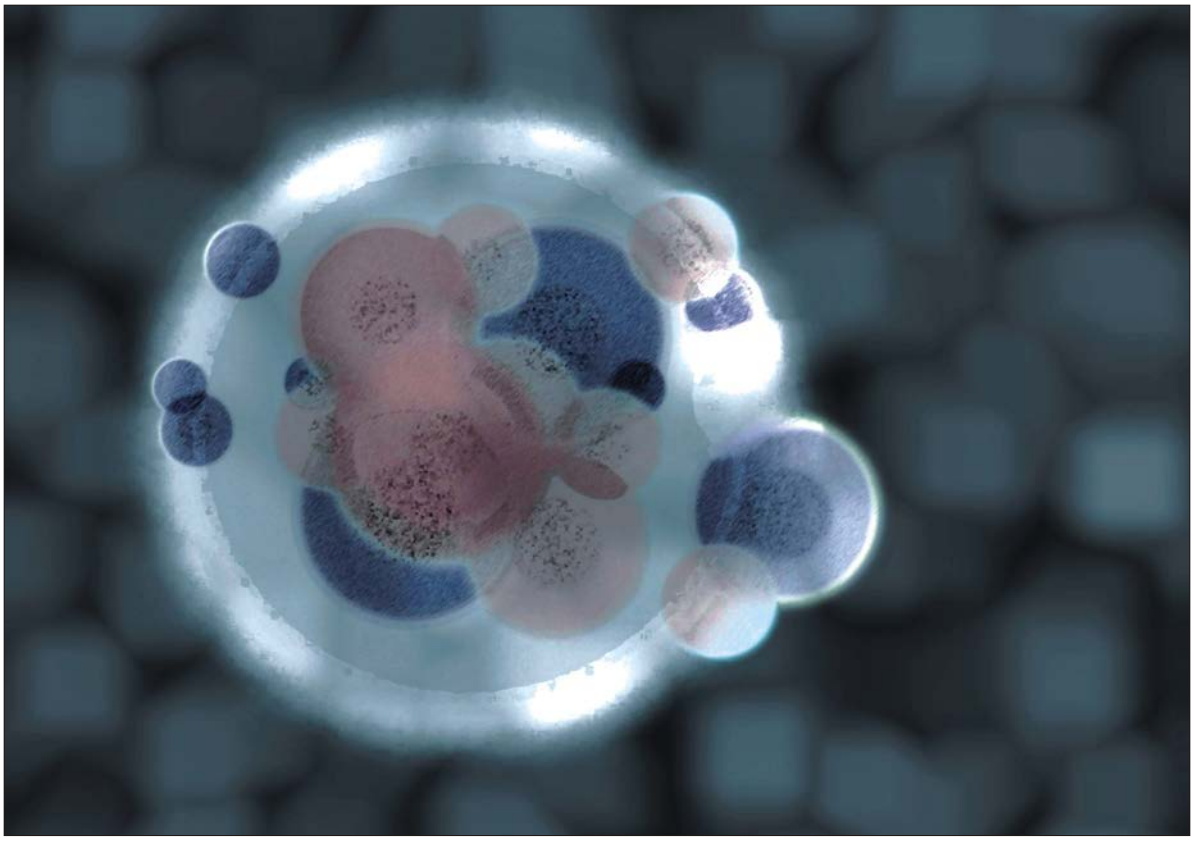

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# Biological Control of Bayoud in the Palm Trees by Strains of *Arthrinium*

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**Abstract:** The main objective of this research project was to investigate the possibility of inhibiting the growth of filamentous fungi, yeasts and bacteria isolated from soil samples, the trunk, leaves and fruit of palm trees infected with bayoud, using strains of the *Arthrinium* genus. *Arthrinium aureum* and *Arthrinium phaeospermum* have notable inhibiting properties on *Fusarium oxysporum* and *F. niveum*, standing out in the literature as the main etiological agents in the bayoud process in oases, meaning that active strains of *Arthrinium* can be applied in the biological control of the process.

**Key words:** Biological control, bayoud, *Arthrinium*, *Fusarium*

## INTRODUCTION

Palm trees constitute the ecological and socio-economic womb of the oasis. They offer a suitable micro-climate for other crops such as fruit, cereals, etc. and they also protect them from the wind. Palm trees represent a basic food source for the people of the Sahara and make a significant economic contribution to the country[1]. Unfortunately, this wealth is increasingly condemned to disappear, due to a vascular disease known as bayoud, whose etiological agent is *Fusarium oxysporum* f. sp. *albedinis*[2]. The methods proposed in order to fight this disease are many and varied, among which one could cite the genetic method, the chemical method and the biological method[3-5].

The biological control of diseases in plants is used as an alternative in cases of pathogens in which it is difficult to apply other methods. The literature quotes several examples of pathogens that have been controlled biologically, using antagonists such as *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp. and some bacteria[3-5].

The main aim of the research that we have carried out is that of studying the possibility of inhibiting the growth of filamentous fungi, yeasts and bacteria isolated from soil samples, the trunk, leaves and fruit of palm trees infected with bayoud, using strains of the *Arthrinium* genus as a biocontrol agent.

*Arthrinium*, is a deuteromycete fungus which is characterised by its great capacity to produce secondary metabolites[6-9] with a marked inhibiting effect on the growth of a wide variety of other filamentous fungi and yeasts that are mainly isolated from crop soil samples.

## MATERIALS AND METHODS

**Strains of *Arthrinium* assayed:** The fungal strains used to carry out this study were:

*Arthrinium aureum* FVB 574.
*Arthrinium phaeospermum* FVB 570.
*Arthrinium serenensis* FVB 548.

**Methodology:** A study was carried out on 50 samples obtained from: soil, trunk, leaves and dates (fruit of palm trees) affected with bayoud from the Rachidia area in the province of Marrakech (Morocco).

The methodology used included the microbiological quantification of samples[10], the isolation and identification of the micro-organisms isolated[10] and a study of the inhibiting properties of strains of the *Arthrinium* genus belonging to the *A. aureum*, *A. serenensis* and *A. phaeospermum* species, in respect of the micro-organisms isolated from the samples[6-9].

## RESULTS AND DISCUSSION

The micro-organism counts detected in the different samples analysed allow one to indicate that the number of UFC/g for the fungi sample varies between $2.70x10^5$ and $1.73x10^4$ and between $1.24x10^6$ and $8.66x10^3$ for bacteria, the soil samples showing the greatest concentration, followed by the leaves, the dates and the trunk in the case of the fungi and the leaves, the dates and the trunk in the case of the bacteria (Table 1).

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Among the species of bacteria identified we found seven from the *Bacillus* genus, along with *Erwinia caratovora* and *Micrococcus varians* (Table 2).

Soil samples were the ones that presented a higher species variability of the genus *Bacillus,* focusing on the fact that from dates samples *Bacillus cereus* was only detected and in a high percentage of the samples (90%) *Micrococcus varians* was isolated.

*Erwinia caratovora* was only detected in those samples from leaves and its microbiota showed a moderate percentage of *Bacillus alvei* (31%) and *Bacillus megaterium* (35%).

From trunk samples, only genus *Bacillus* was detected, being mostly isolated *Bacillus megaterium* (54%) strains.

Table 1: CFU/g of the fungi and the bacteria in the samples analysed

| | Fungi | | | Bacteria |
|---|---|---|---|---|
| | EM | S | PDA | TSA |
| Soil | $2.70x10^5$ | $1.8x10^5$ | $2.70x10^5$ | $1.24x10^6$ |
| Trunk | $1.73x10^4$ | $5.6x10^4$ | $1.50x10^4$ | $8.66x10^3$ |
| Leaves | $2.25x10^5$ | $1.0x10^5$ | $1.18x10^5$ | $1.15x10^5$ |
| Dates | $1.00x10^5$ | $0.8x10^5$ | $0.07x10^5$ | $8.70x10^4$ |

Table 2: Percentage isolation of bacteria isolated and identified in the samples analysed

| Bacteria isolated | Soil | Trunk | Leaves | Dates |
|---|---|---|---|---|
| *Bacillus alvei* | *18% | | *31% | |
| *Bacillus brevis* | *30% | | *1% | |
| *Bacillus cereus* | *14% | | | *6% |
| *Bacillus circulans* | *23% | | | |
| *Bacillus macerans* | | *24% | | |
| *Bacillus megaterium* | *5% | *54% | *35% | |
| *Bacillus subtilis* | *6% | *22% | | |
| *Erwinia caratovora* | | | *33% | |
| *Micrococcus varians* | *4% | | | *90% |

*Presence of bacteria in the samples analysed

Table 3: Percentage of filamentous fungi and yeasts isolated and identified in the samples analysed

| Fungi | Soil | Trunk | Leaves | Dates |
|---|---|---|---|---|
| *Alternaria alternata* | | 3% | 20% | |
| *Aspergillus candidus* | 6% | | | |
| *Aspergillus fumigatus* | 2% | | | |
| *Aspergillus flavus* | 16% | | | |
| *Aspergillus niger* | 8% | 23% | 20% | 52% |
| *Aspergillus ochraceus* | 21% | | | |
| *Aspergillus terreus* | 28% | | | |
| *Cladosporium herbarum* | | | 12% | |
| *C. sphaerospermum* | | 13% | 10% | |
| *Fusarium oxysporum* | 2% | | 4% | |
| *F. oxysporum* Var. *niveum* | 2% | | | |
| *Mucor mucedo* | | 2% | | |
| *Penicillium citreo viride* | 3% | | | |
| *Penicillium chrysogenum* | 1% | | | |
| *Penicillium frequentans* | 7% | 13% | 12% | 10% |
| *Penicillium rubrum* | 1% | | | |
| *Scopulariopsis brevicaulis* | | 24% | | |
| *Trichoderma viride* | 3% | | | |
| *Ulocladium chartarum* | | | 6% | |
| Yeasts | | 22% | 16% | 38% |

Among the fungi, the species identified were: *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus terreus*, *Cladosporium herbarum*, *Cladosporium sphaerospermum*, *Fusarium oxysporum*, *Fusarium oxysperum* Var. *niveum, Mucor mucedo, Penicillium granulosum, Penicillium frequentans, Penicillium rubrum, Scopulariopsis brevicaulis, Trichoderma viride Ulocladium chartarum* and *Saccharomyces cerevisiae*.

The *Aspergillus* and *Penicillium* genera showed the highest isolation percentage in soil, 81 and 12%, respectively. These two genera were isolated in all the samples. The number of colonies of the species *Aspergillus* is higher than that of *Penicillium*. These genera are followed in appearance by *Fusarium* and finally *Trichoderma* (Table 3).

Where the isolated strains met the inhibiting action of the strains of the *Arthrinium* genus, it was observed that *Arthrinium serenensis* was the strain that showed the greatest inhibiting action on the growth of the bacteria isolated, with a notable effect on *Bacillus macerans* and *Bacillus megaterium* followed by *Erwinia caratovora*. *Arthrinium aureum* was active on all the bacteria, the most sensitive strain being *Bacillus subtilis* and *A. phaeospermum* was active on all bacteria except *B. circulans* (Table 4).

The three strains of *Arthrinium* tested have inhibiting properties on all the bacteria isolated, except *Arthrinium phaeospermum* on *Bacillus circulans* (Table 4).

As regards action against filamentous fungi three strains of *Arthrinium* showed the ability to inhibit the development of *Aspergillus candidus, Cladosporium herbarum, Penicillium frequentans* and *Penicillium granulosum*. Of the three strains of *Arthrinium* studied,

Table 4: Antibiotic activity of the three *Arthrinium* strains against the bacteria isolated in the samples analysed

| | Strains | | |
|---|---|---|---|
| Bacteria isolated | *Arthrinium aureum* | *Arthrinium sereneusis* | *Arthrinium phaeospermum* |
| *Bacillus alvei* | ++ | ++ | + |
| *Bacillus brevis* | ++ | ++ | ++ |
| *Bacillus cereus* | ++ | ++ | +++ |
| *Bacillus circulans* | + | ++ | - |
| *Bacillus macerans* | ++ | +++ | +++ |
| *Bacillus megaterium* | ++ | +++ | ++ |
| *Bacillus subtilis* | +++ | ++ | + |
| *Erwinia caratovora* | + | + | + |
| *Micrococcus varians* | ++ | ++ | +++ |

+: Presence of inhibition haloes, shown by the antibiotic activity of the *Arthrinium* strains studies in relation to the bacteria isolated

-: absence of inhibition haloes

Table 5: Antibiotic properties of strains of *Arthrinium* on the filamentous fungi and yeasts isolated from the samples analysed

| Fungi | *A. aureum* | *A. serenensis* | *A. phaeospermum* |
|---|---|---|---|
| *Alternaria alternata* | + | - | - |
| *Aspergillus candidus* | + | + | + |
| *Aspergillus fumigatus* | + | - | - |
| *Aspergillus flavus* | - | - | - |
| *Aspergillns niger* | + | ++ | - |
| *Aspergillns ochraceus* | - | - | - |
| *Aspergillus terreus* | - | + | + |
| *Cladosporium herbarum* | + | + | + |
| *C. sphaerospermum* | - | - | - |
| *Fusarium oxysporum* | + | + | + |
| *F. oxysporum* Var. *niveum* | + | - | + |
| *Mucor mucedo* | - | - | - |
| *Penicillium citreo viride* | - | - | - |
| *Penicillium chrysogenum* | + | + | + |
| *Penicillium frequentans* | + | + | + |
| *Penicillium rubrum* | - | - | - |
| *Scopulariopsis brevicaulis* | - | - | - |
| *Trichoderma viride* | - | - | - |
| *Ulocladium chartarum* | - | - | - |
| Yeasts | - | - | - |

only *A. aureum* displayed properties against *Alternaria alternata* and against *Aspergillus fumigatus*. Only *A. serenensis* and *A. phaeospermum* were active against *A. terreus*. Likewise, we can indicate that *A. aureum* and *A. phaeospermum* displayed activity against *Fusarium oxysporum* and *F. niveum*, while the latter showed a resistance to the action of *A. serenensis* (Table 5).

*Arthrinium aureum* and *Arthrinium phaeospermum* have notable inhibiting properties on *Fusarium oxysporum* and *F. niveum*, standing out in the literature as the main etiological agents in the bayoud process in oases, meaning that active strains of *Arthrinium* can be applied in the biological control of the process.

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