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Antagonistic Activity of Yeast against Post-harvest Diseases of Tropical Fruits *

by

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KEYWORDS. — *Candida oleophila* Strain O ; *Colletotrichum musae, Fusarium moniliforme ; Cephalosporium sp.* ; *Penicillium digitatum ; Penicillium italicum ;* Citrus and Banana.

SUMMARY. — *Candida oleophila* Strain O was previously selected for its high and reliable antagonistic activity against *Botrytis cinerea* and *Penicillium expansum*, two important wound pathogens on post-harvest apples. The evaluation of this antagonistic strain on wound pathogens of tropical fruits has been more recently undertaken with its application at three concentrations (10^6, 10^7, 10^8 cfu/ml), on a fungal complex formed by the association of *Colletotrichum musae* (10^3 conidia/ml), *Fusarium moniliforme* (10^4 conidia/ml) and *Cephalosporium sp.* (10^4 conidia/ml), representing crown rot, the most important post-harvest disease of exported banana. The application of strain O at 10^8 cfu/ml showed the highest protective level (56 %). The influence of incubation period between strain O (10^8 cfu/ml) and the fungal complex incubation has also been studied. Strain O was applied 24 h before the complex, but also 15 min. or 3 h after its inoculation. The highest protective level was observed when strain O was applied 24 h before the complex.

On the other hand, the efficacy of strain O (10^5, 10^6 and 10^8 cfu/ml) was also assessed against two major wound pathogens of citrus, *P. digitatum* and *P. italicum* (10^5, 10^6 and 10^7 spores/ml), on "Clementine" and "Maroc-late" varieties. The highest protective level (up to 100 %) was detected with the application of strain O at 10^8 cfu/ml whatever the pathogen and the citrus variety. The antagonistic activity of *C. oleophila* was also dependent on the incubation time before pathogen inoculation. The protective level increased with time between application of the antagonist and inoculation of fungal spores.

MOTS-CLES. — *Candida oleophila* souche O ; *Colletotrichum musae* ; *Fusarium moniliforme* ; *Cephalosporium sp.* ; *Penicillium digitatum* ; *Penicillium italicum* ; Agrumes et bananes.

RESUME. — *Activité antagoniste de levures vis-à-vis des maladies de post-récolte des fruits tropicaux*. — *Candida oleophila* souche O a été préalablement sélectionnée pour son activité antagoniste élevée et stable face à *Botrytis cinerea* et *Penicillium expansum*, deux pathogènes de blessures importants sur pommes en post-récolte. Le niveau de protection offert par la souche O a été testé plus récemment vis-à-vis d'autres pathogènes de blessures touchant certains fruits tropicaux. L'activité antagoniste de la souche O appli-

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quée à trois concentrations (10^6, 10^7 et 10^8 ufc/ml) a été évaluée par rapport à un complexe parasitaire formé de *Colletotrichum musae* (103 conidies/ml), *Fusarium moniliforme* (10^4 conidies/ml) et *Cephalosporium sp.* (10^4 conidies/ml). Ce complexe est responsable du développement de pourritures de la couronne, la plus importante maladie post-récolte touchant les bananes d'exportation. L'application de la souche O à 10^8 ufc/ml a permis d'obtenir le niveau de protection le plus élevé (56 %). L'étude de l'influence du temps séparant l'application de la souche O de celle du complexe parasitaire a révélé que la protection pouvait être considérablement renforcée lorsque la souche O était appliquée 24 heures avant le complexe, et ce, en comparaison avec une application de la souche O effectuée 15 minutes et 3 heures après l'inoculation du complexe parasitaire.

D'autre part, l'efficacité de la souche O (10^5, 10^6 et 10^8 ufc/ml) a été évaluée vis-à-vis de *P. digitatum* et *P. italicum* (10^5, 10^6 et 10^7 conidies/ml), deux pathogènes importants des agrumes. Deux variétés d'agrumes ont été utilisées: «Clémentine» et «Maroc-Late». L'emploi de la souche O à 10^8 ufc/ml a permis d'atteindre des niveaux de protection de 100 % et ce, quels que soient le pathogène et la variété d'agrumes. De plus, l'efficacité de *C. oleophila* souche O était également dépendante du temps séparant l'application de la levure de celle du pathogène.

TREFWOORDEN. — *Candida oleophila* afstamming O ; *Colletotrichum musae* ; *Fusarium moniliforme* ; *Cephalosporium sp.* ; *Penicillium digitatum* ; *Penicillium italicum* ; Citrusvruchten en bananen.

SAMENVATTING. — *Antagonistische werking van gisten tegen na-oogstziekten van tropische vruchten.* — *Candida oleophila* werd van te voren geselecteerd voor zijn hoge tegenstrijdige en stabiele activiteit tegenover *Botrytis cinerea* en *Penicillium expansum*, twee belangrijke pathogenen van verwondingen, op appels in post-oogst. Het beschermingsniveau dat wordt aangeboden door de afstamming O werd onlangs eveneens getest ten aanzien van andere pathogenen van verwondingen die bepaalde tropische vruchten treffen. De antagonistische activiteit van de afstamming O met drie concentraties (10^6, 10^7 en 10^8 ufc/ml) ten opzichte van een parasitair complex gevormd door *Colletotrichum musae* (10^3 conidies/ml), *Fusarium moniliforme* (10^4 conidies/ml) en *Cephalosporium sp.* (10^4 conidies/ml) werd geëvalueerd. Dit complex is verantwoordelijk voor de ontwikkeling van rotting van de kroon, de belangrijkste post-oogstziekte bij de bananen voor uitvoer. De toepassing van de afstamming O met 10^8 ufc/ml heeft het mogelijk gemaakt om het hoogste beschermingsniveau te verkrijgen (56 %). De studie van de invloed van de tijd die de toepassing van de afstamming O van die van het parasitaire complex scheidt, heeft eveneens aangetoond dat de bescherming aanzienlijk versterkt kon worden wanneer de afstamming O 24 uur voor het complex was toegepast en dit in vergelijking met een toepassing van de afstamming O die 15 minuten en 3 uur na inoculatie van het parasitaire complex wordt uitgevoerd.

Anderzijds werd de doeltreffendheid van de afstamming O (10^5, 10^6 en 10^8 ufc/ml) t.o.v. *P. digitatum* en *P. italicum* (10^5, 10^6 en 10^7 conidies/ml), twee belangrijke pathogenen van de citrusvruchten, geëvalueerd. Twee variëteiten van citrusvruchten werden gebruikt: „Clementine" en „Maroc-Late". Het gebruik van de afstamming O aan 10^8 ufc/ml heeft het mogelijk gemaakt om beschermingsniveaus van 100 % te bereiken en dit ongeacht het pathogeen en de variëteit van citrusvruchten. Bovendien is de doeltreffendheid van *C. oleophila* afstamming O alweer afhankelijk van de tijd die de toepassing van de gist van die van de pathogeen scheidt.

Introduction

The post-harvest diseases cause important losses of fruits. In the United States, these losses are ranged from 1 to 20 %, depending on the commodity (Janisiewicz & Korsten 2002). Post-harvest citrus diseases are responsible for significant economic losses in the world (Droby *et al.* 1989). These losses are provoked by green and blue decays caused respectively by *Penicillium digitatum* and *P. italicum* (Chalutz & Wilson 1990). On the other hand, the quality of exported bananas has also decreased due to various post-harvest diseases and particularly the banana's crown rot disease, which is considered as the most important post-harvest disease of exported bananas, present in all growing countries (Krauss & Johanson 2000). In the rainy season, losses of more than 10 % have been recorded on Windward Islands, bananas arriving in the UK (Krauss & Johanson 2000). Organisms involved in the disease are numerous and vary according to locality, time of year and other factors (Slabaugh 1994). However, the most frequently isolated fungi from the crown rot include *Colletotrichum musae* (major pathogen), *Botryodiplodia theobromae*, *Cephalosporium sp.*, *Ceratocystis paradoxa*, *Verticilium theobromae* and *Fusarium sp.* (Meredith 1971).

The control of these post-harvest diseases on citrus and banana is mainly based on fungicide treatments (de Lapeyre de Bellaire & Nolin 1994, Krauss *et al.* 1998, Jijakli 1999) such as thiabendazole (TBZ) or imazalil (IMZ) sprayed on fruits during packing-house operations. Nevertheless, this practice was criticized because of the appearance of fungicide-resistant strains of post-harvest wound pathogens (Vinas *et al.* 1993, Johanson & Blazquez 1992, East & Kenyon 1998). Furthermore, growing concern for the human safety and protection of environment imposed to develop alternatives to usual synthetic fungicides in order to control post-harvest diseases (Wisniewski & Wilson 1992). In this context, the biological control of post-harvest diseases of fruits has been shown as a realistic alternative to synthetic fungicide because the application sites are limited to the fruits, the environmental factors are defined and stable in the storage rooms, and the harvested commodities are of high value which allows to support costs of biological treatments potentially higher than chemical methods (Fokkema 1991, Wilson & Wisniewski 1992, Jijakli *et al.* 1999). Effective biological control has been reported for post-harvest diseases of citrus (Chalutz & Wilson 1990, El-Ghaout *et al.* 2000). Research on the use of biological antagonists to control crown rot is more recent (Krauss 1996, Krauss *et al.* 1998, East & Kenyon 1998, De Costa & Subasinghe 1998).

Candida oleophila strain O was previously selected and approved for control of wound post-harvest diseases of apples and pears (Jijakli *et al.* 1993). The aim of the present study was to assess *in vivo* the antagonistic activity of the yeast *C. oleophila* strain O against both post-harvest diseases of citrus caused by *P. digitatum* and *P. italicum* and against banana's crown rot disease. This study

evaluates also the influence of relative concentrations of both micro-organisms (antagonist and pathogen) and different incubation periods between strain O treatment and fungal complex inoculation on the protective level.

Effect of Relative Concentrations of Micro-organisms (Pathogen and Antagonist) on Protective Level

In order to study the effect of the relative concentrations of strain O and both citrus pathogens on the protective level, citrus fruits were treated with 50 µl of *C. oleophila* strain O at concentrations of 10^5, 10^6 and 10^8 cfu/ml. The concentrations were determined by the Bürker cell. After disinfecting (sodium hypochlorite, 10 %) and drying, "Maroc-late" fruits were wounded at two equidistant points at the equatorial side. Each wound was 5 mm in diameter and 4 mm in depth. On the other hand, "Clementine" fruits received a single wound with 5 mm in diameter and a depth ranged from 1 to 2 mm. One hour after the application of the antagonist, wounded fruits were inoculated by 50 µl of *P. digitatum* and *P. italicum* (10^5, 10^6 and 10^7spores/ml). Fifty µl of sterile distilled water was applied on the control before the inoculation of the pathogen. Fruits were kept during seven days at 24° C under sixteen hours of photoperiod. Three fruits (six wounds for "Maroc-late" variety and three wounds for "Clementine" variety) were used per treatment and two trials were carried out over time.

In case of bananas the protocol differs, according to disease epidemiology and natural contaminations. Green bananas (*Musa acuminata* AAA, Cavendish, cv Grande Naine) were harvested at the stage recommended by the method set up by Ganry (1978) to form clusters of four bananas. Crowns of these clusters were inoculated by three fungi implicated in the complex, namely : *Colletotrichum musae, Fusarium moniliforme* and *Cephalosporium sp*. Spore concentrations were adjusted to 10^3 conidia/ml for *C. musae* and 10^4 conidia/ml for both others. One hundred µl of conidial suspension were applied to the centre of the freshly exposed crown tissue.

Three hours after pathogen inoculation, strain O was applied at three different concentrations (10^6, 10^7, 10^8 cfu/ml) by submerging banana's crowns into strain O suspension for ten seconds. Two kinds of control were used for each experiment : a "non-treated" control, only inoculated with the same pathogens and a "fungicide" control, submerged in a thiabendazole bath (500mg/L, Mertec 20S) for one minute.

Bananas were then stored to simulate maritime transport for thirteen days long and Percentage of Crown Necrosed Surface (PCNS) was carried out by measuring the internal crown rot surface in comparison with the total crown rot surface.

The analysis of the results expressed in percentage of protection offered by *C. oleophila* strain O against *P. digitatum* and *P. italicum* on both orange varieties

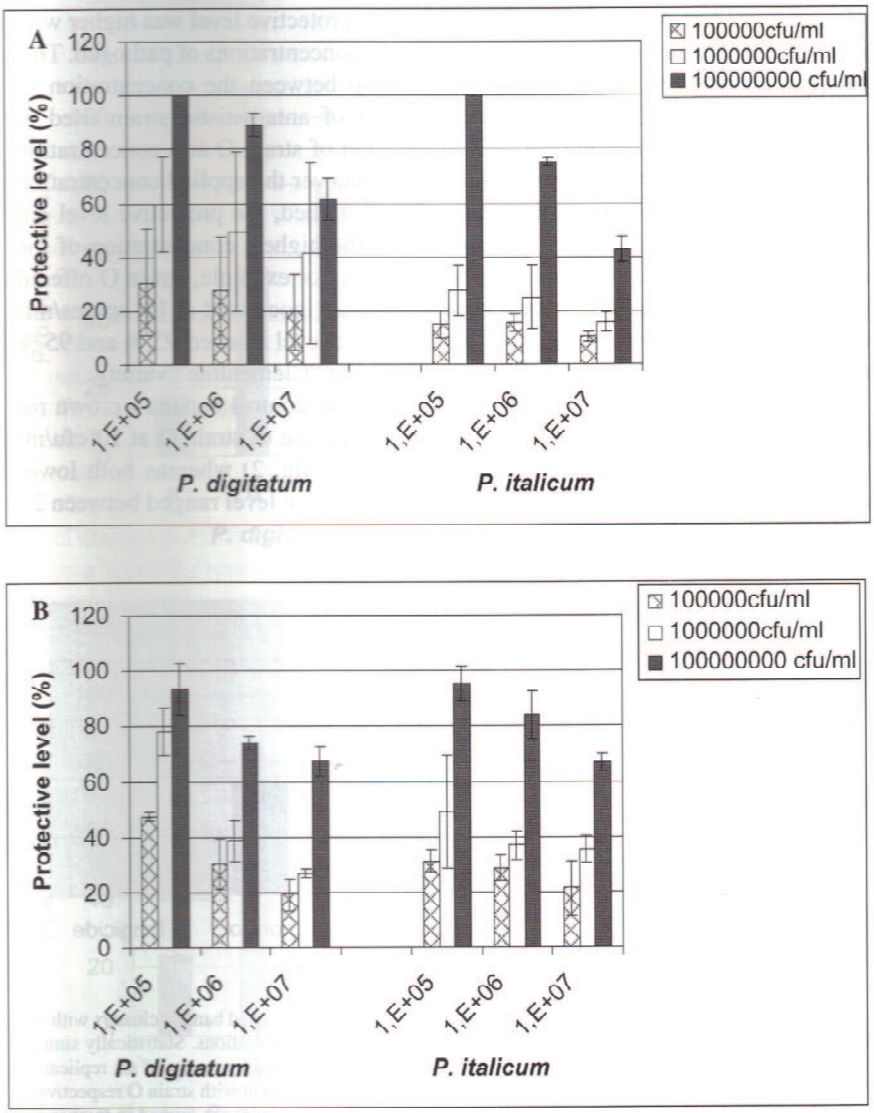

Fig. 1. — Evaluation of the antagonistic activity of *C. oleophila* strain O against *P. digitatum* and *P. italicum* with respect to their relative concentrations. Protective level was observed on citrus varieties "Maroc-late" (fig. 1A) and "Clementine" (fig. 1B) after seven days of incubation at 24° C. Citrus fruits were treated with a suspension of 50 µl of strain O (10^5, 10^6 and 10^8 cfu/ml) and inoculated one hour later by a suspension of *P. digitatum* or *P. italicum* (10^5, 10^6 and 10^7 spores/ml). The average of protective level was calculated from two separate experiments. Each experiment contained three fruits per treatment. Vertical bars represent the standard error associated to their respective average.

"Maroc-late" and "Clementine" showed that the protective level was higher with increasing concentrations of antagonist and low concentrations of pathogen. This observation revealed a quantitative relationship between the concentration of *P. italicum* and *P. digitatum* and cell number of antagonistic strain used to control the disease development. The application of strain O at a concentration of 10^5 cfu/ml offered only a weak protection whatever the applied concentration of both pathogens. Whatever the citrus variety studied, the protective level did not exceed 35 % (fig. 1). The treatment with the highest concentration of the antagonist (10^8 cfu/ml) allowed a higher efficacy. For example, strain O offered a total protection of 100 % against both pathogens (inoculated at 10^5 spores/ml) on the "Maroc-late" variety whereas the protective level reached 93 % and 95 % respectively for *P. digitatum* and *P. italicum* on the "Clementine" variety.

The different concentrations of strain O used to control banana's crown rot allowed to reduce the incidence of the disease. The use of strain O at 10^8 cfu/ml showed also the highest protective level of 56 % (fig. 2) whereas both lower concentrations (10^6 and 10^7 cfu/ml) offered a protective level ranged between 23 and 29 %.

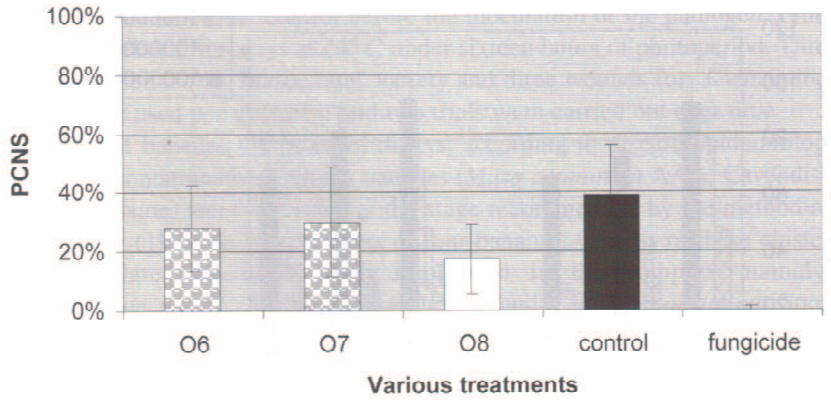

Fig. 2. — Percentage of Crown Necrosed Surface (PCNS) on inoculated banana clusters with the fungal complex and treated with *C. oleophila* strain O at various concentrations. Statistically similar values of PCNS are represented in the same colour. Values are expressed by means of six replicates, vertical bars represent standard errors. O6, O7, O8 correspond to treatment with strain O respectively applied at 10^6, 10^7, 10^8 ufc/ml ; control : no yeast applied ; fungicide : bananas dipped in thiabendazole (500 mg/L) for one minute.

Influence of the Fruit Incubation Period, between Strain O Application and Pathogen Inoculation, on the Protective level

This part was focused on the effect of growing time between the antagonist application and the pathogen inoculation. Both fruits were treated with strain O

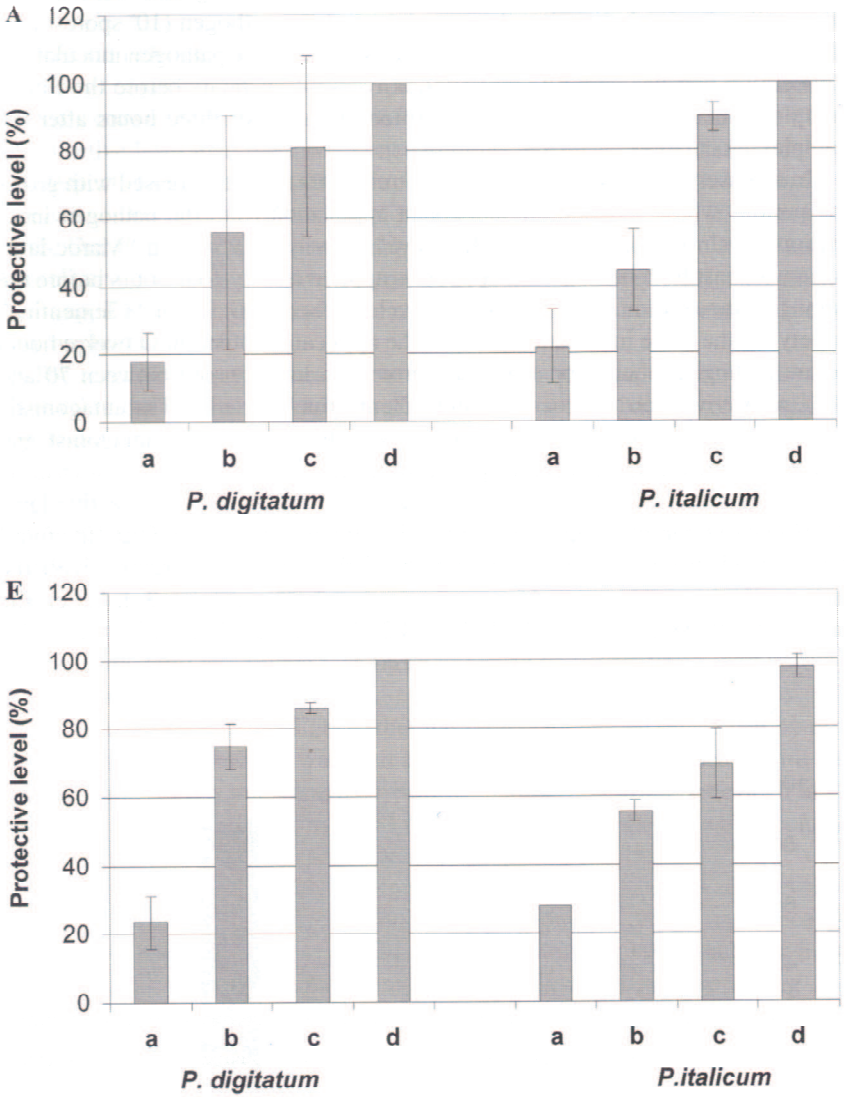

Fig. 3. — Evaluation of the antagonistic activity of yeast *C. oleophila* strain O with respect to incubation time separating the antagonist application and pathogen inoculation (*P. digitatum* and *P. italicum*). Protective level was observed on citrus varieties "Maroc-late" (fig. 3A) and "Clementine" (fig. 3B) after seven days of incubation at 24° C. Citrus fruits were treated with a suspension of strain O (10^8 cfu/ml) and inoculated with a suspension of *P. digitatum* or *P. italicum* (10^6 spores/ml). Strain O was applied on citrus either twelve hours after pathogen inoculation (a), at the same time (b), or twelve (c) or twenty-four (d) hours before pathogen inoculation. The average of protective level was calculated from two separate experiments. Each experiment contained three fruits per treatment. Vertical bars represent the standard error associated to their respective average.

at a concentration of 10^8 cfu/ml. The application of the antagonist was made on citrus either twelve hours after the inoculation of the pathogen (10^6 spores/ ml), at the same time, or twelve or twenty-four hours before the pathogen inoculation. Concerning bananas, strain O was applied twenty-four hours before the fungal complex inoculation on the crown, or fifteen minutes or three hours after the complex inoculation.

In all cases, protection offered by the strain O treatment increased with growing periods which separate the antagonist application from the pathogen inoculation. Strain O showed a protective level superior to 95 % on "Maroc-late" variety against both pathogens when it was applied twenty-four hours before the pathogen inoculation. The protective level reached 100 % on "Clementine" variety for the same inoculation period. The application of strain O twelve hours before pathogen inoculation allowed a protective level ranged between 70 and 80 % respectively on "Maroc-late" and "Clementine" varieties. The antagonistic activity remained also important when both microorganisms (antagonist and pathogens) were simultaneously applied (fig. 3).

Similar results were also observed on banana (fig. 4). The protective level (57 %) was remarkably reinforced, more than twice, when strain O at 10^8 cfu/ml was applied twenty-four hours before the pathogens in comparison with the treatment where the antagonist was introduced 15 min. or 3 h. after the pathogens allowing respectively 24 % and 20 % of protection.

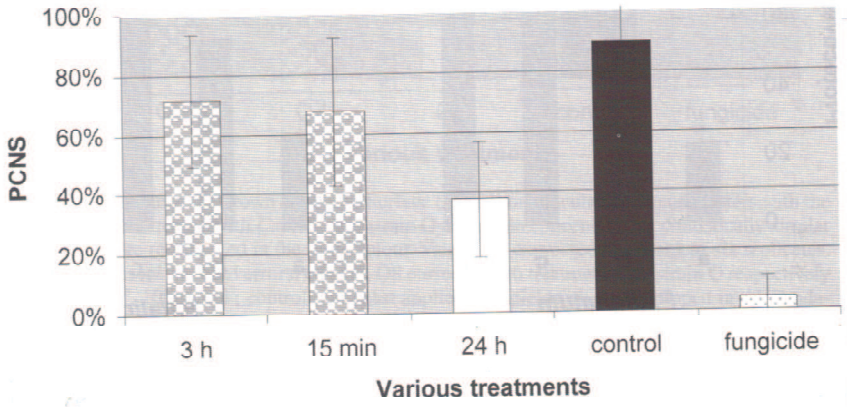

Fig. 4. — PCNS on banana clusters inoculated with the fungal complex and treated with *C. oleophila* strain O (10^8 ufc/ml). Statistically similar values of PCNS are represented in the same colour. PCNS mean is the result of six replicates, and standard errors are represented by vertical bars. 24 h. : strain O applied 24 h. before the fungal complex inoculation ; 15 min. and 3 h. : strain O respectively applied 15 min. and 3 h. after the fungal complex inoculation ; control : no treatment ; fungicide : bananas dipped in thiabendazole (500mg/L) for one minute.

Discussion

This study has showed the potential application of *C. oleophila* strain O as a biological control method against citrus green and blue molds and against banana's crown rot disease. A concentration of 10^8 cfu/ml of strain O gave higher protective level than the lower concentrations whatever the studied model. In this respect, DROBY *et al.* (1989) reported that an increase in *Debaryomyces hansenii* concentration resulted in more effective biocontrol of *P. digitatum* on citrus. Our results are also in accordance with those of EL-GHAOUT *et al.* (2000) who observed a more effective control of post-harvest decay with antagonistic yeasts applied at 10^8 cfu/ml and often no control of decay when biocontrol agents were applied at 10^5 cfu/ml.

The level of efficacy of strain O at 10^8 cfu/ml on post-harvest citrus diseases obtained in our laboratory was high and opens a good probability of transfer in practical conditions. This efficacy on citrus was higher than on banana's crown rot disease, where protection was limited and variable depending on the incidence's disease. Indeed, strict correlation between the severity of the banana's crown rot and the protective level by strain O at a concentration of 10^8 cfu/ml was observed. When the severity of the symptoms increased, protective level by strain O decreased. This observation is in accordance with a more important reduction of banana's crown rot in natural infestation conditions, where symptoms are generally less severe than those observed in our artificial conditions of inoculation.

This study has also highlighted that the protection can be reinforced by different strategies. Protection was increased when strain O was added before the pathogen inoculation in comparison with the addition of strain performed after it. This parameter must be considered in the development of a biological control method against fruit post-harvest diseases using this yeast strain.

Other techniques should also be considered to increase the protective level of biocontrol method. The polybag conditioning allows to reduce incidence of banana's anthracnose (CHILLET & DE LAPEYRE DE BELLAIRE 1996) and crown rot (PACICO 2001) by the establishment of a modified atmosphere during the banana's transport. This physical method should be tested in combination with strain O. The design of the yeast formulation is also important and in some cases allows a higher and more stable efficacy of the antagonist (JIJAKLI *et al.* 2002). Another possibility is the use of antagonist mixtures which probably increase the protective level (KRAUSS & JOHANSON 2000, JANISIEWICZ & KORSTEN 2002, KRAUSS *et al.* 1999). These strategies will be tested for both fruit models and more particularly on banana fruit.

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