Available online: www.notulaebotanicae.ro
Print ISSN 0255-965X; Electronic 1842-4309
Not Bot Horti Agrobo, 2015, 43(2):413-419. DOI:10.15835/nbha4329791

Biochemical Changes in Orange Fruit Due to Plant – *Penicillium italicum* – Antagonism Interactions

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Abstract

One of the most important orange fruit diseases is blue mold which cause by *Penicillium italicum* that is responsible for important economic losses. This study investigated biochemical changes in exo-mesocarp layers of orange fruits related to host-pathogen- yeast interactions. Initial result showed that among eight strains isolated, the most effective antagonist were belonged to two species of *Pichia kluyveri* (M45) and *Rhodotorula mucilaginosa* (M61). These isolates were selected for biochemical evaluation. In order to assessment of biochemical changes, the orange fruits were inoculated with 40 µl of yeast cell suspension and after 24 h, the wounds were inoculated with 20 µl of conidial suspension of *P. italicum*. The analysis of variance showed that all of the measured biochemical characterises were significant in both layers by treatments (yeast isolates; pathogen; yeast isolates + pathogen and control) ($P \leq 0.01$), including POD, CAT and β-1, 3-glucanase activities and total phenolic compounds. Also result showed that when the yeast isolates (M45 or M54) were inoculated into wounds with the pathogen, it stimulated the orange to increase produce of total phenol and enzymes activity (POD, CAT and β-1, 3-glucanase) and these changes were related to incubation time. The result showed that understanding biochemical mechanism derived from plant-pathogen-antagonist interactions is essential for investigating the dynamics of infectious processes.

Keywords: biological control, citrus, exo-mesocarp, Iran, phenolic compounds

Introduction

In Iran, Citrus culture has a very old history. The production of these fruit as a market crop that have almost 300 years old history (Ebrahimi, 2002). *Citrus sinensis* is one of the most important horticultural crops in Iran (Ansari and Feridoon, 2006), which its annual production it is among ten first countries of the world (FAOSTAT, 2013).

Sweet orange are attacked by a wide range of pathogens, which can change host survival, growth and reproduction (Agrios, 2005). Crops defend themselves against pathogens by a combination of structural characters and biochemical reactions, which can be induced by attack (Hanley *et al*., 2007; Wittstock and Gershenzon, 2002).

One of the most prevalent micro-organism of the sweet orange is blue mold, which is created by *Penicillium italicum*. The most important damage factor in postharvest period is *Penicillium* that imposes billions dollars of damage every year upon this market (Pitt *et al*., 2009). *Penicillium* growth typically occurs as a result of wound infections in produce.

To prevent development and limit activity of this pathogen, chemical treatments are widely used. However, such of them may produce serious problems, residues on the fruit (Cabras *et al*., 1999; Palou *et al*., 2008), coming into existence of fungicide-resistant strains (Ben-Yehoshua *et al*., 1994), and harmful to human health (Suwalsky *et al*., 1999). In order to alternative to these fungicides some treatments have obtained successful results controlling postharvest decay (Feliziani *et al*., 2015).

Biological control of fruits has appearance recently as a promising alternative to the use of chemical fungicides (Cwalina-Ambroziak and Nowak, 2012; Youssef *et al*., 2012; Moretto *et al*., 2014).

Treatment of fruit with microbial agents has been performance to be an affect method for control of postharvest decays. Some microbial such as bacteria and yeasts are reported to reduce effectively various postharvest decays of fruits (Chalutz and Wilson, 1990; Janisiewicz and Korsten, 2002; Tian *et al*., 2002). Citrus plants produce volatile organic compounds (VOCs) as secondary metabolites that play an important role in interaction routes with microorganisms (Marques *et al*., 2014).

In several plant species, used of microbial antagonists and chemical inducer was reported to provide inhibitor against

Received: 10 Jan 2015. Received in revised form: 11 Aug 2015. Accepted: 14 Aug 2015. Published online: 10 Dec 2015.

pathogens (Friedrich *et al*., 1996; Van Loon *et al*., 1998). So, it is important to assessment of interactions among pathogen, yeast antagonist and orange fruits during postharvest storage; in particular the enzymes regulating reactive oxygen levels, as there production and accumulation (hydrogen peroxide, superoxide and hydroxyl radical) are the most frequently showed biochemical agents during plant-microbe interaction (Wang *et al*., 2004). Host defence responses expressed systemically involve the synthesis and accumulation of antifungal glucanohydrolases such as chitinase, chitosanase, and β-1, 3-glucanase (Ryals *et al*., 1996; Van Loon *et al*., 1998). The primary objective of this study was to confirm the ability of two isolates of *Pichia kluyveri* (M45) and *Rhodotorula mucilaginosa* (M61) to control postharvest blue mold which cause by *Penicillium italicum* in orange fruits and the other objective was to determine whether the activity of the peroxidase (POD), catalase (CAT), β-1, 3-glucanase and phenolic compound would be affected in orange fruit layers, following the application of two antagonists alone or in combination with the pathogen.

Materials and Methods

Select of fruit, pathogen and biological control yeasts

In this present study, orange fruits (*Citrus sinensis* cv. 'Thomson navel') at maturity and uniform size without wounds were selected. The oranges were obtained from Pakdasht city that these fruits were cultivated in orchard located in North of Iran and kept at 4 °C until needed.

The isolate Pi1 of *Penicillium italicum* obtained from infected orange fruits. The culture was derived from single spore isolate and maintained on potato Dextrose Agar (PDA) at 4 °C in darkness until use.

Biocontrol agents were isolated from the surface of fruits, leaves and shoot of *Citrus limon*, gathered from Bandar-Abbas, Hormozgan, Iran. Isolates consisted of two isolates of *Metschnikowia pulcherrima* (M54) and one isolate of *Pichia kluyveri* (M45). Identification of selected strains was carried out by identification service CBS. YMA and PDA cultures were used to isolate eight yeasts that are as follow: three isolates of *Pichia guilliermondii* (M60, M63 and M47), two isolates of *Metschnikowia pulcherrima* (M43 and M54), one isolate of *Pichia kluyveri* (M45) and one of *Rhodotorula mucilaginosa* (M61).

In vivo biological control studies

P. italicum (Pi1) 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 fruits were washed in 90% ethanol for 5 s followed by dipping in 0.2% sodium hypochlorite solution and rinsed with SDW. The oranges were wounded with 3 mm diameter nail to a depth of 4 mm in quadruplicate.

Extraction and assay of peroxidase activity

The extraction and assay of POX was carried out as per the method described by Lagrimini and Rothstein (1987). The reaction mixture contained 3.5 mL 0.1 M phosphate buffer (pH 6.0), 0.05 mL 12 mM guaiacol, 0.03 mL H_2O_2 and 0.5 mL enzyme. Changes in absorbance were recorded at 470 nm for 1 min with a spectrophotometer (Milton Roy, Spectronic 501, Unterfoehring - Germany). The activity of peroxidase was presented as ΔOD 470nm /min/mg protein.

Extraction and assay of catalase activity

The method of extraction as described by Du and Bramlage (1995) was used in the experiment. CAT activity was determined by following the disappearance of H_2O_2 in the enzyme reaction mixture. The enzyme extract (0.25 ml) was added to 2 ml assay mixture (50 mM Tris-HCl buffer pH 6.8, containing 5 mM H2O2). The reaction was stopped by adding 0.25 ml 20% titanic tetrachloride (in concentrated HCl, v/v) after 10 min at 20°C. A blank was prepared by addition of 0.25 ml 20% titanium tetrachloride at zero time to stop the enzyme activity. The absorbance of the reaction solutions was read at 415 nm against water. CAT activity was determined by comparing absorbance against a standard curve of $H_2O_2$from 0.25 to 2.5 mm. The activity of CAT was presented as H_2O_2mm/min/mg protein.

Extraction and assay of β-1, 3-glucanase activity

The method of extraction as described by Ippolito *et al*. (2000) was used in this experiment. Tissue samples of each fruit were taken from the wounds and individually homogenized at 4 °C in two volumes (w: v) of 50mM sodium acetate buffer, pH 5.0, and the homogenate was centrifuged at 4 °C (20 min, 14000 × g). Proteins in the supernatant were precipitated in 60% acetone (v: v) at −20 °C and the resulting pellet, following centrifugation (20 min, 14000 × g at 4 °C), was washed three times with 60% acetone.

The pellet was suspended in 2 ml of 50 mM sodium acetate buffer (pH 5.0) and assayed for β-1, 3-glucanase activities. β-1, 3-glucanase was determined following the method of Abeles and Forrence (1979). β-1, 3-glucanase activity was assayed by incubating 30 ml of enzyme preparation for 30 min at 40 °C in 30 ml of 4% laminarin. The reaction was terminated by heating the sample in boiling water for 5 min and the amount of reducing sugars was measured spectrophotometrically at 500 nm after reaction with 372 ml of 3,5-dinitrosalicilate. Final activity values are reported as nmol glucose/min/mg of total protein.

Determination of total phenolic compounds

The method of extraction as described by Yamomoto *et al*. (1977) was used in the experiments. Orange fruits (1.0 gr fresh weight of both layers) were ground in a mortar with 10 ml of 80% methanol and filtered through double layers of gauze. The residue was washed twice with 80% methanol (each time with three ml). The filtrate and washing were combined and centrifuged at 4000 g for 5 min at room temperature and the supernatant was assayed. Total phenol was measured with Folin-Ciocalteu's reagent (Merck, Darmstadt, Germany). 0.5 ml extracts were diluted with distilled water to 7 ml in a test tube. The contents were well mixed. 0.5 ml Folin-Ciocalteu's reagent was added and the tubes were thoroughly shaken again. Exactly 3 min later 1 ml of saturated sodium carbonate solution was added and the mixture made up to 10 ml with good mixing. After leaving the samples for one hour at room temperature, the absorbance was measured at 725 nm. Caffeic acid (Fluka, Germany) was used as a reference phenolic compound. The total phenolic compounds of samples were expressed as mg caffeic acid per g of fruit fresh weight.

Table 1. Analysis of variance for the biochemical traits of orange fruit under plant-yeast isolates-pathogen interactions

S.O.V	df	MS (For M45 isolate)							
		POD (Mesocarp)	POD (Exocarp)	CAT (Meso)	CAT (Exo)	β-1, 3-gluc (Meso)	β-1, 3-gluc (Exo)	Phenol (Meso)	Phenol (Exo)
Treatment	3	0.12*	0.32*	0.75*	0.61*	0.05*	0.38*	0.02*	0.02*
Time	4	0.80*	0.46*	0.47*	1.01*	0.99*	4.01*	0.01*	0.08*
Tr*Ti	12	0.08*	0.23*	0.03*	0.44*	0.08*	0.15*	0.02*	0.08*
Error	40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
CV	-	0.89	0.37	1.47	0.5	2.87	4.68	0.7	2.12
S.O.V	df	MS (For M54 isolate)							
Treatment	3	0.19*	0.31*	0.68*	0.24*	0.14*	0.21*	0.01*	0.01*
Time	4	0.89*	0.43*	0.54*	0.71*	1.13*	4.63*	0.00*	0.18*
Tr*Ti	12	0.13*	0.23*	0.10*	0.47*	0.08*	0.18*	0.02*	0.01*
Error	40	0.00	0.02	0.01	0.00	0.00	0.04	0.00	0.00
CV	-	0.77	0.35	4.93	0.45	2.37	15.52	0.67	1.58

*: Significant at 1% probability level. Exocarp and mesocarp layers are orange and white parts of orange fruits, respectively.

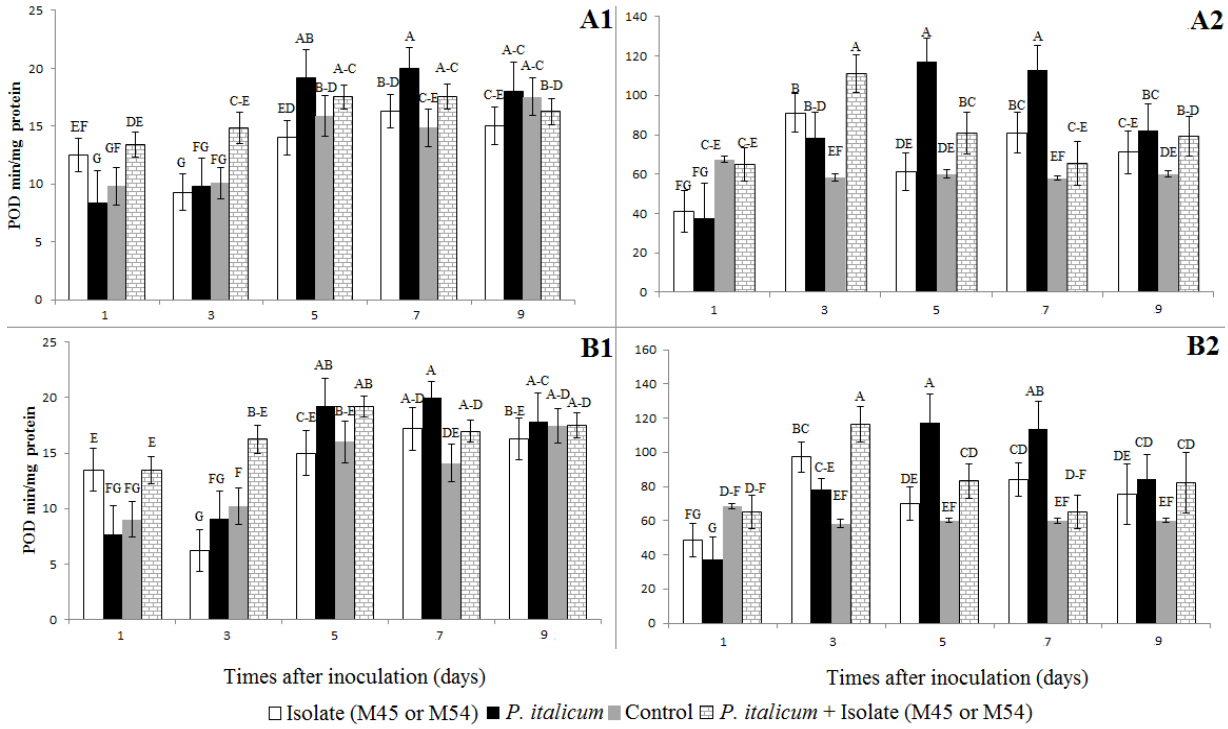

Fig 1. Peroxidase activity in orange fruits tissues treated with yeast isolates {A1 and A2 (M45): white and orange tissues, respectively; B1 and B2 (M54): white and orange tissues, respectively}, *P. italicum*, interaction of them and control. The activity of peroxidase was presented as ΔOD 470 nm/min/mg protein; values are averages of three replicates. Error bars indicate ±SE.

Statistical analysis

Enzymatic assays were carried out twice for each sample and a mean of two assays were used for statistical analysis as value of each replicate. The completely randomized design was used for biocontrol activity experiment.

In order to assessment of enzymatic and phenolic compounds, the experiments were arranged as a 4x5 factorial in a completely randomized design with four replicates and four treatments (yeast isolate; pathogen; yeast isolate with pathogen challenge; control) and after 1, 3, 5, 7 and 9 days of treatments, the characteristics were measured. Analysis of variance was performed on the data and means were separated using Duncan's multiple range test.

Results

Selection of isolates

In the first test, there were eight yeast isolates, three isolates of *Pichia guilliermondii* (M60, M63 and M47), two isolates of *Metschnikowia pulcherrima* (M43 and M54), one isolate of *Pichia kluyveri* (M45) and one of *Rhodotorula mucilaginosa* (M61) to evaluated the biological activity for against *Penicillium italicum*. Dual culture, cell free metabolite and volatile test were used *in vitro* assay. Result of *in vitro* tests showed that two strains (M45 and M54) had a greater impact on disease control. Therefore, for further testing these strains were used (data not shown).

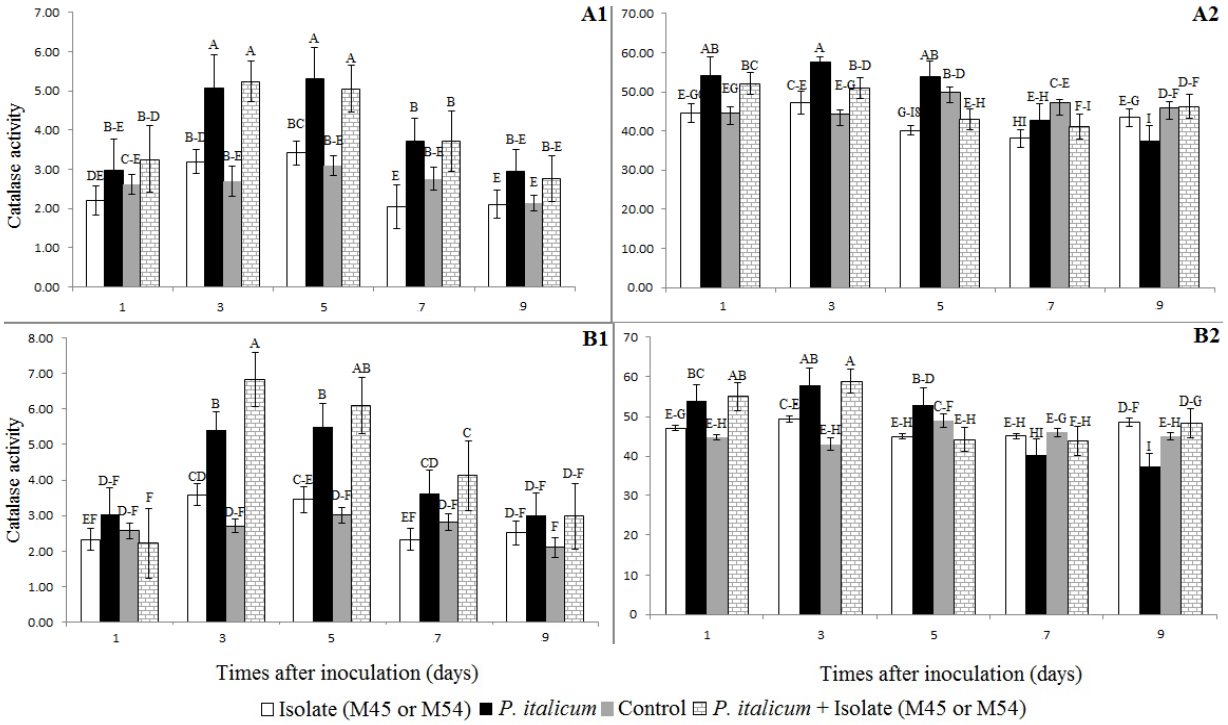

Fig 2. Catalase activity in orange tissues treated with yeast isolates {A1 and A2 (M45): white and orange tissues, respectively; B1 and B2 (M54): white and orange tissues, respectively}, *P. italicum*, interaction of them and control. The activity of catalase was presented as mM H_2O_2/min/mg protein; values are averages of three replicates. Error bars indicate ±SE

Analysis of variance

The analysis of variance showed that all of the measured biochemical traits were variable in both layers by treatments ($P \leq 0.01$), including POD, CAT and β-1, 3-glucanase activities and total phenolic compounds (Table 1), suggesting a high biochemical changes among present treatments.

POD activity

Changes in the POD activity in exo-mesocarp layers and days after inoculation are shown in Fig. 1. POD activity in mesocarp layer of orange fruit treated with pathogen or in combination with isolates showed the increase, reaching maximum level 7 days after storage at 20°C, and then decreased (Fig. 1, A1-B1). In orange fruits exocarp layer treated with pathogen alone indicated enzyme increased of the enzyme, with maximum level 5 days and then decreased, whereas in interaction of pathogen and isolate (M45 or M54) the enzyme was increased in three days after inoculation and then decreased (Fig. 1, A2-B2). Also, in exocarp layer enzyme did not any change in control treatment after inoculation, but in mesocarp layer it was increased.

The results of Duncan test for factors interactions showed that the highest POD activity in exocarp and mesocarp layers in present of M45 isolate was related to fifth and seventh day after inoculation with *P. italicum*, respectively (Fig. 1, A1-A2). The lowest one was related to first day after inoculation with *P. italicum*. Almost same results were observed in present of M54 isolate (Fig. 1, B1-B2).

CAT activity

CAT activity remained unchanged in exo-mesocarp layers in control treatment (Fig. 2). Three days after storage, CAT activity increased in both layers treated with antagonist, pathogen and combination of them, then decreased after 5 days. In mesocarp layer the lowest of CAT activity for factors interactions was related to control treatment after nine days (in both of M45 or M54 isolates) (Fig. 2, A1-B1).

β-1, 3-glucanase activity

Nine days after treatment, β-1, 3-glucanase activity in exocarp layer slowly increased in all treatment (Fig. 3, A2, B2). The enzyme in mesocarpe layer of orange fruit treated with interaction of M45 isolate and pathogen increased, reaching maximum levels 7 days after treatment and then decreased, whereas after 5 days of inoculation, in interaction of another isolate (M54) with pathogen, the enzyme increased, then decreased (Fig. 3, A1 and B1). In both layers isolates alone and control treatments, the enzyme was slowly increased.

Total phenolic compounds

In the first five days of inoculation, total phenolic compounds content in mesocarpe layer in treated with pathogen alone and combination with antagonist, increased slowly, then decreased (Fig. 4, A1, B1). The maximum phenolic content in treated orange with antagonists (M45 or M54) and control were obtained one day after inoculation. In the first three days of inoculation, total phenolic compounds content in exocarpe layer in treated with pathogen alone and combination with M45

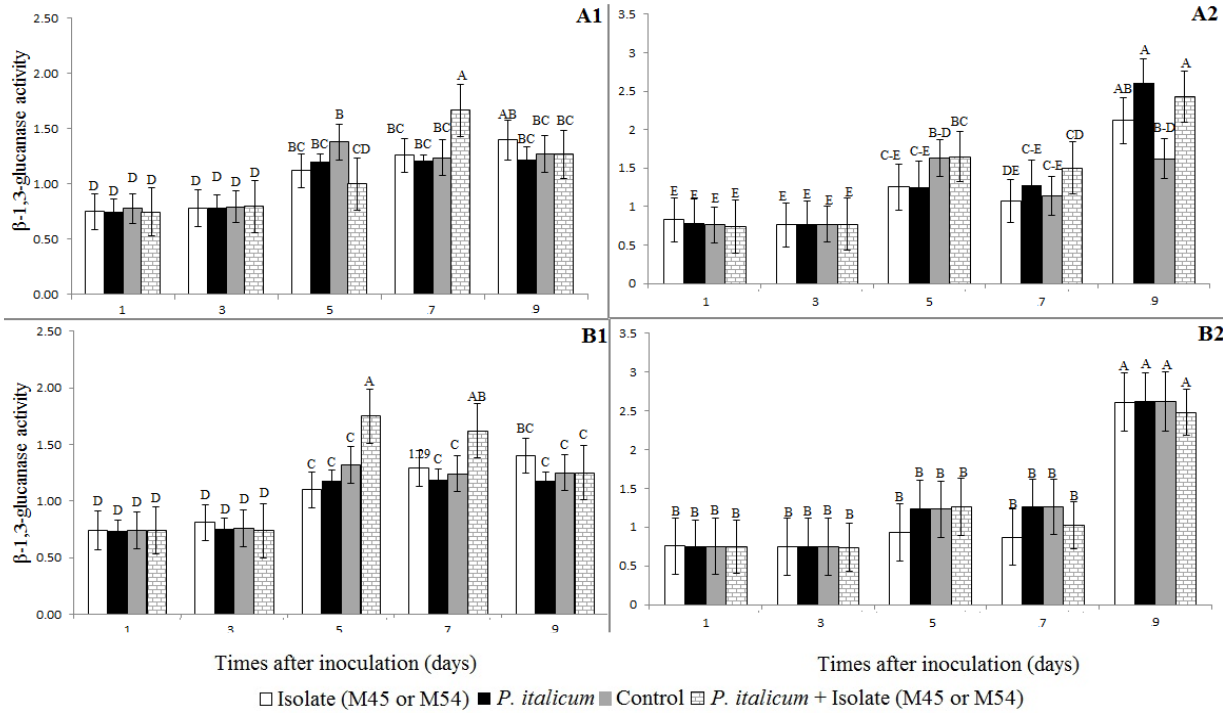

Fig 3. β-1,3-glucanase in orange tissues treated with yeast isolates {A1 and A2 (M45): white and orange tissues, respectively; B1 and B2 (M54): white and orange tissues, respectively}, P. italicum, interaction of them and control. The activity of β-1, 3-glucanase was presented as nm glucose/min/µg protein, values are averages of three replicates. Error bars indicate ±SE

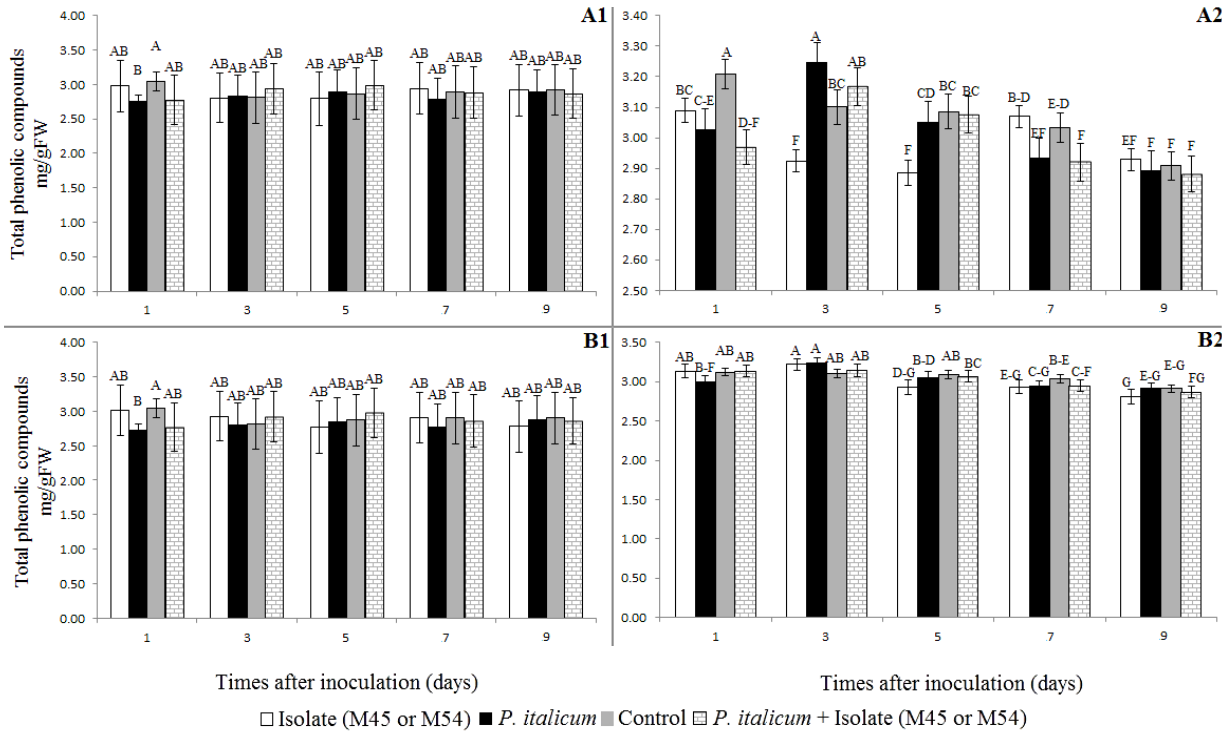

Fig 4. Total phenolic compounds in orange tissues treated with yeast isolates {A1 and A2 (M45): white and orange tissues, respectively; B1 and B2 (M54): white and orange tissues, respectively}, *P. italicum*, interaction of them and control. Total phenolic compounds was presented as mg caffeic acid g^{-1} of fruit weight, values are averages of three replicates. Error bars indicate ±SE

isolate, increased, then decreased (Fig. 4, A2), whereas total phenolic compounds in exocarp layer remained on the same level in all treatments with present of M54 isolate (Fig. 4, B2). The results of Duncan test showed that just two interactions effect had a significant difference in mesocarp layer for Total phenol component (in both of M45 or M54 isolates) (Fig. 3, A1-B1).

Discussion

The selection of effective antagonistic yeasts already is important for their practical uses (Arras *et al*., 1996). Of eight yeasts isolated for their antagonistic activity against the pathogen, two isolates of *Pichia kluyveri* (M45) and *Metschnikowia pulcherrima* (M54) had the most effective. Previously reports showed a number of effective yeasts isolate for the biological control of *Penicillium italicum* on citrus (Arras *et al*., 2002; Lahlali *et al*., 2014). The main mechanism of this biological control is based on induction of host defences (Janisiewicz and Korsten, 2002; Massart and Jijakli, 2007). Our result showed accumulation of enzymes activities (POD, CAT and β-1, 3-glucanase) and slowly increased accumulation of total phenol, few days after inoculation in both layers of the orange fruit. The resistance of fruits to pathogens is mainly due to pre-existent physical and chemical barriers (Arras *et al*., 1996). POD activity in exo-mesocarp layers was increased due to treated with pathogen or in combination with isolates, whereas in control treatment it was unchanged. This suggested that increase of POD activity is related to interaction between orange fruit with microorganisms. Resistance plants for pathogen have high level of POD (Percival, 2001). Peroxidases can eliminate the potentially toxic H_2O_2 with concomitant benefits (Liochev, 2013) and hydrogen peroxide, which are highly toxic to many organisms (Wang *et al*., 2014). Catalase expression after inoculation was similar to peroxidase; it was unchanged in control treatment and increased in both layers treated with antagonist, pathogen and combination of them. Peroxidase and catalase convert potentially dangerous to water through their combined action (Gong *et al*., 2001).

Among the enzymes involved in the defence against oxidative stress, catalase plays a key role by converting H_2O_2 to O_2 and H_2O (Scandalios, 1993). Respond of Some plants to a variety of environmental stresses is catalase increased (Scandalios *et al*., 1997). β-1, 3-glucanase activity in exo-mesocarp layers slowly increased in all treated. β-1, 3-glucanase is capable of hydrolysing fungal cells, and inhibit the growth of phatogen (Jongedijk *et al*., 1995). β-1, 3-glucanase, POD and Chitinase are involve in plant defence responses against fungal infection (Hückelhoven *et al*., 1999). Result of present study indicated that with inoculation of orange fruits with yeast isolates and pathogen, the enzymes were increased. The main reason was related to interaction between plant and microorganisms. Similar result reported on the other plant, such as apple (Alavifard *et al*., 2012; Bordbar *et al*., 2010), tobacco (Ye *et al*., 1992), tomato (Li *et al*., 2008) and rice (Bhattacharya *et al*., 2013).

Slowly accumulation of total phenol was observed, after fruit inoculation with microorganisms. Our result is in contrast with Bordbar et al. (2010) reported, that total phenolic compounds was decreased rapidly 6 days after inoculation, in apple fruits treated with antagonist, pathogen and or in combination. Phenolic substances have several important roles in defence mechanisms of plant against pathogens. Phenolic compounds may resistance to a disease by limiting the growth of the pathogen (Isaac, 1991). According to the plant-yeast isolates- pathogen interaction, it was observed that when the yeast isolates (M45 or M54) were inoculated into wounds with the pathogen, total phenol and enzymes activity (POD, CAT and β-1, 3-glucanase), were increased, these change were related to incubation time.

Conclusion

Our research identified effective of yeast isolates that could be used in postharvest biological control, alternative to the use of chemical fungicides. Also results showed that understanding biochemical mechanism derived from plant-pathogen-antagonist interaction is essential for investigating the dynamics of infectious processes.

Acknowledgments

This work was supported by the University of Tehran.

References

Agrios G (2005). Plant Pathology. Academic Press (5th ed) New York.

Alavifard F, Etebarian HR, Sahebani N, Aminian H (2012). Induction of resistance in apple fruit inoculated with antagonistic *Candida membranifaciens* isolates and *Botrytis mali*. Journal of Crop Protection 1(3):249-259.

Ansari NA, Feridoon H (2008). Postharvest application of hot water, fungicide and waxing on the shelf life of Valencia and the local orange cv. Siavarz. Acta Horticulturae 768:271-277.

Arras G, Ghibellini A, Quadu F, Demontis S, Sussarellu L (1996). Attività inibitrice di lieviti isolati da frutti di agrumi nei confronti di *Penicillium digitatum*. Italus Hortus 3(2):3-7.

Arras G, Scherm B, Migheli Q (2002). Improving biocontrol activity of *Pichia guillermondii* against post-harvest decay of oranges in commercial packing-houses by reduced concentrations of fungicides. Biocontrol Science and Technology 12(5):547-553.

Ben-Yehoshua S, Goldschmidt E, Bar-Joseph M (1994). Citrus fruits. Encyclopedia of Agricultural Science 1:357-378.

Bhattacharya S, Chakraborty K, Pal TK (2013). Induction of systemic resistance in rice by leaf extracts of *Datura metel* against sheath blight disease. Archives of Phytopathology and Plant Protection 46(18):2259-2269.

Bordbar FT, Etebarian HR, Sahebani N, Rohani H (2010). Control of postharvest decay of apple fruit with *Trichoderma virens* isolates and induction of defense responses. Journal of Plant Protection Research 50(2):146-152.

Cabras P, Schirra M, Pirisi FM, Garau VL, Angioni A (1999). Factors affecting imazalil and thiabendazole uptake and persistence in citrus fruits following dip treatments. Journal of Agricultural and Food Chemistry 47(8):3352-3354.

Chalutz E, Wilson C (1990). Postharvest biocontrol of green and blue mold and sour rot of citrus fruit by *Debaryomyces hansenii*. Plant Disease 74(2):134-137.

Cwalina-Ambroziak B, Nowak M (2012). The effects of biological and

chemical controls on fungal communities colonising tomato (*Lycopersicon esculentum* Mill.) plants and soil. Folia Horticulturae 24(1):13-20.

Du Z, Bramlage WJ (1995). Peroxidative activity of apple peel in relation to development of poststorage disorders. HortScience 30(4):205-209.

Ebrahimi Y (2002). Citrus industry in Iran. In: D'Onghia AM (Ed), Djelouah K (Ed), Roistacher CN (Ed). Proceedings of the Mediterranean research network on certification of citrus (MNCC): 1998-2001. Bari: CIHEAM pp 31-35.

FAOSTAT F (2013). Agriculture Database. Retrieved 2015 Jan 10 from

http.faostat3faoorg/faostatgateway/go/to/download/Q/*/E Accessed.

Feliziani E, Landi L, Romanazzi G (2015). Preharvest treatments with chitosan and other alternatives to conventional fungicides to control postharvest decay of strawberry. Carbohydrate Polymers 132:111-117.

Friedrich L, Lawton K, Ruess W, Masner P, Specker N, Rella MG, Meier B, Dincher S, Staub T, Uknes S (1996). A benzothiadiazole derivative induces systemic acquired resistance in tobacco. The Plant Journal 10(1):61-70.

Gong Y, Toivonen PM, Lau O, Wiersma PA (2001). Antioxidant system level in 'Braeburn' apple is related to its browning disorder. Botanical Bulletin of Academia Sinica 42.

Hanley ME, Lamont BB, Fairbanks MM, Rafferty CM (2007). Plant structural traits and their role in anti-herbivore defence. Perspectives in Plant Ecology, Evolution and Systematics 8(4):157-178.

Hückelhoven R, Fodor J, Preis C, Kogel K-H (1999). Hypersensitive cell death and papilla formation in barley attacked by the powdery mildew fungus are associated with hydrogen peroxide but not with salicylic acid accumulation. Plant Physiology 119(4):1251-1260.

Ippolito A, El Ghaouth A, Wilson CL, Wisniewski M (2000). Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. Postharvest Biology and Technology 19(3):265-272.

Isaac S (1991). Fungal-plant interactions. Springer.

Janisiewicz WJ, Korsten L (2002). Biological control of postharvest diseases of fruits. Annual Review of Phytopathology 40(1):411-441.

Jongedijk E, Tigelaar H, Van Roekel JS, Bres-Vloemans SA, Dekker I, van den Elzen PJ, Cornelissen BJ, Melchers LS (1995). Synergistic activity of chitinases and β-1, 3-glucanases enhances fungal resistance in transgenic tomato plants. Euphytica 85(1-3):173-180.

Lagrimini LM, Rothstein S (1987). Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. Plant Physiology 84(2):438-442.

Lahlali R, Hamadi Y, Drider R, Misson C, El Guilli M, Jijakli MH (2014). Control of citrus blue mold by the antagonist yeast *Pichia guilliermondii* Z1: Compatibility with commercial fruit waxes and putative mechanisms of action. Food Control 45:8-15.

Li SM, Hua GG, Liu HX, Guo JH (2008). Analysis of defence enzymes induced by antagonistic bacterium *Bacillus subtilis* strain AR12 towardsRalstonia solanacearum in tomato. Annals of Microbiology 58(4):573-578.

Liochev SI (2013). Reactive oxygen species and the free radical theory of aging. Free Radical Biology and Medicine 60:1-4.

Marques JPR, Amorim L, Silva-Junior GJ, Spósito MB, Appezzato-da Gloria B (2014). Structural and biochemical characteristics of citrus flowers associated with defense against a fungal pathogen. AoB Plants 7:plu090.

Massart S, Jijakli HM (2007). Use of molecular techniques to elucidate the mechanisms of action of fungal biocontrol agents: a review. Journal of Microbiological Methods 69(2):229-241.

Moretto C, Cervantes ALL, Batista Filho A, Kupper KC (2014). Integrated control of green mold to reduce chemical treatment in post-harvest citrus fruits. Scientia Horticulturae 165:433-438.

Palou L, Smilanick JL, Droby S (2008). Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. Stewart Postharvest Review 4(2):1-16.

Percival GC (2001). Induction of systemic acquired disease resistance in plants: potential implications for disease management in urban forestry. Journal of Arboriculture 27(4):181-192.

Pitt JI, Hocking AD, Diane A (2009). Fungi and food spoilage: Springer.

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner H-Y, Hunt MD (1996). Systemic acquired resistance. The Plant Cell 8(10):1809.

Scandalios JG (1993). Oxygen stress and superoxide dismutases. Plant Physiology 101(1):7.

Scandalios JG, Guan L, Polidoros AN (1997). Catalases in plants: gene structure, properties, regulation, and expression. Cold Spring Harbor Monograph Series 34:343-406.

Suwalsky M, Rodríguez C, Villena F, Aguilar F, Sotomayor CP (1999). The pesticide hexachlorobenzene induces alterations in the human erythrocyte membrane. Pesticide Biochemistry and Physiology 65(3):205-214.

Tian S, Fan Q, Xu Y, Jiang A (2002). Effects of calcium on biocontrol activity of yeast antagonists against the postharvest fungal pathogen *Rhizopus stolonifer*. Plant Pathology 51(3):352-358.

Van Loon L, Bakker P, Pieterse C (1998). Systemic resistance induced by rhizosphere bacteria. Annual Review of Phytopathology 36(1):453-483.

Wang L, Zhang, L, Niu Y, Sitia R, Wang CC (2014). Glutathione peroxidase 7 utilizes hydrogen peroxide generated by Ero1α to promote oxidative protein folding. Antioxidants & Redox Signaling 20(4):545-556.

Wang YS, Tian SP, Xu Y, Qin GZ, Yao H (2004). Changes in the activities of pro-and anti-oxidant enzymes in peach fruit inoculated with *Cryptococcus laurentii* or *Penicillium expansum* at 0 or 20 °C. Postharvest Biology and Technology 34(1):21-28.

Wittstock U, Gershenzon J (2002). Constitutive plant toxins and their role in defense against herbivores and pathogens. Current Opinion in Plant Biology 5(4):300-307.

Yamomoto H, Hokin H, Tany T, Kadota G (1977). Phenylalanine ammonia-lyase in relation to the corn rust resistance of oat leaves. Phtopathology 90(2):203-211.

Ye X, Järlfors U, Tuzun S, Pan S, Kuc J (1992). Biochemical changes in cell walls and cellular responses of tobacco leaves related to systemic resistance to blue mold (*Peronospora tabacina*) induced by tobacco mosaic virus. Canadian Journal of Botany 70(1):49-57.

Youssef K, Ligorio A, Sanzani SM, Nigro F, Ippolito A (2012). Control of storage diseases of citrus by pre-and postharvest application of salts. Postharvest Biology and Technology 72:57-63.