Original

In vitro and *in vivo* antifungal activity of natural inhibitors against *Penicillium expansum*

Inibidores naturais no controle in vitro e in vivo de Penicillium expansum

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Abstract

Penicillium expansum is the causative agent of apple blue mold. The inhibitory effects of the capsaicin derived from *Capsicum* spp. fruits and yeast *Hansenula wingei* against *P. expansum* were evaluated in an *in vitro* and in *in vivo* assay using Fuji apples. The minimum inhibitory concentration of capsaicin determined using the broth micro-dilution method was 122.16 μg mL^{-1}. Capsaicin did not reduce blue mold incidence in apples. However, it was able to delay fungal growth in the first 14 days of the *in vivo* assay. The in vivo effect of the yeast *Hansenula wingei* AM2(-2), alone and combined with thiabendazole at low dosage (40 μg mL^{-1}), on the incidence of apple diseases caused by *P. expansum* was also described. *H. wingei* AM2(-2) combined with a low fungicide dosage (10% of the dosage recommended by the manufacturer) showed the best efficacy (100%) up to 7 days of storage at 21 °C, later showing a non-statistically different decrease ($p > 0.05$) after 14 (80.45%) and 21 days (72.13%), respectively. These results contribute providing new options for using antifungal agents against *Penicillium expansum*.

Keywords: *capsaicin; Hansenula wingei; blue mold.*

Resumo

Penicillium expansum é o agente causador da doença em maçã conhecida como mofo azul. O efeito inibitório da capsaicina derivada dos frutos *Capsicum* spp. e da levedura *Hansenula wingei* foi avaliado através de ensaios *in vitro* e *in vivo* em maçã. A concentração inibitória mínima da capsaicina de 122,16 μg mL^{-1} foi determinada usando microdiluição. A capsaicina não mostrou capacidade em reduzir a incidência do mofo azul na maçã. Entretanto, um retardo no crescimento do fungo foi observado nos 14 primeiros dias dos ensaios *in vivo*. Também descrevemos o efeito da levedura *Hansenula wingei* AM2(-2) isolada e em combinação com tiabendazol em baixa dosagem (40 μg mL^{-1}) no controle da doença de maçãs por *P. expansum*. *Hansenula wingei* AM2(-2), em combinação com baixa dosagem de tiabendazol (10% da recomendada pelo fabricante), apresentou 100% de eficácia após 7 dias de estocagem a 21 °C, seguido de 80,45% e 72,13% de eficácia após 14 e 21 dias, respectivamente($p > 0,05$). Estes resultados contribuem com as novas opções de emprego de agentes antifúngicos contra *Penicillium expansum*.

Palavras-chave: *capsaicina; Hansenula wingei; mofo azul.*

1 Introduction

Blue mold disease is one of the most important causes of postharvest losses in apple production in Brazil (COELHO et al., 2007; BRASIL, 2005). *Penicillium expansum* is responsible for fruit infection and patulin production (COELHO et al., 2011; LIMA et al., 2011; PITT, 1997), a mycotoxin that causes genotoxicity in mammalian cells (MOSS; LONG, 2002). This fungus has proven able to proliferate in bags, wooden boxes, and storage chambers causing losses of up to 30% during storage and transportation of apples in Brazil (CANAVER; PIERO, 2011).

Several methods have been used to solve post-harvest losses, such as fungicide treatment and modified controlled atmosphere (MONTERO et al., 2010; ROMANAZZI et al., 2012). Postharvest fungicide treatment is a primary method of control of *Penicillium* infections. Thiabendazole is one of the most commonly used chemical fungicides in the control of postharvest fungal apple decay, but it has led to the emergence of resistant strains (SPOTTS; CERVANTES, 1986; BARALDI et al., 2003).

Public concern regarding eating food grown with the addition of synthetic fungicides and the demand for more natural foods, free of chemical additives, has resulted in the need to find new strategies to develop fungicides to control fruit postharvest diseases. In fact, new approaches have been studied, such as the use of biological control agents, sanitizing products, and naturally-occurring compounds (MARI; BERTOLINI; PRATELLA, 2003; MARTÍNEZ-ROMERO, 2007; ROBIGLIO et al., 2011).

In terms of biological control, yeasts used in fruits are safe antagonists, do not pose risk and with low ecological impact, whose mechanism of antagonistic action is based on competition over space and nutrients (JANISIEWICZ; KORSTEN, 2002). Additionally, some species produce extracellular substances

Received 3/8/2012
Accepted 4/10/2012 (0M5828)
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which act against a variety of filamentous fungi in treated fruits, such as killer toxins, β-1,3-glucanase enzymes, and chitinase (CASSONE et al., 1997; IPPOLITO et al., 2000; BALLESTER et al., 2010). *Hansenula* sp is one of the most often yeast genera described as killer toxin producing yeast (GOLUBEV, 1998) including HK toxin of *Hansenula mrakii* that are targeted to β-1,3-D-glucan skeleton in the cell wall (THEISEN; MOLKENAU; SCHMITT, 2000). In a preliminary study, a killer positive strain of *H. wingei* AM2-2 showed stable in vitro antagonism against *Fusarium verticillioides* 103F for more than 30 days (GASPERINI et al., 2011).

Lima et al. (2011) showed a reduction of *Penicillium* rot in apple fruit by 35 and 52% when low dosages of *Rhodosporidium kratochvilovae* strain Ls11 and *Cryptococcus laurentii* strain LS28, respectively, were used.

In addition, naturally occurring antimicrobial substances have been found to be an alternative for the control of postharvest pathogens (VASANTHA RUPASINGHE et al., 2006). Phytoalexin, phenolic, and essential oils have been shown to have antifungal activity against *Penicillium expansum*; *Botrytis cinerea*, *Aspergillus flavus*, and *Fusarium* ssp. (PEDRAS; PEARSON, 2005; AHUJA; KISSEN; BONES, 2012; SINDHU et al., 2011; NERI et al., 2006; MARTÍNEZ-ROMERO et al., 2007). Capsaicin is an alkaloid responsible for the pungent flavor of hot pepper, and it is known for its high biological activity (LUO; PENG; LI, 2011). Many studies have shown that capsaicin and its analogues exhibit antimicrobial activity against *Bacillus subtilis* (MOLINA-TORRES; GARCIA-CHÁVEZ; RAMIREZ-CHÁVEZ, 1999), *Helicobater pylori* (ZEYREK; OGUZ, 2005), *Streptococcus mutans* (SANTOS et al., 2012), *Escherichia coli* (LIU et al., 2012), and *Colletotrichum capsici* (KRAIKRUAN; SANGCHOTE; SUKPRAKARN, 2008). Moreover, capsaicin micro-capsules were able to inhibit the growth of *Botrytis cinerea* and *Aspergillus niger* (XING; CHENG; YI, 2006).

In the present study, the antifungal activity of capsaicin was measured and whether this naturally occurring compound is efficient in controlling *P. expansum* on apples was investigated. Additionally, the *in vivo* antifungal activity of antagonistic yeast *Hansenula wingei* against *P. expansum* on apples was tested.

2 Materials and methods

2.1 Fruits and chemicals

Fuji apples were selected according to homogeneous size, maturity, color, and absence of injuries from a local market in Francisco Beltrão (Brazil).

Capsaicin from *Capsicum* ssp. was acquired from Sigma [≥50% capsaicin (HPLC) and ~35% dihydrocapsaicin, Sigma-Aldrich, Schnelldorf, Germany] and dissolved in Dimethyl Sulfoxide (DMSO) (≥99.9%, Sigma, Saint Louis, USA) to obtain a stock solution of 2.44 mg.mL^{-1}. Thiabendazole was kindly provided by Syngenta (Chemical group benzimidazole; trade formulate Tecto®, 48.5% w/v a.i., São Paulo, Brazil). All other chemicals used were of analytical grade and were obtained from Vetec (Rio de Janeiro, Brazil).

2.2 Biological control Agent (BCA)

The antagonistic killer yeast *Hansenula wingei* strain AM2(-2), previously isolated from corn at the Laboratory of Food Microbiology of Federal University of Technology - Parana, was the BCA used in this study. The yeast was kept on Potato Dextrose Agar [(PDA) HIMEDIA, Mumbai, India] at 4 °C for subsequent assay; *H. wingei* strain AM2(-2) was activated in Yeast Medium Agar [(YM agar) 2.0% glucose, 0.5% yeast extract; 0.23% NaH_2PO_4; 1.0% NaCl; 0.5% $(NH_4)_2SO_4$; 1.8% agar] at 25 °C overnight, followed by cell suspension in distilled water at 10^8 cells.mL^{-1} (Mac Farland n.3 scale).

2.3 Pathogen

Single-spored *Penicillium expansum* no. 2 was isolated from naturally decaying apple (NELSON; TOUSSON; MARASAS, 1983). The culture stored in PDA at 4 °C was grown on PDA slants at 21 °C for 120 hours before use. A spore suspension was prepared by inoculating the culture into 3 mL sterile YM broth, and the cell number was adjusted to 1×10^5 spores.mL^{-1} (Newbauer chamber).

2.4 In vitro antifungal activity of capsaicin against P. expansum

The antifungal activity was evaluated using a liquid growth inhibition assay, as described by Fehlbaum et al. (1994), with some modifications. Briefly, different volumes of the capsaicin stock solution (0.63, 1.25, 2.5, 5, 10 µL) were added to 80 µL of fungus (1×10^5 spores.mL^{-1}) in YM broth and diluted in sterile distilled water to a final volume of 100 µL. The capsaicin concentrations tested were 15.25, 30.54, 61.08, 122.16, and 244.30 µg mL^{-1}, and the corresponding DMSO concentrations achieved were 0.63, 1.25, 2.5, 5, and 10%, respectively. Thiabendazole was used as positive control at concentrations of 16.10, 8.05, 4.02, 2.01, and 1.00 µg mL^{-1}. For the negative control preparation, capsaicin or thiabendazole were replaced with sterile water. Fungal growth was measured by monitoring the OD increase at 630 nm using a TP Reader NM microplate (Thermoplate, Curitiba, Brazil). The minimal inhibitory concentration (MIC) was determined following incubation at 25 °C for 48 hours. The MIC is the lowest concentration of capsaicin preventing visible antifungal growth, and the determinations were carried out independently 3 times, using triplicate samples each time.

2.5 Ability of H. wingei and capsaicin in controlling P. expansum in vivo

The *in vivo* assay was based on the methods described by Lima et al. (2006, 2011). For the antifungal assay with *H. wingei* AM2(-2), 45 cv Fuji apples were superficially disinfected by dipping the fruits in a sodium hypochlorite solution (2.0% active chlorine) for 15 minutes, rinsing with sterile distilled water, and drying them at room temperature. Four wounds (3 mm wide and 3 mm deep) were made on each fruit. Two treatments were performed: BCA treatment (yeast cell suspension alone); and BCA + thiabendazole at low dosage (40 µg mL^{-1}, 10% of the full suggested dosage). For the BCA treatment, 30 µL of yeast cell

suspension in distilled water (3.0×10^8 cells.mL^{-1}) (Mac Farland n.3 scale) were placed in each wound, while for the combined treatment (BCA + thiabendazole at low dosage), the same yeast cell suspension was placed in each wound, followed by 30 µL of fungicide at low dosage. In each experiment, the controls were represented by the apple fruits in which the wounds were treated with 30 µL of sterile distilled water alone (negative control), 30 µL of thiabendazole at full dosage, as recommended by the manufacturer (400 µg mL^{-1}), or 30 µL of thiabendazole at low dosage (40 µg mL^{-1}), as positive controls.

After two hours at room temperature, each wound was inoculated with 10^5 conidia of *P. expansum* n. 2. The fruits were packed in polypropylene plastic bags (previously disinfected with 70% ethanol) and incubated at 21 °C; the number of wounds showing rot symptoms was assessed after 7, 14, and 21 days. Each treatment included three replicates, and each consisted of 15 fruits (three fruits per treatment).

One treatment was performed for the capsaicin assay, and in addition to the capsaicin solution, it consisted of four wounds in each fruit tested. Twenty-seven apples were used to carry out the capsaicin experiment. The treatments were: A) 30 µL of sterile distilled water (positive control); B) 30 µL of capsaicin stock solution 2.44 mg.mL^{-1}; and C) 30 µL of fungicide 400 µg mL^{-1} (negative control). The number of wounds showing rot symptoms was assessed at 7, 14, and 21 days, and photographic records were performed.

To compare the integrated treatments with those applied separately, the percentage values of infected wounds were transformed into percentages of protection efficacy (PE), as follows: PE = [(C – T)/C] × 100, where C is the number of infected wounds in the control (water + pathogen), and T is the number of infected wounds in the examined treatment (BCA alone or BCA + fungicide). Values of PE ranged from 0 (no PE) to 100 (maximum PE) (LIMA et al., 2011).

2.6 Statistical analyses

The mean values for fungal growth obtained from the *in vitro* antifungal activity assay with capsaicin, represented as the absorbance by ELISA assay, were evaluated by the Tukey test ($p < 0.05$) using the Anova/Manova software (Statistica version 7.0, Inc. Tulska OK, USA, 2005). For the *in vivo* assays, the mean values obtained from the three replicates during the entire experiment (PE values) were evaluated by the Tukey test ($p < 0.05$) using the Anova/Manova software (Statistica version 7.0, Inc. Tulska OK, USA, 2005).

3 Results and discussion

3.1 In vitro antifungal activity of capsaicin against P. expansum

Growth-inhibiting effect of MIC found in the experiments of capsaicin against *Penicillium expansum* was described in Table 1. The maximum inhibitory effect of capsaicin was found at a concentration of 112.16 µg mL^{-1}, and it was statically independent of the DMSO concentration (Figure 1).

Table 1. Minimal inhibitory concentration (MIC) of capsaicin and controls - *in vitro* growth of *P. expansum*.

Thiabendazole Conc. (µg mL^{-1})	DMSO Conc. (%)	Capsaicin Conc. (µg mL^{-1})
16.10 (–)	**10** (–)	244.30 (–)
8.05 (–)	5 (+)	**122.16** (–)
4.02 (+)	2.5 (+)	61.08 (+)
2.01 (+)	1.25 (+)	30.54 (+)
1.00 (+)	0.63 (+)	15.25 (+)

(+): Presence of fungal growth; (–) absence of fungal growth; positive control: thiabendazole; negative control: YM agar + water; DMSO: capsaicin solution control. Measurements were analyzed using Tukey´s test ($P < 0.05$). Value in bold is the MIC.

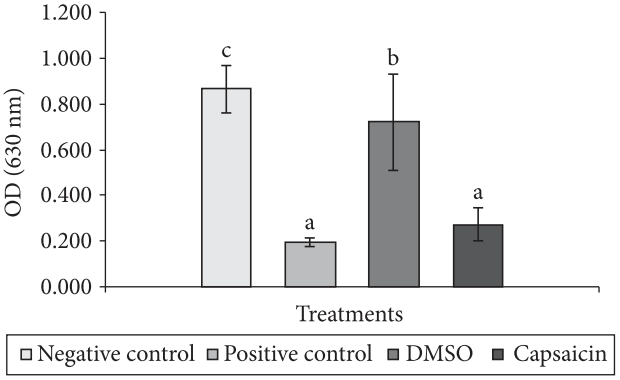

Figure 1. Comparison of the optical density of antifungal assay after 48 hours using: negative control (YM agar + water); positive control [thiabendazole (8.05 µg mL^{-1})]; DMSO (5%); capsaicin (122.16 µg mL^{-1}). Three separate experiments were performed, and in each assay three replicates per treatment were used. Measurements marked by the same letter do not differ statistically according to the Tukey test ($p > 0.05$).

This finding is in accordance with the National Committee for Clinical Laboratory Standards (1997), which states that dilution of insoluble compounds should be done with DMSO, and its concentration must not exceed 10% of the medium. However, incubation with DMSO alone affected the growth of the fungus, but it was not responsible for antifungal activity of capsaicin at its MIC value. Compared with the positive control, capsaicin was 15-fold less active against the microorganism strains studied. On the other hand, Xing, Cheng and Yi (2006) demonstrated that capsaicin microcasules exhibited antifungal activity against filamentous fungi, *B. cinerea* and *A. niger* at a concentration of 590 µg mL^{-1}. Interestingly, the authors believe that the inhibitory effect of the system originated from synergistic actions of capsaicin and tannins. According to the measuring fluorescence polarization of liposomes, capsaicin's changed membrane fluidity could be a result of disruption of the microorganisms' membrane (TSUCHIYA, 2001). Further, DNA microarray analysis suggests that the antimicrobial mechanism of capsaicin is related to the induction of an osmotic stress and key genes for membrane biosynthesis (KURITA et al., 2002).

3.2 Activity of H. wingei and effects of capsaicin against P. expansum in vivo

The results of the experiments carried out on wounded apples with capsaicin did not show protective efficacy after 21 days. Its visual protective efficacy on 7, 14, and 21 days is shown in Figure 2. As can be seen, capsaicin presented low efficacy; the diameters of the infected wounds were lower than those observed in the control, indicating low activity against the fungus. According to Vasantha Rupasinghe et al. (2006) the differences in the *in vivo* and *in vitro* treatments can be related to apple tissue features, such as pH, levels of essential nutrients (vitamins, minerals, and nitrogen-containing compounds) or natural phenolic compounds. Furthermore, Salas et al. (2011) reported that *in vivo* antifungal activity depends on the nature of the fungi and compound used.

The protective efficacy (PE) of *H. wingei* AM2 (-2) alone and in combination with thiabendazole against *P. expansum* on apples is shown in Figure 3. The visual protective efficacy of the biological control agent – BCA *Hansenula wingei* AM2 (-2) alone and in combination with a low dosage of thiabendazole (40 μg mL^{-1}) against *Penicillium expansum* no.2 on apples stored at 21 °C for 7 days is shown in Figure 4.

Figure 2. *In vivo* antifungal assay of capsaicin (2.44.mg.mL^{-1}) against *Penicillium expansum* n. 2 on apples stored at 21 °C. a) 7 days; b) 14 days; c) 21 days.

After 7 days of storage at 21 °C, the control apples (water + *P. expansum*) showed no protective efficacy (zero PE), *i.e.*, 100% percentage of disease incidence (Figure 3). Although the treatment with *H. wingei* alone exhibited no efficacy either (zero PE), as shown in Figure 3, the diameter of the infected wounds (represented as brown rot circumferences) in Figure 4b were smaller in size than those observed in the control (Figure 4a), indicating a slight inhibition of the fungus. In this case, the high initial amount of yeast inoculum used in the experiment (3.0×10^8 cells.mL^{-1}) contributed to some fruit decay by nutrient competition since yeasts are also regarded as deteriorating agents when in contact with the inside of the fruit. In studies carried out by other researchers, including a 10^6-10^8 cells.mL^{-1} initial dosage of antagonistic yeasts, it was reported low efficacy in controlling disease incidence up to 6 or 7 day storage (GENG et al., 2011; LIMA et al., 2011). Another explanation for the *in vivo* ineffectiveness of the yeast alone treatment could be the difficulty of the killer toxin in diffusing into the fruit, consequently, affecting performance; the killer toxin is an extracellular substance that inhibits the development of other microorganisms (WALKER; McLEOD; HODGSON, 1995). In a previous study, *H. wingei* AM2 (-2) showed a positive killer factor against standard yeast isolates used as reference (GASPERINI et al., 2011).

In our preliminary *in vitro* study, 100% of conidia germination of *P. expansum* no. 2 (10^5 conidia.mL^{-1}) was inhibited by cell-free culture supernatant of *H. wingei* AM2 (-2) in a broth medium (data not shown). Thus, it is suggested that the antagonistic activity of *H. wingei* AM2 (-2) is essentially associated with the production of an extracellular substance, not yet reported in the literature for this species.

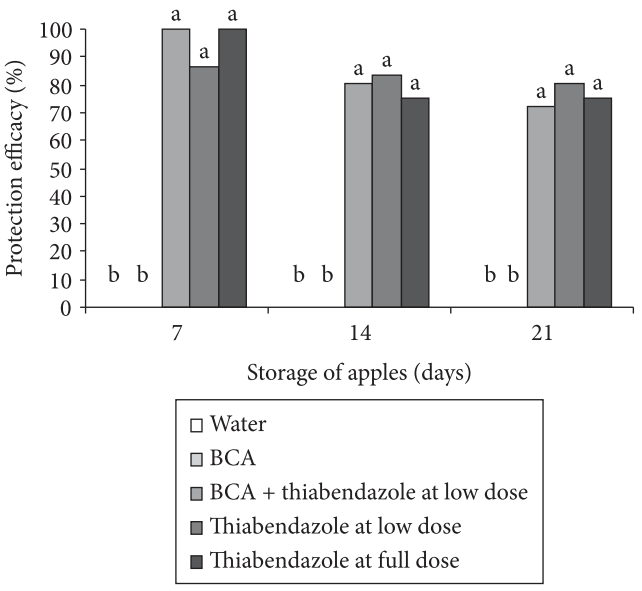

Figure 3. Activity of the biological control agent – BCA *Hansenula wingei* AM2(–2) alone and in combination with a low dosage of thiabendazole (40 μg mL^{-1}) against *Penicillium expansum* n. 2 on apples stored at 21 °C for 7, 14 and 21 days. Values marked by the same letter do not differ statistically according to the Tukey test ($p > 0.05$). BCA: biological control agent.

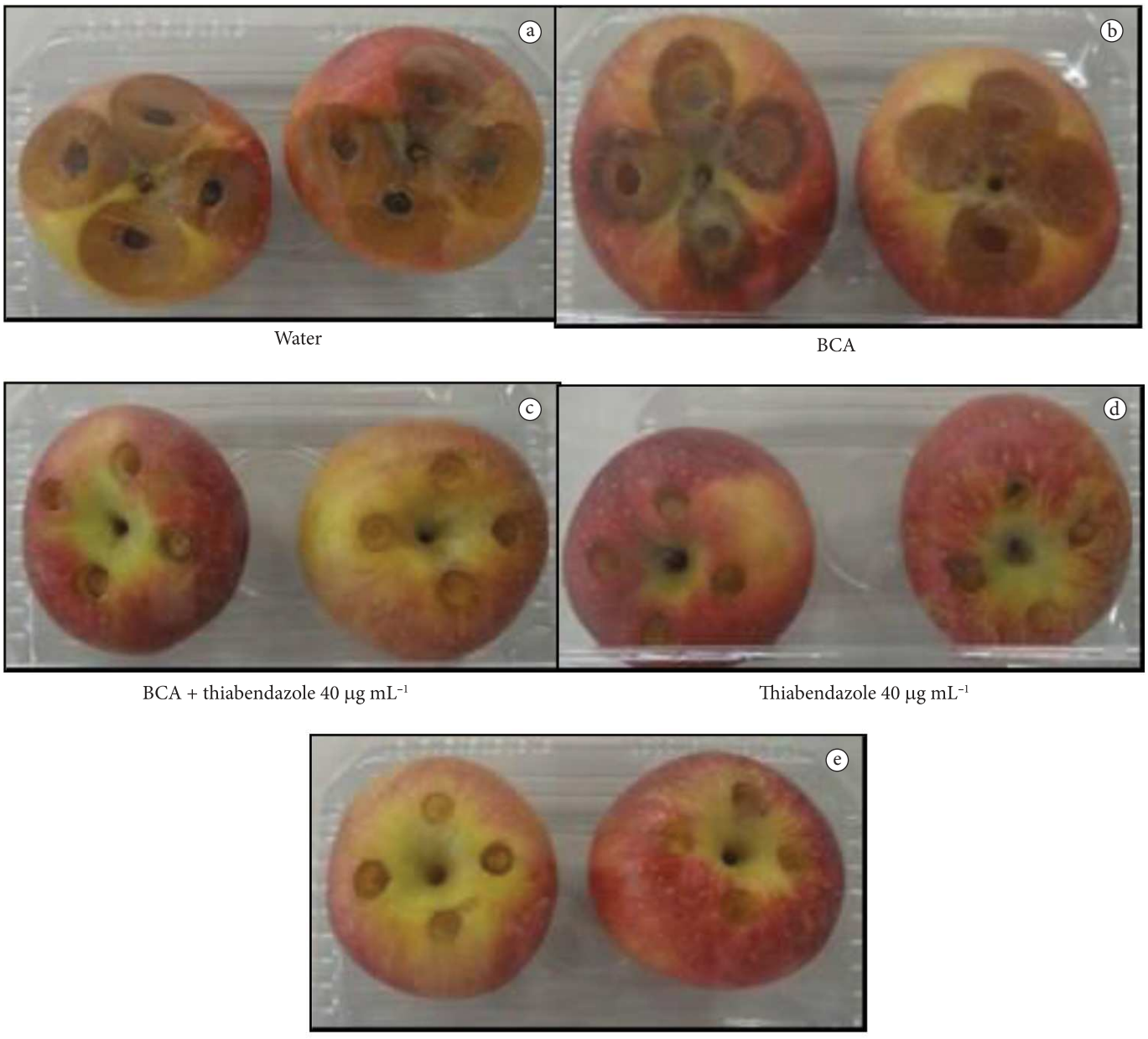

Figure 4. Visual protective efficacy of the biological control agent – BCA *Hansenula wingei* AM2(–2) alone and in combination with a low dosage of thiabendazole (40 µg mL^{-1}) against *Penicillium expansum* n. 2 on apples storage at 21 °C for 7 days. a) water; b) *H. wingei* AM2(–2) alone; c) *H. wingei* AM2(–2) in combination with thiabendazole (40 µg mL^{-1}); d) thiabendazole alone at low dose (40 µg mL^{-1}); E: thiabendazole alone at full dose recommended by manufacturer (400 µg mL^{-1}).

In addition, PE in the treatments BCA with thiabendazole at 40 µg mL^{-1}, thiabendazole at 40 µg mL^{-1} alone, and thiabendazole at 400 µg mL^{-1} alone were significantly effective against the fungus with PE higher than 85% (Figure 3). Figure 4c-e showed no incidence of disease in the treatments BCA in combination with thiabendazole at 40 µg mL^{-1}, thiabendazole at 40 µg mL^{-1} alone, and thiabendazole at 400 µg mL^{-1} alone, respectively. On the other hand, the incidence of disease was clearly visible in Figure 4a (positive control, water), represented as a brown rot circumference. Comparing the protection efficacy between BCA + thiabendazole at a low dosage and thiabendazole at a low dosage alone, the former showed the maximum effect (100%), while the fungicide alone, in this study, controlled 86.15%, showing antagonistic activity of yeast against fruit decay (Figure 3). It is important to mention that the optimization of biological control efficacy also depends on the survival

and colonization of biological agents in wounded fruits in the presence of low amounts of fungicides. Lima et al. (2011) reported a significant rot reduction of 98% after 7 days of storage by using an integrated treatment based on antagonistic yeasts with chemical fungicides, such as boscalid or cyprodinil, both at low dosage. The combination of *Kluyveromyces marxianus* with sodium bicarbonate was as effective as the imazalil treatment in natural infection trials, which gave about 90% control of green mold (GENG et al., 2011).

After 14 and 21 days of storage, BCA + thiabendazole at low dosage and thiabendazole at low dosage alone showed no significant PE ($p > 0.05$), 84.45-72.13% and 83.3-80.50%, respectively, while the fungicide at full dosage maintained 75% PE. In this study, the application of yeast in combination with a low dosage of thiabendazole was as effective in the control of *P. expansum* rot after 21 days of storage as the chemical fungicide applied separately, at low and full dosage (Figure 3, $p > 0.05$).

Although thiabendazole is one of the most important synthetic fungicides still allowed for postharvest treatment of fruit in many countries, the low efficacy or inefficacy against *P. expansum* reported by Lima et al. (2011) could be related to the widespread diffusion of fungal pathogen isolates that have become resistant to fungicides used for a long time in packing houses.

According to the results obtained, the combination of BCA with thiabendazole at a low dosage showed the best efficacy (100%) up to 7 days of storage at 21 °C, followed by a non- statistically significant decrease after 14 (80.45%) and 21 days (72.13%). Several studies on storage of fruits at 20-25 °C have reported efficacy by combining antagonistic yeasts with chemical substances up to 7 days (LIMA et al., 2006, 2011; GENG et al., 2011). However, Fazio, Gonçalves and Hoffmann (2012) reported a PE of 87.5% for the control of *P. expansum* on apples stored at 21 °C for 21 days when a combination of *Dekkera bruxellensis* and thiabendazole 40 μg mL^{-1} was used.

Therefore, the application of antagonistic yeasts in combination with fungicides at low dosage (10% of that recommended by the manufacturer) becomes a very promising alternative to control postharvest spoilage fungi, reducing a large amount of toxic waste in the treated fruits.

4 Conclusion

In conclusion, the present study describes, for the first time, the antifungal effect exhibited by capsaicin against *P. expansum*. This study expands the existing knowledge of antimicrobial activity of natural compounds suggesting that it is likely that hot pepper could be used to control blue mold disease. In *in vivo* experiments, the yeast combined with fungicide at low dosage showed satisfactory efficacy in the control of fruit decay up to 14 days, becoming a control strategy from an economic and technical point of view, such as better control of fruit rot diseases caused by prolonged fruit storage. Future investigations will focus on the synergistic effect of *Hansenula wingei* with other naturally occurring antimicrobial substances on the growth of filamentous fungi.

Acknowledgements

The authors gratefully acknowledge the National Council of Scientific and Technological Development (CNPq, Brazil), CAPES-Brazil and Araucaria foundation for the financial support provided. We are sincerely grateful to Dr Elisabete Hiromi Hashimoto of the Universidade Tecnológica Federal do Paraná, Laboratory of Food Microbiology, for providing the yeast *Hansenula wingei* strain AM2(-2) used in this study.

References

AHUJA, I.; KISSEN, R.; BONES, A. M. Phytoalexins in defense against pathogens. **Trends in Plant Science**, v. 17, n. 2, p. 73-90, 2012. http://dx.doi.org/10.1016/j.tplants.2011.11.002

BALLESTER, A. R. et al. Biochemical and molecular characterization of induced resistance against *Penicillium digitatum* in citrus fruit. **Postharvest Biology and Technology**, v. 56, n. 1, p. 31-38, 2010. http://dx.doi.org/10.1016/j.postharvbio.2009.10.002

BARALDI, E. et al. Studies on thiabendazole resistance of *Penicillium expansum* of pears: pathogenic fitness and genetic characterization. **Plant Pathology**, v. 52, p. 362-370, 2003.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento – MAPA. **Brasil deve iniciar exportação de manga para o Japão em junho**. MAPA, 2005. Disponível em: <http://extranet.agricultura.gov.br/pls/pubacs_cons/!ap_detalhe_noticia_cons?p_id_publicacao=3536>. Acesso em: 17 dez. 2011.

CANAVER, B. S.; PIERO, R. M. Di. Quitosana e adjuvantes para o controle preventivo do mofo azul da macieira. **Tropical Plant Pathology**, v. 36, n. 6, p. 419-423, 2011.

CASSONE, A. et al. Antibodies, killer toxins and antifungal immunoprotection: a lesson from nature? **Immunology Today**, v. 18, n. 4, p. 164-169, 1997. http://dx.doi.org/10.1016/S0167-5699(97)84662-2

COELHO, A. R. et al. *Penicillium expansum* versus antagonist yeasts with perspectives of application in biocontrol and patulin degradation. **Brazilian Archives of Biology and Technology**, v. 50, n. 4, p. 725-733, 2007.

COELHO, A. R. et al. Avaliação do potencial antagônico de leveduras, visando biocontrole de deterioração por *Penicillium expansum*. **Semina Ciências Agrárias**, v. 32, p. 1879-1892, 2011.

FAZIO, M. L. S.; GONÇALVES, T. M. V.; HOFFMANN, F. L. Potencial antagônico de levedura sobre fitopatógenos micotoxigênicos em frutas. **Higiene Alimentar**, v. 26, p. 147-151, 2012.

FEHLBAUM, P. et al. Insect immunity. Septic injury of Drosophila induces the synthesis of a potent antifungal peptide with sequence homology to plant antifungal peptides. **Journal of Biological Chemistry**, v. 269, p. 33159-33163, 1994.

GASPERINI, A. M. et al. Leveduras killer visando biocontrole de fungo micotoxigênico In: CONGRESSO BRASILEIRO DE MICROBIOLOGIA, 26., 2011, Foz do Iguaçu. **Anais**... Foz do Iguaçu: Sociedade Brasileira de Microbiologia, 2011. v. 1, p. 1.

GENG, P. et al. Combination of *Kluyveromyces marxianus* and sodium bicarbonate for controlling green mold of citrus fruit. **International Journal of Food Microbiology**, v. 151, n. 2, p. 190-194, 2011. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.08.023

GOLUBEV, W. I. Mycocins (killer toxins) In: KURTZMAN, C. P.; FELL, J. W. (Eds.). **The yeasts, a taxonomic study.** London: Academic Press, 1998. p. 55-62.

IPPOLITO, A. et al. Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. **Postharvest Biology and Technology**, v. 19, n. 3, p. 265-272, 2000. http://dx.doi.org/10.1016/S0925-5214(00)00104-6

JANISIEWICZ, W. J.; KORSTEN, L. Biological control of postharvest diseases of fruits. **Annual Review Phytopathology**, v. 40, p. 411-441, 2002.

KRAIKRUAN, W.; SANGCHOTE, S.; SUKPRAKAN, S. Effect of capsaicin on germination of *Colletotrichum capsici* conidia. **Kasetsart Journal - Natural Science**, v. 42, n. 3, p. 417-422, 2008.

KURITA, S. et al. Studies on the antimicrobial mechanisms of capsaicin using yeast DNA microarray. **Bioscience, Biotechnology and Biochemistry**, v. 66, n. 3, p. 532-536, 2002.

LIMA, G. et al. Integration of biocontrol yeast and thiabendazole protects stored apples from fungicide sensitive and resistant isolates of *Botrytis cinerea*. **Postharvest Biology and Technology**, v. 40, n.1, p. 341-349, 2006. http://dx.doi.org/10.1016/j.postharvbio.2006.01.017

LIMA, G. et al. Integrated control of blue mould using new fungicides and biocontrol yeasts lowers levels of fungicide residues and patulin contamination in apples. **Postharvest Biology and Technology**, v. 60, n. 2, p. 164-172, 2011. http://dx.doi.org/10.1016/j.postharvbio.2010.12.010

LIU, X. et al. Antibacterial activity of capsaicin-coated wool fabric. **Textile Research Journal**, v. 82, n. 6, p. 584-590, 2012. http://dx.doi.org/10.1177/0040517511426608

LUO, X. J.; PENG, J.; LI, Y. I. J. Recent advances in the study on capsaicinoids and capsinoids. **European Journal of Pharmacology**, v. 635, n. 1-3, p. 1-8, 2011. http//10.1016/j.ejphar.2010.09.074

MARI, M.; BERTOLINI, P.; PRATELLA, G.C. Non conventional methods for the control of post-harvest pear diseases. **Journal of Applied Microbiology**, v. 94, n. 5, p. 761-766, 2003.

MARTÍNEZ-ROMERO, D. et al. Influence of carvacrol on survival of *Botrytis cinerea* inoculated in table grapes. **International Journal of Food Microbiology**, v. 115, n. 2, p. 144-148, 2007. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.10.015

MOLINA-TORRES, J.; GARCÍA-CHÁVEZ, A.; RAMÍREZ-CHAVÉZ, E. Antimicrobial properties of alkamides present in flavouring plants traditionally used in Mesoamerica: affinin and capsaicin. **Journal of Ethnopharmacology**, v. 64, n. 3, p. 241-248, 1999. http://dx.doi.org/S0378-8741(98)00134-2

MONTERO, C. R. S. et al. Complementary physical and chemical treatments as an alternative to fungicide use to control postharvest decay incidence and fruit quality of Montenegrina tangerines. **Crop Protection**, v. 29, p. 1076-1083, 2010. http://dx.doi.org/10.1016/j.cropro.2010.06.014

MOSS, M. O.; LONG, M. T. Fate of patulin in the presence of the yeast *Saccharomyces cerevisiae*. **Food Additives and Contaminants**, v. 19, n. 4, p. 387-399, 2002. http://dx.doi.org/10.1080/02652030110091163

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS – NCCLS. **Performance standards for antimicrobial disk susceptibility test**. 6th ed. Wayne, 1997. Approved Standard M2-A6.

NELSON, P. E.; TOUSSON, T. A.; MARASAS, W. F. O. ***Fusarium* species an illustrated manual for identification**. Pennsylvania: Pennsylvania State University Press, 1983. 193 p.

NERI, F. et al. Control of *Penicillium expansum* in pears and apples by *trans*-2-hexenal vapours. **Postharvest Biology and Technology**, v. 41, n. 1, p. 101-108, 2006. http://dx.doi.org/10.1016/j.postharvbio.2006.02.005

PEDRAS, M. S. C.; PEARSON, W. K. A. Metabolism and detoxification of phytoalexins and analogs by phytopathogenic fungi. **Phytochemistry**, v. 66, n. 4, p. 391-411, 2005. http://dx.doi.org/10.1016/j.phytochem.2004.12.032

PITT, J. I. Toxigenic *Penicillium* species. In: DOYLE, M. P.; BEUCHAT, L. B.; MONTVILLE, T. J. (Eds.). **Food Microbiology**: Fundamentals and Frontiers. Washington: ASM Press, 1997. p. 411.

ROMANAZZI, G. et al. Recent advances on the use of natural and safe alternatives to conventional methods to control postharvest gray mold of table grapes. **Postharvest Biology and Technology**, v. 63, n. 1, p. 141-147, 2012. http://dx.doi.org/10.1016/j.postharvbio.2011.06.013

ROBIGLIO, A. et al. Yeast biocontrol of fungal spoilage of pears stored at low temperature. **International Journal of Food Microbiology**, v. 147, n. 3, p. 211-216, 2011. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.04.007

SALAS, M. P. et al. Antifungal activity of natural and enzymatically-modified flavonoids isolated from citrus species. **Food Chemistry**, v. 124, n. 4, p. 1411-1415. 2011. http://dx.doi.org/10.1016/j.foodchem.2010.07.100

SANTOS, M. M. P. et al. Antibacterial activity of *Capsicum annuum* extract and synthetic capsaicinoid derivatives against Streptococcus mutans. **Journal Natural Medical**, v. 66, n. 2, p. 354-356, 2012. http://dx.doi.org/10.1007/s11418-011-0579-x

SINDHU, S. B. et al. Chemoprevention by essential oil of turmeric leaves (*Curcuma longa* L.) on the growth of *Aspergillus flavus* and aflatoxin production. **Food and Chemical Toxicology**, v. 49, n. 5, p. 1188-1192, 2011. http://dx.doi.org/10.1016/j.fct.2011.02.014

SPOTTS, R.; CERVANTES, L. A. Population, pathogenicity and benomyl resistance of *Botrytis* spp., *Penicillium* spp. and *Mucor piriformis* in packinghouses. **Plant Disease**, v. 70, p. 106-108, 1986.

THEISEN, S.; MOLKENAU, E.; SCHMITT, M. J. Wicaltin, a new protein toxin secreted by the yeast *Williopsis californica* and its broad-spectrum antimycotic potential. **Journal of Microbiology and Biotechnology**, v. 10, p. 547-550, 2000.

TSUCHIYA, H. Biphasic membrane effects of capsaicin, an active component in *Capsicum* species. **Journal of Ethnopharmacology**, v. 75, p. 295-299, 2001. http://dx.doi.org/10.1016/S0378-8741(01)00200-8

VASANTHA RUPASINGHE, H. P. V. et al. Vanillin inhibits pathogenic and spoilage microorganisms in vitro and aerobic microbial growth in fresh-cut apples. **Food Research International**, v. 39, n. 5, p. 575-580, 2006. http://dx.doi.org/10.1016/j.foodres.2005.11.005

XING, F.; CHENG, G.; YI, K. Study on the antimicrobial activities of the capsaicin microcapsules. **Journal of Applied Polymer Science**, v. 102, n. 2, p. 1318-1321, 2006. http://dx.doi.org/10.1002/app.23766

ZEYREK, F. Y.; OGUZ, E. *In vitro* activity of capsaicin against *Helicobacter pylori*. **Annals of Microbiology**, v. 55, n. 2, p. 125-127, 2005.

WALKER, G.; McLEOD, A.; HODGSON, V. Interactions between killer yeast and pathogenic fungi. **FEMS Microbiology**, v. 127, n. 3, p. 213-222, 1995.