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Botrytis cinerea Pers. en manzana poscosecha, control con *Candida oleophila* Montrocher y/o fungicidas sintéticos
Botrytis cinerea Pers. in postharvest apple fruit, control with *Candida oleophila* Montrocher *strains* and/or synthetic fungicides

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Palabras clave: control de *Botrytis cinerea*; combinación fungicida-levadura; manzana en poscosecha
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Resumen

Un método alternativo de control, para incrementar el control y para reducir el uso de fungicidas sintéticos, tres cepas de *Candida oleophila* y/o cuatro fungicidas sintéticos comerciales fueron utilizados para el control del daño provocado por *Botrytis cinerea* en manzana en poscosecha. Los fungicidas sintéticos; Cyprodinil+Fludioxonil, Tiabendazol y Benomilo, permitieron el crecimiento de las cepas de *Candida oleophila* cuando fueron sometidas a la presión de éstos. El fungicida Captan no permitió el crecimiento de ninguna de las cepas de *Candida oleophila*. El control del daño provocado por *Botrytis cinerea* expresado en % y reducción de éste, promedió un control del; 100% para el Cyprodinil+Fludioxonil; Captan, 97.5%; Tiabendazol, 94.1 y Benomilo, 93.7%. Todas las cepas de *Candida oleophila*, individualmente, controlaron al 100%. El Tiabendazol y el Benomilo mejoraron su control contra *Botrytis cinerea* cuando se combinaron con *Candida oleophila*. Un control del 100% del daño provocado por *Botrytis cinerea* en manzana Golden Delicious en poscosecha se logra utilizando las cepas de *Candida oleophila* de manera individual o con Cyprodinil+Fludioxonil solo. El uso de *Candida oleophila* como un método alternativo para el control del daño provocado por *Botrytis cinerea* en manzana en poscosecha significa una reducción del uso de fungicidas sintéticos, evitando también la presencia de residuos del fungicida en la manzana tratada y en el medio ambiente, reduciendo así el riesgo de daño para humanos.

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Abstract

As an alternative control method, to improve control and to reduce synthetic fungicide use, three *Candida oleophila* strains and/or four commercial synthetic fungicides were used to control *Botrytis cinerea* damage on postharvest apple fruit. Synthetic commercial fungicides; Cyprodinil+Fludioxonil, Thiabendazole and Benomyl, allowed *Candida oleophila* strains colony growth when challenged to the pressure of these fungicides. Synthetic commercial fungicide Captan did not allow any *Candida oleophila* strains colony growth. Control of *Botrytis cinerea* expressed in % of damage and damage reduction, gave an average control of; 100% for Cyprodinil+Fludioxonil; Captan, 97.5%; Thiabendazole, 94.1% and Benomyl, 93.7% All *Candida oleophila* strains, individually, gave a 100% control. Thiabendazole and Benomyl improved their efficiency to control *Botrytis cinerea* when combined with *Candida oleophila*. Control of *Botrytis cinerea* damage on postharvest Golden Delicious apple fruit can be achieved up to 100% either with *Candida oleophila* strains individually and/or with Cyprodinil+Fludioxonil alone. The use of *Candida oleophila* as an alternative method to control *Botrytis cinerea* damage on postharvest apple fruit means a reduction of synthetic fungicide use, plus avoiding fungicide residues on the treated apple fruit and on the environment, thus reducing the risk for human health damage.

Introduction

Apple fruit production in 2016 worldwide was 89.3 million ton. Mexico contributed with 0.716 million ton in the same year (FAO, 2016). Within Mexico, Chihuahua State is the number one producer of apples with an average production of 0.586 million of ton per year (SIAP, 2017). Golden Delicious is the most important cultivar grown in Chihuahua State. One of the main problems that occurs during the postharvest storage of apple fruit is the damage due to *Botrytis cinerea* (Yu *et al*., 2007, 339; Calvo *et al*., 2007, 251; Williamson *et al*., 2007, 561; Xiao and Kim, 2008, 1; Li *et al*., 2011, 151; Guerrero-Prieto *et al*., 2011, 91). , which is still mainly controlled by using chemical synthetic fungicides (Chand-Goyal and Spotts, 1996, 253; Droby *et al*., 2009, 137, 138; Eshel *et al*., 2009, 48; Quaglia 2011, 307; Feliziani *et al*., 2013, 133; Wisniewski *et al*., 2016, 3; Sandoval-Flores *et al*., 2018, 207) that contribute to subsequent human health and environmental risks and the development of microorganism resistance (Mari *et* *al*., 2014, 1). *Botrytis cinerea* remains quiescent in the host and grows at different temperatures, 0°, 4°, 12°, 15°, 25° and 28°C (Fernandez *et al*., 2014, 541, 542) and starts damage at different number of days, depending on the storage conditions and the host physiology (Williamson *et al*., 2007, 561). The damage caused by *Botrytis cinerea* in different produce is of a significative economical importance worldwide (Williamson *et al*., 2007, 561). In developing countries, like Mexico and the apple growing area of Chihuahua, Mexico, (Guerrero-Prieto *et al*., 2013, 76) reports a loss of postharvest fruit of 50% of damage due microorganisms, including *Botrytis cinerea* on postharvest apples. The damage caused on apple fruit because of *Botrytis cinerea* starts in the physically damaged areas of the fruit, showing symptoms with brown color of rotten soft pulp tissue, which increases in size as the fruit stays longer in the cold storage (Williamson *et al*., 2007, 562, 563; Xiao and Kim, 2008, 2, 3, 4). The use of yeast as a biocontrol agent is an alternative to the use of fungicides (Chand-Goyal and Spotts, 1996, 253; Spotts *et al*., 2002, 252; Droby *et al*., 2009, 138; Eshel *et al*., 2009, 48; Quaglia 2011, 308; Sowndhararajan, *et al*., 2013, 1492; Mari *et al*., 2014, 2; Droby *et al*., 2016, 22, 23; Sui *et al*., 2016, 34; Wisniewski *et al*., 2016, 4). In the apple growing area of Chihuahua, Mexico, several of the most commonly used fungicides are; Thiabendazoles, Benomyl, and Captan (Ramírez-Legarreta and Jacobo-Cuéllar, 2002, 172) and can be applied to the fruit by a dip in water, which is used for a bath before it is either sorted to be packed and before being cold stored (Sastre *et al*., 1999, 182). A study like the present one, will be helpful to the apple growers in the Mexico's number one apple producing area, since biological control agents, like *Candida oleophila*, work better when obtained from the same fruit, as in the present work (Guerrero-Prieto *et al*., 2004, 223). *Candida oleophila* (*Co*) strains used in the present research work were isolated from apple fruit grown in the Chihuahua apple production area by (Guerrero-Prieto *et al*., 2004, 223) and this is the reason for using this yeast, which is common to apple fruit and other fruits in the world (Droby *et al*., 2009, 138). *Co* can grow at different temperatures and is able to grow at 0° C, which is a key characteristic since apple fruit is stored at 0° C and can control pathogens such as *Botrytis cinerea* (Guerrero-Prieto *et al*., 2004, 225; 2011, 97). One of the modes of action used by *Co* to control *Botytis cinerea* is nutrient competition (Guerrero-Prieto *et al*., 2011, 97) and production of exo-ß,1-3 glucanase production (Guerrero-Prieto *et al*., 2014, 431). With the hypothesis that the combination of *Candida oleophila* and the synthetic fungicides, will allow to reduce the use of synthetic fungicides, the objectives for this research work were, to explore the resistance of the three

Candida oleophila (*Co*) strains to the synthetic fungicides, *in vitro,* by measuring colony growth, alone and in combination with the fungicides being tested, and to evaluate the control *in vivo* of *Botrytis cinerea,* in postharvest Golden Delicious apple using the strains, to improve control efficiency and reduce fungicide use.

Method

Candida oleophila strains L06, L07 smooth and L07 rugose used in this research, as well as *Botrytis cinerea*, were obtained from "Temperate zone microorganism's collection" at Centro de Investigación en Alimentación y Desarrollo, A. C. (CIAD, A. C. www.ciad.mx) Cuauhtémoc Unit, Chih. México. Both, *Candida oleophila* and *Botrytis cinerea* were not checked morphologically because they came from the "Temperate zone microorganism's collection" and this is a guarantee for the authenticity of the microorganisms used. Inoculum concentrations were determined based on previous works from (Guerrero-Prieto *et al*., 2011, 92) and it is common to use higher inoculum concentrations than the natural concentration found in the original yeast and fungus concentration in the fruit. Concentrations used for each one of the three *Candida oleophila* strains (L06, L07 smooth and L07 rugose) were $1.1x10^9$ cfu/mL (colony forming units per ml) and for *Botrytis cinerea* $1x10^6$ conidia/ml, in both cases using a Neubauer chamber for determining inoculum concentration (Renping *et al*., 2011, 152). *In vitro Candida oleophila* colony growth. Growing media used was PDA (potato, dextrose, agar, 39 g/lt) and the fungicides were mixed with the PDA. Once the Petri dishes with PDA plus the fungicide were inoculated, they were incubated at 22°C for one week. Based on fungicide manufacturer recommendations, three doses for each commercial fungicide were used; low (lower than recommended), medium (manufacturer´s recommendation) and high (higher than recommended), as follows; Cyprodinil+Fludioxonil (Switch ® 62.5 WG Syngenta), 0.75, 1.0, 1.5 g/l; Benomyl (Benlate ® 50 PM Du Pont), 0.25, 0.5, 0.75 g/l; Thiabendazole (Tecto ® 60 Syngenta) 0.25, 0.5, 0.75 g/l; and Captan (Captan ® 50 WP Adama Mexico), 1.5, 2,0, 2.5 g/l. All the fungicides used in the present research work are used in the apple growing area of the study and all of them are authorized for their use in apple fruit (Ramirez-Legarreta and Jacobo-Cuéllar, 2002, 172). *In vitro* tests had four replications per treatment (one Petri dish was considered as one replication) and experiments, under a completely randomized design, were run three times. Control *in vivo* of *Botrytis cinerea*. Each one of twelve postharvest Golden Delicious apple fruits were wounded on the stem end of the fruit, as is a common technique used for this kind of studies (Janisiewicz *et al*., 2000, 1197), making a 0.5 cm diameter and 3.0 mm depth well. Wells were inoculated, before inoculation with *Botrytis cinerea,* with 20 µl of a $1.1x10^9$ cfu/mL suspension of each one of the three *Candida oleophila* strains, depending on treatment, and then 20 µl of each fungicide doses were applied in the well, of those used, according with treatment. After 15 min of the two inoculations, each well was inoculated again with 20 µl of a $1x10^6$ conidia/ml *Botrytis cinerea* suspension. Plastic bagged fruit was cold stored during 75 days at 0.0 °C, since is the temperature at which apple fruit is cold stored and can be stored for up to 12 months (Xiao and Kim, 2008, 1). After this, the fruit was revised daily, and when damage was detected, the diameter of the lesion caused by the pathogen, in each well, was measured in millimeters. Degree of control was reported as % damage and reduction of damage due to the treatments. Experiments were run three times. The experimental design was a completely randomized one. Statistical analysis. Results from both, *in vitro* and *in vivo* treatments, were analyzed by ANOVA, under a completely randomized design using SAS (Statistical Analysis System, Version 6.12. Cary, NC USA). Tukey test (α =0.05) was used when statistical differences among treatments were detected (Guerrero-Prieto *et al*., 2017, 76).

Results

When treated with the Benomyl, Thiabendazole and Cyprodinil+Fludioxonil, the *Candida oleophila* strains grew with different amounts of reduction in colony growth. The *Candida oleophila* strains did not grow, at all, at any dose when treated with Captan. Benomyl, at all doses, did not reduce the colony growth of *Candida oleophila* strain L06. However, Thiabendazole and Cyprodinil+Fludioxonil did reduce the colony growth at all three doses used with statistically significant differences (α = 0.05) among them. No dose of Benomyl reduced the colony growth of L07 smooth *Candida oleophila* strain, but all doses of Thiabendazole and Cyprodinil+Fludioxonil resulted in a reduction with statistically significant differences (α = 0.05) among them. The colony growth for *Candida oleophila* strain L07 rugose was reduced by Cyprodinil+Fludioxonil at the three doses used showing statistically significant differences. Only the medium dose of Benomyl reduced the colony growth of L07 rugose. A high dose of Thiabendazole clearly resulted in the colony growth reduction of L07 rugose. Benomyl reduced the colony growth of the three *Candida oleophila* strains to a lesser degree (0.33 mm of damaged area when used alone, against 0.22 mm of damaged area when used combined with the *Co* strains), followed by Thiabendazole that had a higher rate of reduction than Benomyl and then Cyprodinil+Fludioxonil that reduced growth to a greater degree than the previous two fungicides. When the three strains of *Candida oleophila* were treated with Captan, none of the colonies grew (Table 1).

Table 1. *In vitro* colony growth of *Candida oleophila* strains after one week. Cuauhtémoc, Chih. Mexico, 2010

Treatments	**Dose and colony growth diameter (mm)**		
L06 alone, and plus fungicide treatment	Low	Medium	High
Control (L06 alone)	7.1a*	7.1a*	7.1a*
Thiabendazole, Benomyl, Cyprodinil+Fludioxonil, Captan	6.8b, 7.1a, 5.2c, 0.0d	6.7b, 7.2a 5.2c, 0.0d	7.0a, 7.0a 5.3c, 0.0d
L07 smooth alone, plus fungicide treatment	Low	Medium	High
Control (L07 smooth alone)	7.3a*	7.2a*	7.3a*
Thiabendazole, Benomyl, Cyprodinil+Fludioxonil, Captan	6.8a, 7.0a, 5.2b, 0.0c	6.5b, 7.3a, 5.2c, 0.0d	6.9b, 7.3a, 5.2c, 0.0d
L07 rugose alone, plus fungicide treatment	Low	Medium	High
Control (L07 rugose alone)	7.2ab*	7.2a*	7.2a*
Thiabendazole, Benomyl Cyprodinil+Fludioxonil, Captan	7.1b, 7.4a, 5.0c, 0.0d	7.1ab, 6.9b, 4.8c, 0.0d	6.3b, 7.3a, 5.0c, 0.0d

Means with different letters, in the same column, indicate significantly different values, Tukey ($\alpha = 0.05$).

Candida oleophila (*Co)* strains *in vitro* colony growth reduction, was reduced, at different degree of reduction, thiabendazole, 5.3%; Benomyl, 0.3%; Cyprodinil+Fludioxonil, 28.7% and Captan, 100%, because of the fungicides and doses evaluated in the present research work. Captan did not allow any growth of any of the *Candida oleophila* strains at all. The mode of action of Captan, which is based on the chemical reaction between the active ingredient and sulphydric enzymes, which produces a toxic compound, thiophosgene, that interferes with the respiration of the fungi cells (Rusell, 2005, 16; Lima *et al*., 2006, 301; National Pesticide Information Center, 2018; Lima *et al*., 2011, 164), resulted in a 100% reduction of the growth of the *Co* strains evaluated. The different amounts of colony growth reduction because of the treatments used, are also due to the mode of action of the fungicides evaluated. The fungicides evaluated in the present work, Thiabendazole, Benomyl, Cyprodinil+Fludioxonil and Captan, each one represents one of the four chemical groups of the fungicides used commercially to control fruit pathogens (Rusell, 2005, 16).

In vivo Botrytis cinerea control with *Candida oleophila* strains and/or fungicide. Table 2 shows the results for the use of Cyprodinil+Fludioxonil and the three *Candida oleophila* strains. Cyprodinil+Fludioxonil and the three *Candida oleophila* strains resulted in a 100% control of *Botrytis cinerea*. When combining the strains and the fungicide, the control of *Botrytis cinerea* was also 100%, which indicates that the colony growth reduction shown for the *Candida oleophila* strains due to the combination with Cyprodinil+Fludioxonil (Table 1) did not have a reduction effect on the *Botrytis cinerea* damage control after treatment with Cyprodinil+Fludioxonil. Cyprodinil+Fludioxonil (Switch ® 62.5 WG Syngenta) includes two different fungicides, with different mode of action, which makes it more efficient to control, in this case, *Botrytis cinerea*. Cyprodinil inhibits methionine synthesis and the production of hydrolytic enzymes, which inhibits spore germination, spore germination tubes and fungi mycelia growth. Fludioxonil works on the contact surface with a preventive activity for a long time, also works on spore germination, inhibiting the penetration process and mycelia growth of the fungi and has a limited effect on fungi sporulation (National Pesticide Information Center, 2018).

Table 2. *Botrytis cinerea* (*Bc)* damaged tissue in postharvest Golden Delicious apple fruit, after treated with Cyprodinil+Fludioxonil, as indicated. Cuauhtémoc, Chih. Mexico, 2010

Cyprodinil+Fludioxonil (CF) treatments and doses		**Damaged Tissue Diameter (mm) at 75 days of cold storage**	**% Damaged Tissue at 75 days of cold storage**
Bc	$1x10^6$ conidia/mL	2.78a	51
Co L06, L07 smooth, and rugose	$1.1x10^9$ cfu/mL	0.0b for all treatments	0 for all treatments
CF alone, plus L06, L07 smooth, rugose	0.25 g/L	0.0b for all treatments	0 for all treatments
CF alone, plus L06, L07 smooth, rugose	0.50 g/L	0.0b for all treatments	0 for all treatments
CF alone, plus L06, L07 smooth, rugose	0.75 g/L	0.0b for all treatments	0 for all treatments
CF alone, plus L06, L07 smooth, rugose	1.00 g/L	0.0b for all treatments	0 for all treatments
CF alone, plus L06, L07 smooth, rugose	1.50 g/L	0.0b for all treatments	0 for all treatments

Means with different letters in the same column are significantly different, Tukey (α =0.05).

Table 3 includes the results when apple fruit was treated with Benomyl. Benomyl did not control *Botrytis cinerea* to 100%. The statistically significant ($\alpha = 0.05$) reduction of *Botrytis cinerea* damage was 92% at a dose of 0.25 g/L and 96% when combined with L07 rugose at the same dose. At doses of 0.50 g/L, Benomyl reduced *Botrytis cinerea* damage by 93%. When combined with L07 smooth, the degree of reduction of damage was 98.3%. Both treatments were statistically significantly different than the *Botrytis cinerea* control. When the doses for Benomyl were 0.75 g/L, the greatest amount of reduction of damage for *Botrytis cinerea* was with Benomyl alone that resulted in an 89.5% reduction. Control of *Bc* damage was increased when Benomyl, from 11.8% to 8.1%, Table 3, was combined with all three *Candida oleophila* strains, and the degree of reduction was 94% when combined with L07 smooth, 92% when combined with L06 and 91.4% when used alone with L07 rugose. All these values were statistically equal. All three *Candida oleophila* strains resulted in 100% control. At doses of 1.0 g/L, Benomyl alone reduced *Botrytis cinerea* damage by 95.2%. However, when combined with L06 and L07 smooth, the control was 100%. An 84.7% control was obtained when combined with L07 rugose. These values were statistically equal. Treatment with Benomyl at the highest doses of 1.5 g/L, alone and combined with L06 and L07 smooth, gave 100% control. An exception occurred when Benomyl was used in combination with L07 rugose. In this case, the degree of control was 83.4%. The response of the control of the damage when *Botrytis cinerea* was treated with Benomyl may be due to a degree of synergism between the fungicide and the *Candida oleophila* strains, since the % of control was increased with an average of 67% in the different treatments used when combining Benomyl-*Candida oleophila* strain. The possible synergism between the *Co* strains and Benomyl, increased the percentage of *Bc* control on apple fruit, because of the modes of action on both, Benomyl, which works through carbendazim, who attaches to microtubules, interfering with several cell functions, such cell division and intracellular transport, (National Pesticide Information Center, 2018) and *Co* strain, which use nutrient competition to control *Bc* (Guerrero-Prieto *et al*., 2011, 96) and exo-ß,1-3 glucanase production (Guerrero-Prieto *et al*., 2014, 431).

Table 3. *Botrytis cinerea* (*Bc)* damaged tissue in postharvest Golden Delicious apple fruit, after treated with Benomyl, as indicated. Cuauhtémoc, Chih. Mexico, 2010

Benomyl (B) treatments and doses		Damaged Tissue Diameter (mm) at 75 days of cold storage	% Damaged Tissue at 75 days of cold storage
Bc	$1x10^6$ conidia/mL	2.7a	51
Co L06, L07 smooth, and rugose	$1.1x10^9$ cfu/mL	0.0b for all treatments	0 for all treatments
B alone, plus L06, L07 smooth, rugose	0.25 g/L	0.46b, 0.0b, 0.0b, 0.26b	8, 0, 0, 4
B alone, plus L06, L07 smooth, rugose	0.50 g/L	0.39b, 0.0b, 0.09b, 0.0b	7, 0, 1.7, 0
B alone, plus L06, L07 smooth, rugose	0.75 g/L	0.57b, 0.45b, 0.32b, 0.47b	10.5, 8, 6, 8.6
B alone, plus L06, L07 smooth, rugose	1.00 g/L	0.26b, 0.0b, 0.0b, 0.83b	4.8, 0, 0, 15.3
B alone, plus L06, L07 smooth, rugose	1.50 g/L	0.0b, 0.0b, 0.0b, 0.91b	0, 0, 0, 16.6

Means with different letters in the same column are significantly different, Tukey (α =0.05).

Table 4 shows the results of the *Bc* control when Thiabendazole was used. Thiabendazole results were like those of Benomyl since Thiabendazole alone was less effective when controlling damage of *Bc*, as compared when combined with the *Candida oleophila* strains. There was an 88.8% reduction of damage when the dose of Thiabendazole was 0.25 g/L. The degree of control increased to 100% when Thiabendazole was applied with L06 and L07 rugose and up to 91.8% when combined with L07 smooth. These values were statistically equal. Thiabendazole alone at a dose of 0.50 g/L and with all combinations, had a 100% control of the damage. A 100% of control was obtained when Thiabendazole was applied at a dose of 0.75 g/L. This was the case for both, Thiabendazole alone, and when combined with L06. However, the degree of control was 96.4% with L07 smooth and 96.8% with L07 rugose, a high degree of control, though. Treatment of Thiabendazole alone at 1.0 g/L resulted in 92% control, and a 100% control was achieved when the treatments were combined. Finally, the degree of control was 100% with Thiabendazole at doses of 1.5 g/L when L06 or L07 rugose were combined with it. Treatment with Thiabendazole alone resulted in a 90.3% control, while the degree of control was 95.2% when combined with L07 smooth. Treatments with all three *Candida oleophila* strains resulted in 100% control. The response of Thiabendazole was like that of Benomyl since the efficiency of control was greater when the fungicide was combined with the *Candida oleophila* strains. This suggests that there may be some degree of synergism between fungicide and strains, because of the modes of action on both, Benomyl, that inhibits mitosis, attaching to tubulin, avoiding fungi cell division (National Pesticide Information Center, 2018) and *Co* strain, which use nutrient competition to control *Bc* (Guerrero-Prieto *et al*., 2011, 96) and exo-ß,1-3 glucanase production, which degrades fungi cell wall and reduces spore and mycelium growth (Guerrero-Prieto *et al*., 2014, 430, 431).

Table 4. *Botrytis cinerea* (*Bc)* damaged tissue in postharvest Golden Delicious apple fruit, after treated with Thiabendazole, as indicated. Cuauhtémoc, Chih. Mexico, 2010

Thiabendazole (T) treatments and doses		**Damaged Tissue Diameter (mm) at 75 days of cold storage**	**% Damaged Tissue at 75 days of cold storage**
Bc	$1x10^6$ conidia/mL	2.7a	51
Co L06, L07 smooth, and rugose	$1.1x10^9$ cfu/mL	0.0b for all treatments	0 for all treatments
Thiabendazole (T) alone, plus L06, L07 smooth, rugose	0.25 g/L	0.6b, 0.0b, 0.5b, 0.0b	11.2, 0, 9.2, 0
T alone, plus L06, L07 smooth, rugose	0.50 g/L	0.0b for all treatments	0 for all treatments
T alone, plus L06, L07 smooth, rugose	0.75 g/L	0.0b, 0.0b, 0.19b, 0.17b	0, 0, 3.6, 3.2
T alone, plus L06, L07 smooth, rugose	1.00 g/L	0.45 b, 0.0b, 0.0b, 0.0b	8, 0, 0, 0
T alone, plus L06, L07 smooth, rugose	1.50 g/L	0.53b, 0.0b, 0.26b, 0.0b	9.7, 0, 4.8, 0

*Means with different letters in the same column are significantly different, Tukey (*α =0.05).

Results for the control of damage due to *Botrytis cinerea* when using Captan, Table 5, either alone or when combined with the *Candida oleophila* strains, indicate that the results observed with some of the Captan-*Candida oleophila* treatments in this experiment may be due solely to the use of Captan (Rusell, 2005, 16, Lima *et al*., 2006, 301, Lima *et al*., 2011, 164). All three *Candida oleophila* strains when applied alone gave a 100% control. Captan was not compatible with the three *Co* strains, since did not allow any *Co* colony growth, as shown in Table 1. The mode of action of Captan, did not allow any colony growth of the *Co* strains, being toxic for the yeasts. Droby *et al*., 2009, 138, mention some of the key characteristics of a biocontrol agent, like to be resistant to chemicals used in the postharvest environment, however, the three *Candida oleophila* strains used in the present research work, were not resistant to Captan, but were resistant to the other three synthetic fungicides evaluated.

Table 5. *Botrytis cinerea* (*Bc)* damaged tissue in postharvest Golden Delicious apple fruit, after treated with Captan, as indicated. Cuauhtémoc, Chih. Mexico, 2010

Captan (C) treatments and doses		**Damaged Tissue Diameter (mm) at 75 days of cold storage**	**% Damaged Tissue at 75 days of cold storage**
Bc	$1x10^6$ conidia/mL	2.78a	51
Co L06, L07 smooth, and rugose	$1.1x10^9$ cfu/mL	0.0b for all treatments	0 for all treatments
Captan (C) alone, plus L06, L07 smooth, rugose	0.75 g/L	0.0b f for all treatments	0 for all treatments
C alone, plus L06, L07 smooth, rugose	1.00 g/L	0.05b, 0.07b, 0.41b, 0.0b	0.9, 1.3, 7.6, 0
C alone, plus L06, L07 smooth, rugose	1.50 g/L	0.56b, 0.0b, 0.09b, 0.49b	10.3, 0, 1.6, 9
C alone, plus L06, L07 smooth, rugose	2.00 g/L	0.05b, 0.0b, 0.0b, 0.04b	0.94 0, 0, 0.82
C alone, plus L06, L07 smooth, rugose	2.50 g/L	0.0b, 0.0b, 0.0b, 0.9b	0, 0, 0, 16.7

Means with different letters in the same column are significantly different, Tukey (α =0.05).

Discussion and conclusion

The use of the *Candida oleophila* L06, L07 smooth and L07 rugose strains as biocontrol agents on postharvest fruit, specifically in Golden Delicious apple fruit, can be considered as safe for humans who may eat the apple fruit treated with *Candida oleophila* (Droby *et al.*, 2009, 138). This is due to the fact of the origin of the yeasts, which are originally obtained from fresh apple fruit, since yeast belong to the natural epiphytic microflora of fruit and vegetables (Guerrero-Prieto *et al*., 2004, 224). When yeast is used as biocontrol agents, before being applied to the produce, yeast is obtained and isolated from the fruit, then they are identified, reproduced and applied in a greater number of colonies forming units than they were originally present in the fruit surface (Chand-Goyal and Spotts, 1996, 254, Guerrero-Prieto *et al*., 2004, 224). These yeasts can be applied to the fresh apple fruit, either by spraying them on the fruit, when still in the orchard or by immersion in the water right before cold storage and/or before fruit sorting and packing (Lahlali *et al*., 2009, 39). This same author (Lahlali *et al*., 2009, 42), had better control results when *Pichia anomala* was sprayed early in the season when the fruit was still in the apple trees in the orchard, before harvest to control *Penicillium expansum*.

The *Candida oleophila* L06, L07 smooth and L07 rugose strains can grow and can be combined with Cyprodinil+Fludioxonil, Benomyl and Thiabendazole. Treatment with the *Candida oleophila* strains alone and Cyprodinil+Fludioxonil alone resulted in 100% control of *Botrytis cinerea*. *Candida oleophila* L06, L07 smooth and L07 rugose, when combined with Benomyl, Thiabendazole and Cyprodinil+Fludioxonil may have some degree of synergism between each one of them to control the damage due to *Botrytis cinerea* on postharvest Golden Delicious apple fruit, which gives them an advantage of using a lower amount of fungicide. *Candida oleophila* strains could substitute the use of synthetic fungicides to control *Botrytis cinerea* in Golden Delicious postharvest apple fruit, thus reducing the use of synthetic fungicides, which implies a reduction on human health risk and the environment because of the use of fungicides. The contribution of the present research work is that the region's apple growers can control *Botrytis cinerea* damage on postharvest Golden Delicious apple fruit using *Candida oleophila* instead of synthetic fungicides (Filonow *et al*., 1996, 212; Santos *et al*., 2004, 332; Guerrero-Prieto *et al*., 2014, 427-428, 431).

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