EVALUATION OF *BACILLUS* SPP. AS POTENTIAL BIOCONTROL AGENT FOR POSTHARVEST GRAY MOLD CONTROL ON GOLDEN DELICIOUS APPLE IN IRAN

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Abstract: Biocontrol of postharvest decay on apples could provide an alternative approach for chemical control. With the effort to look for effective biocontrol agents, *Bacillus pumilus* (B19), *B. subtilis* (B11), *B. cereus* (B16), *B. subtilis* (B11), *B. cereus* (B17), *B. brevis* (EN63-1) and *B. licheniformis* (EN74-1) were tested for their potential activity against gray mold caused by *Botrytis mali* on Golden Delicious apple in Iran. Dual culture, cell free metabolite and volatile compounds tests showed that all seven isolates inhibited growth of the pathogen. All isolates prevented development of *B. mali* lesion diameter on apples stored at 40°C from 9 to 32.2 mm as compared to 41.6 to 51.4 mm in control. At 20°C the lesion size was from 7 to 24.9 mm for treatments with antagonistic bacteria while in control it ranged from 42.2 to 46.6.

Key words: antagonists, *Botrytis mali*, mycelial growth

INTRODUCTION

Gray mold due to *Botrytis cinerea* Pers. ex Fr. and *B. mali* Ruehle is one of the most important postharvest rot of apples in Iran. *B. mali* was recently described as a separate species from *B. cinerea* based on morphological characteristics and DNA sequence differences (O'Gorman *et al*. 2005). The use of fungicides immediately before or after harvest to prevent rots is being increasingly limited because of environmental, toxicological and technical risks (Jamalizadeh *et al*. 2008). Moreover, the onset of resistance towards the few authorized fungicides is a frequent phenomenon in the population of fungal pathogens (Spotts and Cervantes 1986; Guizzardi *et al*. 1995; Stehmann and DeWard 1996). Biocontrol may also provide a good tool to solve the problems with chemical residues in fruit and juice and pathogen resistance development. Compared with root or foliar diseases, the application of biocontrol agents against postharvest rots of biotic origin is more suitable since the environmental factors are more stable and can be controlled. Considerable research effort has been devoted to select organisms that effectively control postharvest diseases of fruit, vegetables, and grains (Wilson *et al*. 1996). Several strains of fungi and bacteria control postharvest diseases of fruits (Pusey and Wilson 1984; Wilson *et al*. 1987; Janisiewicz and Roitman 1988; Roberts 1990; Janisiewicz and Marchi 1992; McLauglin *et al.* 1992; Bonaterra *et al.* 2003). *Bacillus* spp. produce spores which are resistant to desiccation, heat, UV irradiation, and organic solvents, and have long been evaluated for disease control, including postharvest diseases (Singh and Daverall 1984; Huang *et al.* 1992). Several strains of the genus *Bacillus* have received much attention as biological control agents. *Bacillus subtilis* isolated from citrus fruit surface was successfully evaluated for control of citrus green and blue moulds caused by *Penicillium digitatum* and *P. italicum* respectively (Obagwu and Korsten 2003), and *Bacillus licheniformis* was reported effective against tomato gray mould caused by *B. cinerea* (Lee *et al.* 2006). The present study was conducted to determine: (1) the potential of *Bacillus* spp. for control of postharvest decay caused by *B. mali* on apple in cold storage and non cold storage conditions at 4 and 20°C respectively (2) survey of changes in the population dynamics of the antagonist *Bacillus* spp. in term biocontrol experiments.

MATERIALS AND METHODS

Causal agents

Two isolates of *B. mali* (19JR and 20JR) obtained from infected apple fruits were used. Pathogens were grown on potato dextrose agar (PDA) plates for 7–14 days and conidia were harvested by pouring a few milliliters of sterile water (0.05% Tween 20) on the plate. The conidial suspension was adjusted to 1×105 spores/ml by counting the number of cell with a hemocytometer.

Biocontrol agents

B. pumilus (B19), *B. cereus* (B16), *B. subtilis* (B11), *B. cereus* (B17) and *B. subtilis* (1J) were obtained from Department of Plant Protection of Abourayhan College,

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Tehran University. *B. brevis* (EN63-1) and *B. licheniformis* (EN74-1) from Pacific Agri-Food Research, Summerland, Canada. The bacterial suspension was prepared from the growth in potato dextrose broth on a rotary shaker at 150 r/min for two days at 25°C. The bacterial cells were collected by centrifugation at 6500×g for 5 min and resuspended in water. Cell concentrations were determined by dilution plating on PDA.

Antagonistic and toxic activity of bacteria and their metabolites to *B. mali*

Dual culture test was used to examine the antagonisitic effect of seven *Bacillus* spp. isolates on mycelial growth of *B. mali* (Dennis and Webster 1971). The bacterial isolates were grown at 20°C in nutrient broth for 24 h on a rotary shaker at 150 r-min-1. Three 9-cm diameter plates per treatment containing 10–15 ml of nutrient agar were used for each treatment. Bacterial suspension (0.1 ml; 1.0x109 CFU/ml, culture) was streaked on half plates. After incubation at 20°C in the dark for 24 h, a plug (8 mm diam.) cut from the leading edge of a 10 day-old culture of *B. mali* on potato dextrose agar (PDA) medium, was placed on the other half of the plate 1.5 cm from the edge of the plate. As the control, PDA was inoculated with the pathogen alone. Plates were incubated at 20°C for 20 days when colony diameters and inhibition zones were measured. The percent growth-inhibition was calculated using the formula $n = (a–b)/a×100$, where n is the % growth inhibition; a is the colony area of uninhibited *Botrytis* spp. and b is the colony area of treated *B. mali* (Etebarian *et al*. 2005). Antifungal activity of volatile compounds from *Bacillus* spp. isolates was assessed by inoculating a Petri plate containing PDA medium with a 5 mm plug of *B. mali*. A second petri plate containing PDA cultured by *Bacillus* isolates. The two plates sealed together with parafilm and incubated at 25°C for 9 days. The percent growth inhibition was determined as above. Control consisted of pure culture without *Bacillus* (Fiddaman and Rossall 1993). Cell free metabolite based on the method of Weller (1988) was conducted to determine if the metabolites produced by *Bacillus* spp. are able to inhibit *B. mali*. Petri plates containing PDA medium were streaked with test bacterial isolates and incubated at 25°C for 72 h. Plates were then placed in a sealed dessicator containing cotton wool soaked with chloroform and the lids of the plates were opened for 1 h. The plates were removed from the dessicator in a laminar flow hood with their lids left partially open for evaporation of chloroform. After 30 min in the hood the plates were inoculated with 5 mm plugs of *B. mali* and incubated at 25°C for 9 days. The surface areas of the *B. mali* colonies were recorded daily, compared with the control treatment, and the percent growth inhibition was calculated (Weller 1988).

Biological control experiment on apples

Biocontrol assays were performed at 20 and 4°C. Golden Delicious apples that had been harvested at commercial maturity were used for biological experiments. The apples were washed in 70% ethanol for 30 s, followed by dipping in 0.1% sodium hypocholorite solution, and rinsed with sterile distilled water. The fruit were wounded in triplicate with a 2.5 mm diameter nail to a depth of 3 mm. Then, 20 µl of bacterial suspension (108 CFU/ml) in sterile distilled water was put in each wound. After 24 h, the treated wounds were inoculated with 20 µl of *B. mali* spores (1×105 CFU/ml). The treated apples were placed on cardboard trays that were then enclosed in plastic bags. The inside of the bags were sprayed with strile distilled water to maintain high relative humidity in the bags (Sholberg *et al.* 1995). The lesion diameters were determined 7 and 14 days after storage at 20°C and 10, 20 and 30 days after storage at 4°C. Each apple constituted a single replicate and each treatment was replicated three times (Jamlizadeh *et al.* 2008).

Population dynamics

The population dynamics of three *Bacillus* spp. isolates were evaluated after 10, 20 and 30 days at 4°C. Fruit samples were taken to measure antagonist populations. The antagonist was recovered by removing the wound tissue with a cork borer. The resulting cylinder (1 cm diameter×1 cm deep) was ground with mortar and pestle with 1ml of 0.05 M phosphate buffer at pH 7.0. Serial 10-fold dilutions were made in phosphate buffer, and 0.1 ml of each dilution was plated on PDA medium. Plates were incubated at 24°C for 24 h and colony counts were made. The number of bacteria and fungi was presented as Log10 CFU/ml per wound.

Statistical analysis

Data on the percent inhibition of colony area were subjected to arcsin square root transformation, and data on decay lesion diameter area were subjected to plus 0.5 square root transformation before conducting analysis of variance. The completely randomized design was used for all experiments. Analysis of variance was performed on the data and means were separated using for all experiments. Analysis of variance was performed on the data and means were separated using Duncans̀ multiple Range Test at $p < 0.05$ (Gomez and Gomez 1984).

RESULTS

Effect of *Bacillus* spp. isolates on mycelial growth of 19JR and 20JR on *B. mali* isolates

All seven isolates of *Bacillus* spp. inhibited mycelial growth of both *B. mali* isolates at average 42.3% for 19JR and 44.7% for 20JR in the dual culture test (Table 1). *B. cereus* (B16) and *B. subtilis* (B11) were more effective than other isolates in dual culture test. Percentage of *B. mali* growth inhibition by volatile metabolites were at an average 25% for 19JR and 32.6% for 20JR. Mycelial growth at higher degree of *B. mali* isolates (19 and 20JR) was reduced more by *B. pumilus* (B19) than the other isolates tested. Cell free metabolites produced by all isolates of *Bacillus* spp. reduced mycelial growth of both *B. mali* isolates for average 40.1% for 19JR and 40.2% for 20JR (Table 1). The highest antifungal activity was demonstrated by cell free metabolites of isolates B16 and B19 (Table 1).

Table 1. Percentage of growth inhibition of *B. mali* isolates 19JR and 20JR by *Bacillus* spp. isolates and treir metabolites. Each treatment was replicated three times. Within columns, means followed by the same letter do not differ significantly at $p < 0.05$ according to Duncan's MRT.

Antagonists	Dual culture		Volatile metabolite		Cell free Culture	
	19JR	20JR	19JR	20JR	19JR	20JR
B. brevis (EN63-1)	45	41.6	12	45.2	42	51
B. licheniformis (EN74-1)	23	31.3	21	20.9	20	15.5
B. pumilus (B19)	27.1	32.6	45.2	47	45.6	67.7
B. subtilis (1J)	51.3	55.8	34.5	25.2	39	26
B. cereus (B16)	74	58.6	28.6	53	87	76
B. subtilis (B11)	62.6	72.1	17	11	35	20.3
B. cereus (B17)	13.6	21.6	17.3	26	12.3	25

Table 2. Efficacy of *Bacillus* spp. in controlling gray mold rot on apple under cold storage temperature (4°C). Each treatment was replicated three times. Columns labeled with a common letter do not differ significantly according to Duncan's MRT at $p = 0.05$

Treatment	10 day	20 day	30 day
	lesion diameter [mm]	lesion diameter [mm]	lesion diameter [mm]
B. mali 20JR (Control)	7.6	26.6	51.4
B. mali (20JR)+EN63-1	4.6	8.2	9.6
B. mali (20JR)+EN74-1	1.8	15.8	19.4
B. mali (20JR)+B19	2.2	12	18.8
B. mali (20JR)+1J	3.9	20.6	12.2
B. mali (20JR)+B16	3.0	19.6	32.2
B. mali (20JR)+B11	5.4	11.4	15
B. mali (20JR)+B17	1.8	5.8	7.2
B. mali 19JR(Control)	7.8	24.4	41.6
B. mali (19JR)+EN63-1	4.6	8.6	11
B. mali (19JR)+EN74-1	1	14.4	20.2
B. mali (19JR)+B19	3	11.2	24.6
B. mali (19JR)+1J	3.9	19.6	13.4
B. mali (19JR)+B16	2.6	20.6	32

Effect of *Bacillus* spp. on gray mold lesion development

After one month in cold storage at 4°C all isolates of *Bacillus* spp. prevented the development of *B. mali* lesion B17 and EN74–1 appeared to be most effective (Table 2). In case of bacterial protection the lesion size ranged from 9 to 32.2 mm while diameter those of unprotected was 41.6 to 51.4 mm. After incubation at 20°C for 15 days, both isolates showed also best efficacy in control of gray mold on apple fruits (Table 3). At 20°C the lesion size was from 7 to 24.9 mm for antagonistic treatments and 46.2 to 46.6 for the control treatment.

Number of *Bacillus* spp. during antagonist-pathogen (20JR) interaction

Number of three isolates of *Bacillus* spp. bacteria in wounds sourounding tissue of apples stored at 4°C increased linearly over 30 days (Fig. 1). The log number of bacterial cells for each wound (20 μl) increased and closely followed ($R2 = 0.9657$ for B17; $R2 = 0.9627$ for EN63–1; and $R2 = 0.8909$ for 1J) a linear function when challenged against *B. mali*. The linear equation for each isolate is $y = 0.39x + 7.7$ for B17, $y = 0.19x+7.85$ for EN63–1, and $y = 0.07x+7.9$ for 1J, where y is the log number of bacteria, and x is the number of days after inoculation.

Table 3. Efficacy of *Bacillus* spp. in controlling gray mold rot on apple under supermarket conditions (20°C). Each treatment was replicated three times. Columns labeled with a common letter do not differ significantly according to Duncan's MRT at p = 0.05

Treatment	7 day	14 day
	lesion diameter [mm]	lesion diameter [mm]
B. mali 20JR (Control)	13.4	46.2
B. mali (20JR)+EN63-1	3.1	6.7
B. mali (20JR)+EN74-1	7.7	15.1
B. mali (20JR)+B19	6.3	22.2
B. mali (20JR)+1J	4.9	20.2
B. mali (20JR)+B16	4.4	7.8
B. mali (20JR)+B11	7.5	15.8
B. mali (20JR)+B17	2.4	11.4
B. mali 19JR (Control)	11.2	46.6
B. mali (19JR)+EN63-1	3.4	5.7
B. mali (19JR)+EN74-1	8	15.1
B. mali (19JR)+B19	6.4	24.9
B. mali (19JR)+1J	5	21.1
B. mali (19JR)+B16	4.3	9
B. mali (19JR)+B11	7.8	16.7
B. mali (19JR)+B17	2.7	7

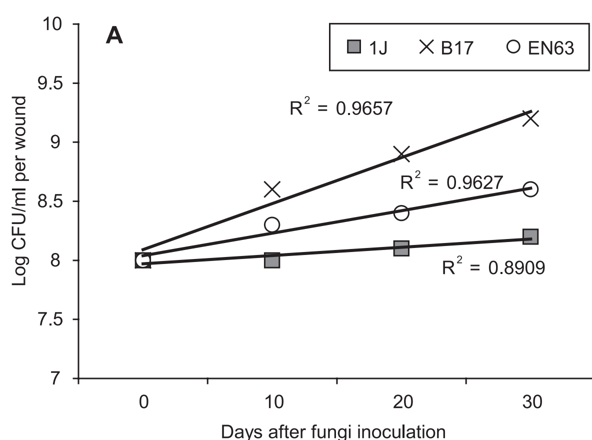

Fig. 1. Growth curve of *Bacillus* spp. 1J, B17 and EN63-1 in apple wounds containing *B. mali* (20JR) over 30 days at 4°C

DISCUSSION

In *in vitro* experiments all the *Bacillus* spp. isolates reduced mycelial growth of pathogen. Zone of inhibition observed between the colonies of pathogen and bacteria could be due to the effect of diffusible inhibitory substances produced by bacteria, which suppressed the growth of pathogen. Another possibility is that the bacterial isolates depleted the nutrients in the agar surrounding them and thereby inhibited the growth of *B. mali*. Hsu and Lockwood (1969) found that the inhibition zone produced by non antibiotic-producing *Streptomyces* were due to the rapid depletion of glucose and glutamic acid to suboptimal level in agar media adjacent to the *Streptomyces* colonies. The presence and size of the zone of inhibition have been used as evidence of the production of antibiotics by the bacteria (Rothrock and Gottlieb 1981; Jackson *et al.* 1991; Crawford *et al.* 1993). For example, Sadfi *et al.* (2001) indicate that halophilic isolate of *B. cereus* X16 produced metabolites against *Fusarium roseum* var. *sambucinum* after a dual culture incubation on PDA (Sadfi *et al.* 2001). Also *in vitro* experiments results showed that *B. brevis* by production antibiotic (gramicidin S) caused decline of mycelial growth of *B. cinerea* (Sadfi *et al.* 2001). *B. cereus* (UW85) by production antibiotic suppress damping-off disease of alfalfa caused by the oomycete pathogen, *Phytophthora medicaginis*, in a laboratory bioassay (Handelsman *et al.* 1990). In cell free culture tests, *B. cereus* (B16) produced large inhibition zones on PDA indicating that this isolate synthesized compounds that are highly active against *B. mali*. However, it should be noted that the size of the inhibition zone is not a reliable indicator of bacteria activity because is based on the mobility of the antifungal compounds. This can be influenced by the polarity of the compound moving through the agar or the molecular size of the compound and because of complexity and number of antifungal metabolites that are produced by antagonistic isolates (Etebarian *et al.* 2005). Significant inhibition in the growth of *B.mali* suggests that one or more soluble metabolite were responsible for biocontrol effect exhibited by *Bacillus* spp. isolates. This study indicates that all tested isolates of *Bacillus* spp. have demonstrated protective activity against gray mold caused by *B. mali*. The isolates varied in biocontrol potential of the disease on 'Golden Delicious' apples. Pusey and Wilson (1984) found similar variation in effectiveness when *B. subtilis* was sprayed onto different stone fruits (Pusey and Wilson 1984). Isolates B17 and EN63-1 were the most effective in control decay at 4 and 20°C. Also other authors reported that *Bacillus* spp. showed good potential for biological control of postharvest diseases causal agent because it survived at high and low temperature (Singh and Daverall 1984). For example *B. licheniformis* survived in hot water of 45°C and in the low temperature storage for a 21 days period (Korsten *et al*. 1991). In this research *Bacillus* spp. isolates were able to survive and multiply inside wounding site (Fig. 1). Growth curves of the antagonists demonstrated that *Bacillus* spp. could colonize and grow in apple wounds. Even after a period of 30 days at 4°C, the number of viable cells was greater than that originally introduced into the wound. The high total microbial populations on fruit were similar to the densities reported by De Jager (1999) on mature untreated mango (*Mangifera indica* L.) fruit. Other workers also found that *Pseudomonas syringae* pv. *lachrymans* populations on wounded pear fruit inoculated with *Penicillium expansum* or *B. cinerea* increased 10 to 100 fold at the wound site over 30 days in storage at 1°C (Janisewicz and Marchi 1992). Also Etebarian *et al.* (2005) found that populations of *P. fluorescens* isolate 1100–6 in wounded apple inoculated with *Penicillium expansum* increased over 20 days (Etebarian *et al.* 2005). In this study, *B. cereus* (B17) which gave the best result in control of gray mold on apple fruits, therefore holds great promise in our search for alternative control measures for apple gray mold, and will be tested further on a commer-

cial scale. Furthermore, a comprehensive study of their exact mode of action and any non-target effects will have to be carried out before they can be recommended for commercial use.

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REFERENCES

Bonaterra A., Mari M., Casalini L., Montesinos E. 2003. Biological control of *Monilinia laxa* and *Rhizopus stolonifer* in postharvest of stone fruit by *Pantoea agglomerans* EPS125 and putative mechanisms of antagonism. Int. J. Food Microbiol. 84: 93–104.

Crawford D.L., Lynch J.M., Whips J.M., Ousley M.A. 1993. Isolation and characterization of actinomycetes antagonists of fungal root pathogen. Appl. Environ. Microbiol. 59: 3899–3909.

De Jager E.S. 1999. Microbial ecology of the mango flower, fruit and leaf surfaces. M.Sc. Thesis, Department of Microbiology and Plant Pathology, University of Pretoria: 2–4.

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

Etebarian H.R., Sholberg P., Eastwell C.J., Sayler R. 2005. Biological control of apple blue mold with *Pseudomonas fluorescens*. Can. J. Microbiol. 51: 591–598.

Fiddaman P.J., Rossall S. 1993. The production of antifungal volatile by *Bacillus subtilis*. J. Appl. Bacteriol. 74: 119–126.

Gomez K.A., Gomez A.A. 1984. Statistical Procedures for Agricultural Research. John Wiley and Sons, New York, 680 pp.

Guizzardi M., Caccioni D.R.L., Pratella G.C. 1995. Resistance monitoring of *Monilinia laxa* (Aderh. et Ruhl.) honey to benzimidazoles and dicarboximides in postharvest stage. J. Plant Dis. Protect. 102: 86–90.

Handelsman J., Raffel S. J., Mester E. H., Wunderlich L., Grau C. R. 1990. Biological control of damping-off of alfalfa seedlings with *Bacillus cereus* UW85. Appl. Environ. Microbiol. 56: 713–718.

Hsu S.C., Lockwood J.L. 1969. Mechanism of inhibition of fungi in agar by *Streotomyces violaceoniger*. Can. J. Bot. 72: 480–485.

Huang Y., Wild B.L., Morris S.C. 1992. Postharvest biological control of *Penicillium digitatum* decay on citrus fruit by *Bacillus pumulus*. Ann. Appl. Biol. 130: 367–372.

Jackson A.M., Whips J.M., Lynch J.M. 1991. *In vitro* screening for identification of potential biocontrol agent of *Allium* white rot. Mycol. Res. 95: 430–434.

Jamalizadeh M., Etebarian H.R., Aminian H., Alizadeh A. 2008. Biological control of gray mold on apple fruits by *Bacillus licheniformis* (EN74-1). Phytoparasitica 36: 23–29.

Janisiewicz W.J., Marchi A. 1992. Control of storage rots on various pear cultivars with a saprophytic strain of *Pseudomonas syringae*. Plant Dis. 76: 555–560.

Janisiewicz W.J., Roitman J. 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. Phytopathology 78: 1697–1700.

Korsten L.,Van Harmelen M.W.S., Heitmann A., DeVilliers E., De Jager E.S. 1991. Biological control of postharvest mango fruit diseases. S.A. Mango Growers' Assoc. Yearbook. 11: 65–67.

Lee J.P., Lee S.W., Kim C.S., Son J.H., Song J.H., Lee K.U., Kim H.J., Jung S.J., Moon B.J. 2006. Evaluation of formulation of *Bacillus licheniformis* for the biological control of tomato gray mold caused by *Botrytis cinerea*. Biol. Control 37: 329–337.

McLauglin R.J., Wilson C.L., Droby S., Ben Arie R., Chalutz E. 1992. Biological control of postharvest diseases of grape, peach, and apple with the yeasts *Kloeckera apiculata* and *Candida guilliermondi*. Plant Dis. 76: 470–473.

O'Gorman D.T., Stokes S., Sholberg P.L. 2005. Identification of *Botrytis mali* within the *Botrytis* population causing fruit decay on apple in the orchards of British Columbia. Can. J. Plant Pathol. 27: 468–469.

Obagwu, J., Korsten, L., 2003. Integrated control of citrus green and blue molds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water. Postharvest Biol. Technol. 28: 187–194.

Pusey P.L., Wilson C.L. 1984. Postharvest biological control of stone fruit brown rot by *Bacillus subtilis*. Plant Dis. 68: 753–756.

Roberts R.G. 1990. Postharvest biological control of gray mold of apple by *Cryptococcus laurentii*. Phytopathology 80: 526–530.

Rothrock C., Gottlieb D. 1981. Importance of antibiotic production in antagonism of selected *Streptomyces* species to two soil-borne plant pathogen. J. Antibiot. 34: 830–835.

Sadfi N., Cherif M., Fliss I., Boudabbous A., Antoun H. 2001. Evaluation of *Bacillus* isolates from salty soils and *Bacillus thuringiensis* strains for the biocontrol of *Fusarium* dry rot of potato tubers. J. Plant. Pathol. 83: 101–118.

Singh V., Daverall S.J. 1984. *Bacillus subtilis* as a control agent against fungal pathogens of citrus fruit. Trans. Br. Mycol. Soc. 84: 487–490.

Sholberg P.L., Marchi A., Bechard J. 1995. Biocontrol of postharvest diseases of apple using *Bacillus* spp. isolated from stored apples. Can. J. Microbiol. 41: 247–152.

Spotts R.A., Cervantes L.A. 1986. Population, pathogenicity and benomyl resistance of *Botrytis* spp., *Penicillium* spp. and *Mucor piriformis* in packing house. Plant Dis. 70: 106–108.

Stehmann C., De Ward M.A. 1996. Sensitivity of population of *Botrytis cinerea* to triazoles, benomyl and vinclozolin. Eur. J. Plant Pathol. 102: 171–180.

Weller D.M. 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopath. 26: 379–407.

Wilson C.L., Franklin J.D., Pusey P.L. 1987. Biological control of Rhizopus rot of peach with *Enterobacter cloacae*. Phytopathology 77: 303–305.

Wilson C.L., Wisniewski M., El Ghaouth A., Droby S., Chalutz E. 1996. Commercialization of antagonistic yeasts for the biological control of postharvest diseases of fruits and vegetables. J. Ind. Microbiol. Biotech. 46: 237–242.

POLISH SUMMARY

OCENA *BACILLUS* SPP. JAKO POTENCJALNEGO CZYNNIKA BIOLOGICZNEGO ZWALCZANIA WYSTĘPUJĄCEGO PO ZBIORZE SZAREJ PLEŚNI NA JABŁKACH GOLDEN DELICIOUS W IRANIE

Biologiczne zwalczanie pozbiorowego gnicia jabłek może być alternatywnym podejściem do zwalczania chemicznego. Biorąc pod uwagę efektywne czynniki biologicznego zwalczania, badano: *Bacillus pumilus* (B19), *B. subtilis* (B11), *B. cereus* (B16), *B. subtilis* (B11), *B. cereus* (B17), *B. berevis* (EN63-1) i *B. licheniformis* (EN74-1) pod względem ich potencjalnej aktywności przeciwko szarej pleśni wywołanej przez *Botrytis mali* na jabłkach Golden Delicious w Iranie. Testy uwzględniające podwójne kultury, bezkomórkowe metabolity i związki lotne wykazały, że wszystkie z siedmiu izolatów inhibowało wzrost patogena. Wszystkie izolaty zapobiegały rozwojowi *B. mali* i ograniczały średnicę ran na jabłkach magazynowanych w 4°C, w zakresie od 9 do 32,2 mm w porównianiu do kontroli, gdzie ich średnica wynosiła 41,6 do 51,4 mm. W 20°C wielkość ran wynosiła od 7 do 14 mm dla zabiegu, w którym użyto antagonistyczne bakterie, podczas gdy w kombinacji kontrolnej wielkość ran wynosiła od 42,2 do 46,6 mm.