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Efficacy of biocontrol of postharvest diseases of apple caused by *Penicillium expansum*

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In this study two genus of biocontrol yeasts were isolated from healthy apple fruits surface. Three strains of *Candida membranfaciens* (A_2, A_4 and A_5) and two strains of *Rhodotorula mucilaginosa* (A_1 and A_7) were evaluated for the control of the blue mold of apple caused by *Penicillium expansum*. One isolate of *P. expansum* was used in these experiments. Dual culture, cell free metabolite and volatile test were used for *in vitro* assays. Yeast strains of two genus inhibited growth of *P. expansum*, inhibition varied from 20.6 - 61.4%, in dual culture, in volatile metabolite from 57.2 - 89.1% and from 54.6 - 86% in cell free metabolite test. Apple fruit wounds were inoculated with 40 µl of yeast cell suspension (10^7 cell ml^{-1}), and after 24 h, inoculated by *P. expansum* (10^5 conidia ml^{-1}). Inoculated apples were incubated at room (20°C) and cold (5°C) temperature. All strains of two genus reduced lesion area at two temperatures. The A_2 (*C. membranfaciens)* was the most effective strain at both temperatures, significantly. Effect of $CaCl_2$ mixed with yeast (A_1), was evaluated at 20°C. The different concentration of $CaCl_2$ reduced the lesion area from 185.07 - 1738.037 mm^2 reduction compared to 2452.84 mm^2 in control after incubation for 15 days at 20°C. At the same time, the other antagonistic yeast conditions such as yeast concentration and inoculation time were studied, and the best conditions for antagonistic yeast were determined.

Keywords: biocontrol of postharvest diseases, *Penicillium expansum.*

INTRODUCTION

Fruits and vegetables suffer significant losses from fungal diseases after harvest (Eckert et al., 1994). Post harvest losses of fruits and vegetables are high, ranging from 10 and 40% depending on the commodity and technologies used in the packinghouses.

Postharvest fungal diseases of apple are mainly caused by *Penicillium expansum* (Gholamnejad and Etebarian, 2009). Traditionally, this disease is controlled by the application of synthetic fungicides. However, the potential impact on environment as well as human health largely limits their application (Wisniewski et al., 1991). It is reported that some pathogens become to fungicide resistant (Spotts and Cervantes, 1986), and thus a fungicide's effect on controlling fungal growth may be greatly reduced. Considering the human health and pollution risks, the use of some fungicides is prohibited in many developed countries (e.g. USA and EU countries).

Recently, biological control has been developed as an alternative to synthetic fungicides (Wilson et al., 1993), and has been achieved considerable success by using antagonistic microorganisms for controlling post harvest diseases. Roberts (1990) discovered that *Cryptococcus laurentii* has antagonistic activity against many post-harvest pathogens. He added that the competition for nutrients may play a role in the antagonism of *Cryptococcus laurentii*. Decay caused by *Rhizopus* spp. is reduced up to 70% when strawberries are treated with *Aureobasiduim pullulans* before storage (Lima et al., 1997).

A few studies were reported in Iran concerning the advantages of utilizing antagonistic micro-organisms include reducing environmental pollution, effectively controlling postharvest diseases, and producing high quality and safe food.

In this study, the efficacy of some yeast species in controlling postharvest diseases of apple caused by *Penicillium expansum* was tested, and one strain with the

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best biocontrol effect was identified. The antagonistic conditions of that yeast and the methods of antagonism were also discussed.

MATERIALS AND METHODS

Fruit

The apple (*Malus domestica* var. Golden Delicious) at uniform size and maturity without wounds or rot, were used in this study. Apples were harvested at commercial maturity and kept at 4°C until use. The apples were obtained from organically grown position in orchard in Damavand, Tehran.

Pathogen

The isolate P_{11} of *P. expansum* obtained from rotted *Malus domestica* 'Golden Delicious' from Damavand, Iran, was used in this study. The culture was derived from single spore isolate and maintained on Potato Dextrose Agar (PDA) at 4 °C in darkness until needed.

Biocontrol agents

Biocontrol agents were isolated from the surface of apple healthy fruits collected from organic production orchards, following methodology described by Wilson et al., (1993). Isolates consisted of *C. membranfaciens* (A_2, A_4 and A_5) and two strains of *R. mucilaginosa* (A_1 and A_7). Identification of selected strains was carried out by "Identification Service CBS" by molecular and morphological methods. Optimum growth temperature for each strain in PDA was determined for each strain. Temperatures assayed were 5, 25, 30, and 37°C.

In vitro biological control studies

Dual culture was carried out according to Dennis and Webster (1971). Yeast strains were grown at 20°C in nutrient broth yeast dextrose for 48 h on a rotary shaker at 250 rpm. Aliquots of 200 µL yeast suspension (1.0×10^7 CFU L^{-1}) were streaked on half plates. After incubation at 20°C in the dark for 48 h, a plug (10 mm diameter) cut from the leading edge of a 14 day old culture of *P. expansum* on PDA medium was placed on the other half of the plate. Potato dextrose agar inoculated with the pathogen alone served as the control. Plates were incubated at 20°C for 18 d then the colony diameters were measured.

Cell- free culture was carried out according to Weller (1988) and volatile metabolite tests were conducted according to Lillbro (M.Sc. thesis, Swedish University of Agricultural Sciences, 2005). These tests were used to observe the effect of yeast strains on mycelial growth of *P. expansum*. All antagonist–pathogen combinations were examined on PDA in 90 mm petri plates with four replications. The plates were incubated 18 days at 25°C for both dual culture and cell free culture and 25 days for volatile metabolite. All tests were carried out in four replicates.

The percent of the growth inhibition was calculated using the formula $n = (a - b)/a \times 100$, where n is the percent growth inhibition, "a" is the colony area of *P. expansum* growth inhibition and "b" is the colony area of treated , as described previously by Etebarian et al. (2005).

In vivo test

The pathogen (P_{11}) was grown on PDA plates for 14 days. *Conidia* were harvested by pouring a few ml of sterile distilled water (SDW) containing 0.05% tween 20 on the plates. The conidia suspension was adjusted to 1×10^5 conidia ml^{-1}. The apple fruits were washed in 70% ethanol for 30 s followed by dipping in 0.1% sodium hypochlorite solution and rinsed with sterile distilled water. The apples were wounded with 2.5 mm diameter nail to a depth of 3 mm in triplicate (Gholamnejad et al., 2009).

Evaluation antagonistic effect of yeasts at different storage temperatures (5 and 20°C): The yeast inoculum was prepared in nutrient yeast dextrose broth NYDB on a rotary shaker at 150 rpm for 48 h at room temperature. The produced yeast cell were collected by centrifugation at 6500 × g for 5 min and resuspended in water to obtain the desired concentration. Concentration was adjusted to 1×10^7 conidia ml^{-1} with hemacytometer (Gholamnejad et al., 2009). The inoculum of *P. expansum* (P_{11}) was prepared as described above.

The apple fruits were wounded as described above, and inoculated with 40 L of yeast cell suspension.

The treated apples were placed on cardboard trays that were then enclosed in plastic bags. The inside of the bags were sprayed with SDW to maintain high relative humidity (up to 90%) in the bags.

After 24 h, the wounds were inoculated with 20 µl of conidial suspe-nsion of the pathogen (10^5 conidia ml^{-1}).Treated apples were incubated at two temperatures, room (20°C) cold (5°C) in humid condition for 15 and 32 days, respectively. The lesion diameter was calculated after 15 days, using calipers. Each apple constituted a single and each treatment was replicated 4 times.

The antagonistic effect of different inoculation times: Wounds were inoculated with 40 L of *C. membranfaciens* (A_5) that was prepared as described above, the cell suspension at a concen-tration of 1×10^7 conidia ml^{-1}. After 2, 14, 32 h and 48 a 20 L *P.expansum* (P_{11}) suspension in a concentration of 1×10^5 conidia ml^{-1} was added to each wound, and the apples were stored at 20°C.

The antagonistic effect of the different yeast concentrations: Wounds were inoculated with 20 l of *C. membranfaciens* (A_4) that was prepared as described above, the cell suspension at the concentrations of 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia ml^{-1}. After 24 h, a 20 µL *P.expansum* (P_{11}) suspension at a concentration of 1×10^5 conidia ml^{-1} was added to each wound. The apples were then stored at 20°C.

Evaluation antagonistic effect of $CaCl_2$ concentration on biocontrol activity of yeast: Aliquots of 50 ml distilled water with different concentration of $CaCl_2$: 0 Mm, 173 Mm (2%), 347 Mm (4%), and 521 Mm (6%) $CaCl_2$, in 250 ml conical flasks were autoclaved (121°C, 15 min) prior to adding suspension of antagonistic yeast to some flask. The cell suspensions of antagonists were adjusted to concentrations of 1×10^7 cfu ml^{-1} in this solution. The same percentages of $CaCl_2$ without antagonistic yeast were prepared as described above.

Wounds were inoculated with a 40 l of the yeasts with and without $CaCl_2$ were prepared as described above. After 24 h, a 20 l of *P. expansum* (P_{11}) suspension in a concentration of 1×10^5 spores ml^{-1} was added to each wound, and the apples was stored for 15 days at 20°C. The lesion diameter was calculated after 15 days, using calipers. Each apple constituted a single and each treatment was replicated 4 times (Tian et al., 2002).

Population dynamics of yeast on apple surface: Growth curves were monitored in fruit wounds at 20 and 5°C. The wounds were inoculated with 40 µl of yeast (A_1 and A_2 strains) suspension of known concentration (10^7 cfu ml^{-1}), and after 24 h wounds were inoculated with 20 µl of conidial suspension of the pathogen (P_{11}) , then apples were incubated for 15 days at 20°C and for 32 days at

Table 1. *P. expansum* (P_{11}) growth inhibition percentage by *C. membranfaciens* (A_2, A_4 and A_5) and *R. mucilaginosa* (A_1 and A_7) strains *in vitro*[a].

Yeast isolates	Percentage of inhibition[b]		
	Dual culture	Volatile test	Cell free metabolite
C. membranfaciens (A5)	20.59 b	73.5 c	84.3 a
C. membranfaciens (A2)	28.48 b	57.18c	84.38 a
C. membranfaciens (A4)	30.93 b	78.94 b	72.49 b
R. mucilaginosa (A1)	61.4 a	71.86 c	83.36 b
R. mucilaginosa (A7)	34.51b	89.14 a	86.03 a

[a]Values are the mean inhibition growth percentage from four replication. [b]Values in the same column followed by the same letter are not statistically different by Duncan's Multiple Range Test ($P < 0.05$).

5°C. Controls were inoculated with SDW.

Apples were removed a block of tissue (1gr). The block was removed and shaken in 9 ml sterile water on a rotatory shaker for 20 min at 150 rpm. Serial dilutions of the washings were made and one mL was plated on NYDA containing 0.5 g/l streptomycin sulphate as a bacteriostat. After incubation at 25°C for 48 h the isolated viable colonies per gram of fresh weight of fruit (cfu/g) were counted for each sample. This study was carried out with four replicates per treatment and sample unit was four apples. (Teixido et al., 1999)

Statistical Analysis

The *in vitro* and *in vivo* assays were analyzed by an analysis of variance (ANOVA) with SAS Software (SAS Institute, version 9.0, Cary, NC). All assays were carried in a completely randomized design with four replicates. Statistical significance was judged at the level $p < 0.05$. When the analysis was statistically significant, Duncan's Multiple-Range Test (SSR Test) was used to test mean separations among mean values of each treatment.

RESULTS

Effects of the antagonists *in vitro*

The two antagonist tested yeasts could inhibit significantly the *P. expansum* (P_{11}) mycelia growth of in dual culture, compared among. However there were significant differences among yeast strains. Growth inhibition of *P. expansum* (P_{11}) by strain A_1 (*R. mucilaginosa*) was significantly greater than calculated by of other strains, whereas strain A_4 (*C. membranfaciens*) had less effect on the growth of the pathogen (Table 1).

Results shown in Table 1 indicated that antifungal activity of volatile metabolites of the *C. membrane-faciens* strain A_2 was lower compared with other strains. Cell free metabolite of *C. membranfaciens* reduced growth of *P. expansum* (P_{11}) by 72.5 - 84.4%, whereas *R. mucilaginosa* reduced the colony area of pathogen from 83.36 - 86.03% (Table 1).

Effect of different storage temperatures on decay area

All of strains of two genus yeasts significantly controlled blue mold on apples at 20 and 5°C (P 0.05). Strains of *C. membranfaciens* reduced decay area from 201.76 to 973.23 mm^2, whereas *R. mucilaginosa* reduced from 1584.14 - 1647.19 mm^2 compared to 3257.44 mm^2 in, after incubation for 15 days at 20 °C (Table 2).

At 5°C, *C. membranfaciens* strains reduced decay area from 298.4 to 881.8 mm^2, whereas *R. mucilaginosa* reduced 1631.9 - 1908 mm^2 compared with 3151.1 mm^2 in control, after 32 days incubation at 5°C (Table 2). Optimum temperature growth for both two antagonistic yeasts was 25°C (data not shown).

Effects of different inoculation times on decay area

P. expansum (P_{11}) was added 2, 14, 32 and 48 h after the inoculation of the antagonistic yeast. Then the apples were stored at 20°C, and the decay area was observed every 24 h after inoculation (Figure 1).

The Figure 1 shown, it can be seen that the later the path-ogen were introduced after the inoculation of the yeast, the better the antagonism was. The reason may be that with enough time the yeast had reproduced and used the nutrient (competition for space and nutrition), or the yeast had secreted enough antagonistic substances. So yeast should been applied the antagonist early after the harvest to prevent the pathogens from infecting.

Effects of different concentration of yeast on decay area

High, middle and low concentrations of yeast (LC = 1×10^5 and 1×10^6 conidia ml^{-1}; MC = 1×10^7 conidia ml^{-1}; HC = 1×10^8 conidia ml^{-1}) were inoculated into wounds of apple. The apple fruits were stored at 20°C. Decay area were recorded every 24 h after the treatment (Figure 2). It was obvious from Figure 2 that the antagonism was related to the concentration of antagonist. When concentration of the yeast reached 1×10^7 conidia ml^{-1} and spore suspension of the pathogens was 1×10^5 conidia ml^{-1}, distinct antagonism was observed. The postharvest biocontrol capability of the apple fruits was

Table 2. Lesion area on Golden delicious apples with *P. expansum* (P_{11}) challenged with *C. membranfaciens* (strains A_2, A_4 and A_5) and *R. mucilaginosa* (strains A_1 and A_7) and incubated for at 20 and 5°C *in vivo*.

Treatment	Lesion area[b] (cm^2)					
	A_5	A_2	A_4	A_7	A_1	Control
20°C	973.23c	309.23d	201.76d	1584.14b	1647.19b	3257.44a
5°C	298.4f	881.84d	490.57e	1631.94c	1907.96b	3151.16a

[a]Values are the mean lesion area from four apples. [b]Values in the same row followed by the same letter are not statistically different by Duncan's Multiple Range Test ($P < 0.05$).

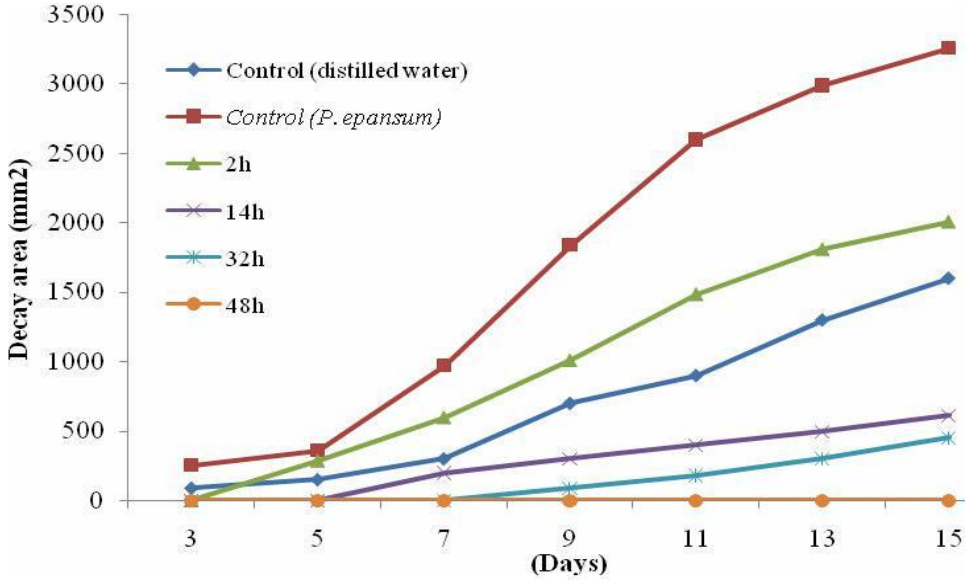


Figure 1. Effect of inoculation time of *P. expansum* on apple decay area.

Table 3. Effect of different concentrations of $CaCl_2$ and *Candida membranfaciens* (A_5) on lesion area on apples[a].

Treatment (%)	Main inhibition (%)[b]	
($CaCl_2$)	A_5[c]+$CaCl_2$	$CaCl_2$ (without yeast)
0	61. 9c	0a
2	70.5d	29b
4	86f	54.8c
6	92.4f	61.1c

[a]Values are the mean lesion area percentage from four apples. [b]Values in the table followed by the same letter (in the row and column) are not statistically different by Duncan's multiple range test. ($P < 0.05$). [c]One *C. membranfaciens* strain

enhanced with the increase of the antagonist concentration.

Effectiveness of $CaCl_2$ on biocontrol activity of two strains of yeasts

There was a significant reduction in the diseases after $CaCl_2$ was added to the yeasts treatments, as controlled to $CaCl_2$ alone (P 0.05) (Table 3 and 4). The results showed that all concentrations of Ca^{2+} had significant effect on *P. expansum* lesion diameter. The control of pathogen was positively correlated to Ca^{2+} concentrations; $CaCl_2$ at 521 Mm provided better control than 347 mmol L^{-1} but wasn't significant. Finally, 347 Mm was

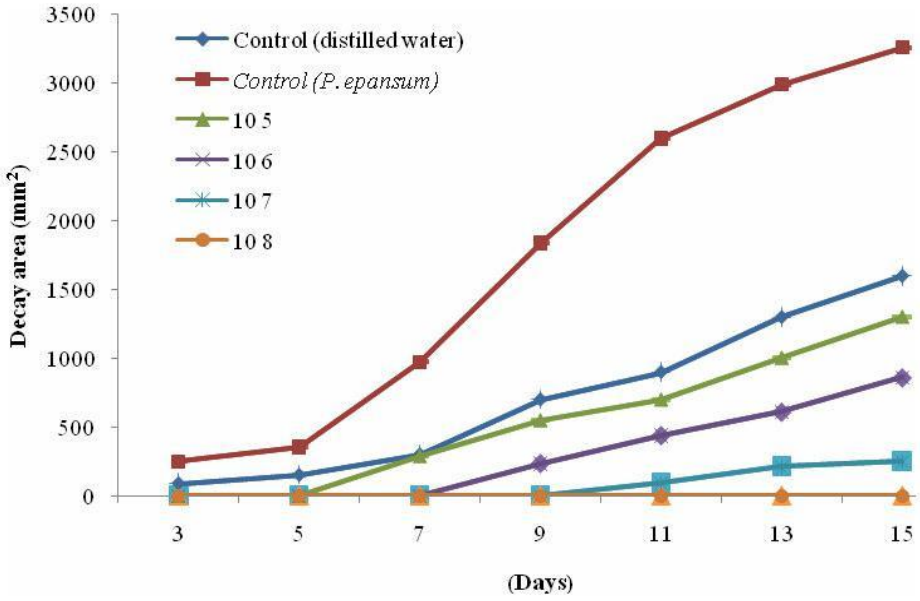


Figure 2. Effects of *C. membranfaciens* (A5) concentration on apple decay area.

Table 3. Effect of different concentrations of $CaCl_2$ and *C. membranfaciens* (A_5) on lesion area on apples[a].

Treatment (%)	Main inhibition (%)[b]	
($CaCl_2$)	A_5[c]+$CaCl_2$	$CaCl_2$ (without yeast)
0	61. 9c	0a
2	70.5d	29b
4	86f	54.8c
6	92.4f	61.1c

[a]Values are the mean lesion area percentage from four apples. [b]Values in the table followed by the same letter (in the row and column) are not statistically different by Duncan's multiple range test ($P < 0.05$).[c]One *C. membranfaciens* strain.

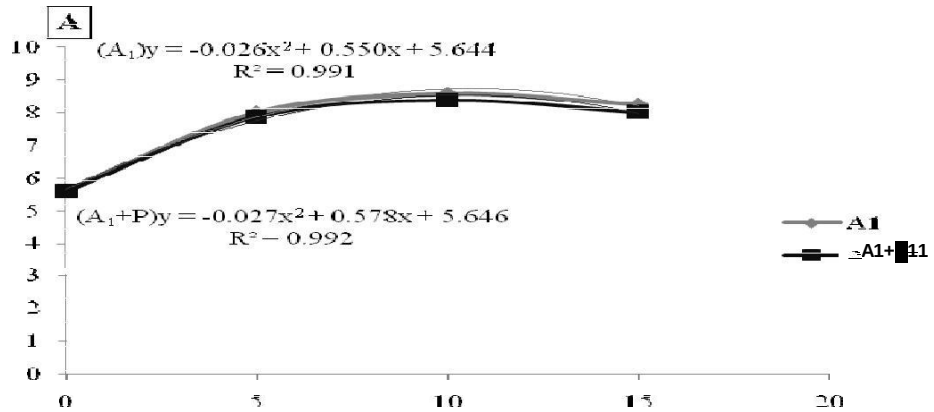
Table 4. Effect of different concentrations of Ca^{2+} and *Rhodotorula mucilaginosa* (A_1) on lesion area in apples[a].

Treatment (%)	Main inhibition (%)[b]	
($CaCl_2$)	A_1 [c]+ $CaCl_2$	$CaCl_2$ (without yeast)
0	56.5c	0a
2	61c	29.1b
4	77.9d	54.8c
6	88.2e	60c

[a]Values are the mean lesion area percentage from four apples. [b]Values in the table followed by the same letter are not statistically different by Duncan's multiple range test ($P < 0.05$). [c]One *R. mucilaginosa* strain.

Population dynamics

The population dynamics of two yeasts were evaluated after 5, 10 and 15 days at 20°C and 10, 20 and 30 at 5°C. The maximum population was recovered 10 and 20 days after the yeasts application at 20°C and 5°C, respectively. Initially, 4×10^5 cfu g^{-1} inoculated on the wounds, 10 days after applications of R. *mucilaginosa* (strain A_1) at 20°C, population were maximum, about 3.7×10^8 CFU g^{-1}, after that decrease population, until end of the experiment



Population sizes of *R. mucilaginosa* strain A_1 in apple wounds stored at 20°C

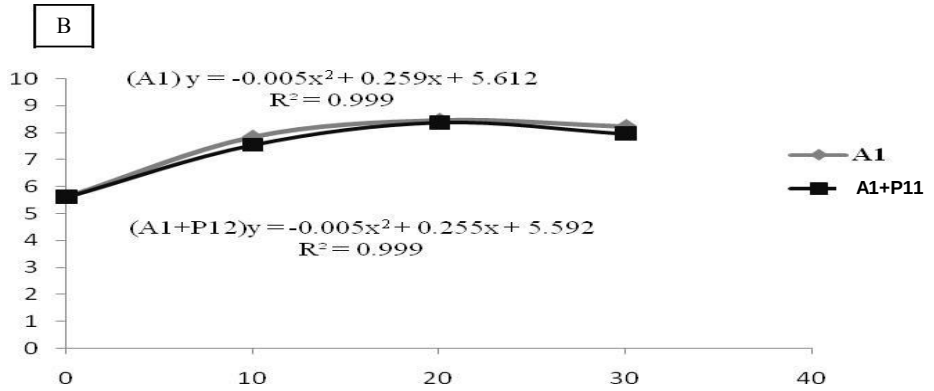


Figure 3. Population dynamics of *R. mucilaginosa* strain A_1 ( ); and strains A_1 to added pathogen ( ), in wounds of apple fruit stored at 20°C (A) , and 5°C (B).

(Figure 1.) . At 5°C, in the twentieth day population was maximum, about 3.1×10^8 cfu g^{-1}, after that decrease population, until end of the experiment (Figure 3).

DISCUSSION

The use of biocontrol agents to manage postharvest decay of fruits has been explored as an alternative to the use of synthetic fungicides (Wilson and Wisniewski, 1989) and several commercial products are now available (Droby et al., 1998). Further identification of new antagonists is desirable because antagonists identified in specific geographic areas may be more effective against the pathogen isolates present in that locale. In the present research, we have identified two yeast antagonists that exhibit biocontrol efficacy against blue mold of apples caused by *P. expansum*. This is the first report of antagonists *Candida membranfaciens* and *Rhodotorula mucilaginosa* as antagonists of blue mold of apples.

The biocontrol activity of yeasts antagonist such *Cryptococcus laurentii* and *Candida ciferrii* (Vero et al., 2002) haven't been attributed to the production antifungal metabolite. Regardless, our data showed better inhibition, in dual culture. All antagonistic yeast strains reduced mycelia growth of pathogen by means of dual culture, cell free metabolite and volatile metabolite test. In cell free culture test all strains of yeast produced high inhibition zone on PDA indicating that they synthesize compounds that are highly active against pathogen.

Growth curves of the antagonists demonstrated that all of strains of yeast could colonize and grow in apple wounds. Even after a period of 32 days at 5°C, and 14 days at 20ëC the number of viable microorganisms was greater than that originally introduced into the wound. Etebarian et al. (2005) demonstrated that *Pseudomonas fluorescens* population on wounded apple inoculated with *P. expansum* increased with 10-100 fold at wound site over 20 days at 20°C. In our case, the wound carrying capacity was approx. 3.7×10^8 cfu ml^{-1} per wound after only 10 days. This may indicate that *R. mucilaginosa* strain A_1 has a high capacity for colonizing apple wounds and could be potentially a better biocontrol agent. These data indicate that only one application of the antagonists may be enough to prevent blue mold rot for at least a period of 32 days at 5°C, and 14 days at 20°C.

In this study result for apples stored at 5°C for 32 days

and at 20°C for 14 days showed that these 5 strains reduced growth of *P. expansum* and was effective for control of pathogen. These strains have also ability for suppressing other pathogen such as green mold of oranges (*Penicillium digitatum*), grey mould on tomato (*Botrytis cinerea*) (Dal Bello et al. 2008). The adaptation of these five strains to a wide range of temperature provides great market potential for this product for control of postharvest diseases on apples in storage and transportation, as well as under arbitrary temperature in the market places and consumers' home.

The activity of both yeast strains and $CaCl_2$ against pathogen provided an opportunity to combine these agents at lower concentration while mainting a high level of effectiveness and consistency of performance are two major factors limiting the use of biocontrol agent in plant disease control (Chalutz and Wilson, 1990). Yeasts and $CaCl_2$ had a significant synergistic effect on reduction of blue mold on golden delicious apple. $CaCl_2$ no suppressive effect on yeasts until 6% (w/v*) in vitro*. A combination of a biocontrol agent with a salt both significantly reduced at concentration from what would be needed if used alone may greatly reduce their residue on fruit (Gholamnejad and Etebarian, 2009).

In conclusion, five strains of yeasts tested here reduced disease severity of blue mold of apple at 5 and 20°C. The combination of these strains and $CaCl_2$ were most effective on controlling of blue mold of apple. These strains warrant further investigation for their ability to control blue mold in commercial situation.

Further identification of new antagonists is desirable because antagonists identified in specific geographic areas may be more effective against the pathogen strains present in that locale.

REFERENCES

Chalutz E, Wilson CL (1990). Postharvest biocontrol of green and blue mold and sour rot of citrus fruits by *Debaryomyces hansenii*. Plant Dis. 74: 134-137.

Dal Bello G, Monaco C, Rollan MC (2008). Biocontrol of postharvest grey mould on tomato by yeasts. Phytopathol., 156: 257-263.

Dennis C, Webster J (1971). Antagonistic properties of species groups of *Trichoderma*. III. Hyphal interaction. Trans. Br. Mycol. Soc., 57: 363-369.

Droby S, Cohen L, Daus A, Wisniewski ME, Wiess B (1998). Commercial testing of Aspire: a yeast preparation for the biological control of postharvest decay of citrus. Biol. Control, 12: 97-101.

Eckert JW, Sievert JR, Ratnayake M (1994). Reduction of imazalil effectiveness against citrus green mold in California packinghouse by resistant biotypes of *Penicillium digitatum*. Plant Dis., 78: 791-794.

Etebarian HR, Sholberg PL, Eastwell KC, Sayler RJ (2005). Biological control of apple blue mold with *Pseudomonas fluorescens*. Can. J. Microbiol., 51: 591-598.

Gholamnejad J, Etebarian HR (2009). Effect of calcium chloride on the biocontrol efficacy of two antagonistic yeasts against *Penicillium expansum* on apple fruit. Phytopara., 37: 255-267.

Gholamnejad J, Etebarian HR, Roustaee A, Sahebani NA (2009). Biological control of apples blue mold by isolates of *Saccharomyces cerevisiae*. J. P. Prot. Rea., 49: 270-275.

Gholamnejad J, Etebarian HR, Roustaee A, Sahebani NA (2009). Characterization of Biocontrol Activity of Two Yeast Strains from Iran against Blue Mould of Apple in Order to Reduce the Environmental Pollution. J. Int. Environ. Appl. Sci., 4(1): 28-36.

Lillbro M (2005). Biocontrol of *Penicillium roqueforti* on grain-acomparison of mode of action of several yeast species. Master theisis for the Agriculture Programme, animal science, performed at the Department of Microbiology. Swedish University of Agricultural Sciences pp. 21.

Lima G, Ippolito A, Nigro F, Salerno M (1997). Effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against postharvest strawberry rot. Postharvest Biol. Technol., 10: 169-178.

Roberts RG (1990). Postharvest biological control of gray mold of apple by *Cryptococcus laurentii*. Phytopathol., 80: 526-529.

Spotts RA, Cervantes LK (1986). Populations, pathogenicity, and benomyl resistance of *Botrytis* spp., *Penicillium* spp. and *Mucor piriformis* in packinghouses. Plant Dis., 70: 106-108.

Teixido N, Usall J, Vinas I (1999). Efficacy of preharvest and postharvest *Candida sake* biocontrol treatments to prevent blue mould on apples during cold storage. International J. Food Microbiol., 50: 203-210.

Tian SP, Fan Q, Xu Y, Jiang AL (2002). Effects of calcium on biocontrol activity of yeast antagonists against the postharvest fungal pathogen *Rhizopus stolonifer*. Plant Dis., 51: 352-358.

Vero S, Mondino P, Burgaeno J, Soubes M, Wisniewski M (2002). Characterization of biological activity of two yeast strains from Uruguay against blue mold of apple. Postharvest Biol. Technol. 26: 91-98.

Weller DM (1988). Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol., 26: 379-407.

Wilson CL, Wisniewski ME (1989). Biological control of postharvest diseases of fruits and vegetables. An emerging technology. Annu. Rev. Phytopathol., 27: 425-441.

Wilson CL, Wisniewski ME, Droby S, Chalutz E (1993). A selection strategy for microbial antagonists to control postharvest diseases of fruits and vegetables. Sci. Horti., 53: 183-189.

Wisniewski ME, Biles C, Droby S, McLaughlin R, Wilson C, Chalutz E (1991). Mode of the postharvest biological yeast, *Pichia guilliermondii* . I. Characterization of attachment to *Botrytis cinerea*. Physiol. Mol. Plant Pathol., 39: 245-258.