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CONTROL OF POSTHARVEST DECAY OF APPLE FRUIT WITH *TRICHODERMA VIRENS* ISOLATES AND INDUCTION OF DEFENSE RESPONSES

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Abstract: The biocontrol activity of two isolates of *Trichoderma virens* against blue mould of apple fruits caused by *Penicillium expansum* and their ability to induce biochemical defense responses in apple tissue were investigated. Apple fruit (*Malus domestica*) wounds were inoculated with 20µl antagonist suspension (10^7 conidia/ml) of *T. virens* and 4 h later with 20 µl of conidial suspension of *P. expansum* (10^5 conidia/ml). The apples were then incubated at 20°C for 8 days. Lesion diameters were measured 4 and 8 days after inoculation with the pathogen. Two isolates of *T. virens* were effective in controlling decay of apple fruits caused by *P. expansum*. Six days after treatment peroxidase activity increased by more than three-fold in apple treated fruit in combination of antagonist and pathogen, in comparison with in wounded, non-inoculated control tissue. Catalase (CAT) activity increased in inoculated fruits in combination of *T. virens* and *P. expansum* in comparison with healthy control at all days and maximum activity level was noted at 6 days after inoculation. The results indicated that T6 and T8 isolates of *T. virens* caused the increase in β-1,3-glucanase activity that reached maximum levels 4 and 6 days after inoculation with pathogen, respectively. The increase in β-1,3-glucanase activity was triggered by wounding although the level of increase was markedly lower than detected in treated fruits.
Phenolic compounds accumulation showed the highest levels 2–4 days after inoculation and then decreased. The ability of *T. virens* to increase the activity of peroxidase, catalase, β-1,3-glucanase and levels of phenolic compounds may be one of mechanisms responsible for its biocontrol activity.

Key words: apple fruit, *Trichoerma virens*, *Penicillium expansum*, peroxidase, catalase, β-1,3-glucanase

INTRODUCTION

Blue mould, caused by *Penicillium expansum* Link. is a major postharvest disease of apple fruits. The most effective control strategy is the postharvest treatment with fungicides. However, fungicide toxicity, along with development of fungicide resistance by pathogens, caused the public considerable concern requiring the development of alternatives to synthetic fungicides (Janisiewicz and Korsten 2002; Droby *et al.* 2003).

The genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents, the antagonistic properties of which are based on the activation of multiple mechanisms. *Trichoderma* strains exert biocontrol activity against fungal phytopathogens either indirectly, by competing for nutrients and space, modify mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism (Benitez *et al*. 2004). The effectiveness of *Trichoderma* sp. as biological control agents (BC-agents) against postharvest disease was proved in a number of studies (Sivakumar *et al*. 2000; Barbosa *et al*. 2001; Wantoch-Rekowski 2004).

At the same time, considerable attention was also placed on the induction of resistance as an important management form of plant protection and control of postharvest diseases of fruit and vegetables (Wilson and others 1994; Kuc 2001; Kogel and Gregor 2005).

The induction of disease resistance following treatment with active microbial and chemical inducer, respectively, was reported to provide protection against invasion of pathogens in several plant species (Friendrich *et al*. 1996; Van Loon *et al*. 1998). Obviously, it is valuable to study the interactions among pathogen, antagonist and apple fruits during postharvest storage, in particular the enzymes regulating reactive oxygen levels, as there production and accumulation (superoxide, hydrogen peroxide, and the hydroxyl radical) are the most frequently observed biochemical agents during plant–microbe interaction (Wang *et al*. 2004). These molecules may play a dual role in defense, including direct antibiotic activity such as cross-linking of cell walls or antimicrobial agents and an indirect effect as signals that mediate the systemic activation of gene expression in response to pathogen (Low and Merida 1996; Hancock *et al*. 2002), also host defense responses expressed

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systemically involve the synthesis and accumulation of antifungal glucanohydrolases such as chitinase, chitosanase, and β-1,3-glucanase (Ryalls *et al*. 1996; Van Loon *et al*. 1998). The primary objective of this study was to confirm the ability of two isolates of *T. virens* to control postharvest blue mould rot caused by *P. expansum* in apple 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 apple fruit tissue, following the application of two antagonists alone or in combination with the pathogen.

MATERIALS AND METHODS

Blue mould pathogen

P. expansum isolated from *Malus domestica Borck* cv. Golden delicious in cold storage of Mehrshahr, Iran was used in this study. All cultures were derived from single spores and maintained on potato dextrose agar at 4°C in darkness until needed.

Biological control isolates

Two isolates *T. virens* (T6 and T8) obtained from Plant Pathology laboratory of Aburaihan Campus, University of Tehran were used in this study. These isolates were obtained from apple surface and identified by Dr. Rohani.

Fruit samples

Malus domestica Golden delicious apples were harvested at commercial maturity and kept at 1.0±0.5°C until used. The apples were washed in 70% ethanol for 30 s, followed by dipping in 0.1% sodium hypochlorite solution, and rinsed three times with sterile distilled water (SDW).

***In vivo* biological control studies**

The fruit were wounded with a 2 mm – diameter nail to depth of 2 mm. *P. expansum* and *Trichoderma* isolates were grown on PDA plates (9 cm diameter) for 7 to 14 d. Conidia were harvested by pouring few milliliters of SDW containing 0.05% Tween 20 in the plates. The conidial suspension was adjusted with a hemacytometer to $1.0x10^5$ and $1.0x10^7$ conidia /ml for *P. expansum* and *Trichoderma*, respectively.

Aliquots of 20 µl of conidial suspension of *Trichoderma* or SDW were applied to each wound, after 4 h, 20 µl conidial suspension of *P. expansum* was applied in to each wound. The treated apples were placed on cardboard trays enclosed in plastic bags. The inside of bags was sprayed with SDW to maintain high relative humidity. The apples were incubated at 20°C for 8 days. Lesion diameters were measured after 4 and 8 days after pathogen inoculation, using digital calipers and lesion area was recorded.

Extraction and assay of peroxidase activity

The method of extraction as described by Gong *et al*. (2001) was used in the experiment.

Fresh apple tissue (5 g) was homogenized in 15 ml of 0.05 M phosphate buffer (pH 7.0) containing 10% polyvinil pyrrolidone (Merck, Darmstadt, Germany) and 0.1 M ethylene diamine tetra acetic acid (EDTA) (Merck, Darmstadt, Germany).

The homogenate was centrifuged at 14 000 rpm for 16 min at 4°C. The supernatant was used for the peroxidase assay. Peroxidase activity was measured by the method of Vetter *et al*. (1958) as modified by Gorin and Heidema (1976). The assay mixture contained 0.1 ml enzyme extract, 1.35 ml 100 mm MES buffer (2-morpholinoethanesulfonic acid, monohydrate) (Sigma, St. Louis, USA) (pH 5.5), 0.05% H_2O_2 (Merck, Darmstadt, Germany) and 0.1% ϱ-phenylenediamine (Merck, Darmstadt, Germany). Changes in absorbance were recorded at 485 nm for 1 min with a spectrophotometer (Milton Roy, Spectronic 501, Unterfoehring-Germany). The activity of peroxidase was presented as ΔOD_{485nm}/min/mg protein.

Extraction and assay of CAT activity

The method of extraction as described by Gong *et al*. (2001) was used in the experiment.

Fresh apple flesh tissue (5 g) was homogenized in 15 ml of Tris-HCl buffer (pH 8.5) including 2 mm EDTA (Merck, Darmstadt, Germany), 10% (w/v) PVPP (Merck, Darmstadt, Germany). The homogenate was centrifuged at 16 000 rpm for 14 min at 4°C. Supernatant was used for the activity measurement. CAT activity was determined by following the disappearance of H_2O_2 in the enzyme reaction mixture (Brennan and Frenkel 1977; Du and Bramlage 1995). The enzyme extract (0.25 ml) was added to 2 ml assay mixture (50 mM Tris-HCl buffer pH 6.8, containing 5 mM H_2O_2). 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_2 mm/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 the experiment. From each fruit, tissue samples were taken from the wounds and individually homogenized at 4°C in two volumes (w/v) of 50 mm sodium acetate buffer, pH 5.0, and the homogenate was centrifuged at 4°C (15 min, 10 000 x *g*). Proteins in the supernatant were precipitated in 60% acetone (v/v) at –20°C and the resulting pellet, following centrifugation (30 min, 12 000 rpm 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 activity. B-1,3-glucanase was determined following the method of Abeles and Forrence (1979). B-1, 3-glucanase activity was assayed by incubating 62.5 ml of enzyme preparation for 2 h at 40°C in 62.5 ml of 4% laminarin (Fluka, Buchs, Switzerland). The reaction was terminated by heating the sample in boiling water for 10 min and the amount of reducing sugars was measured spectrophotometrically at 492 nm after reaction with 372 µl of 3, 5-dinitrosalicilate (Merck, Darmstadt, Germany). Final activity values are reported as nmol glucose/min/µg of total protein.

Assay of protein content

Total protein was determined by the method of Bradford (1976) using bovine serum albumin as standard.

Determination of total phenolic compounds

The method of extraction as described by Yamomoto *et al*. (1977) was used in the experiments. Apple fruits (1.0 g fresh weight) were ground in a mortar with 8 ml of 80% methanol (Merck, Darmstadt, Germany) 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 1 000 rpm for 5 min at room temperature and the supernatant was assayed.

Total phenol was determined 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 compound of samples were expressed as mg caffeic acid per g of fruit fresh weight.

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 enzymatic and phenolic compounds assays, the experiments were arranged as a 4x4 factorial with four treatments (*T. virens* ± pathogen challenge, healthy control and pathogen control) and four sampling time in a completely randomized design with four replicates Analysis of variance was performed on the data and means were separated using Duncan's multiple range test at $p < 0.05$ (Little and Hill 1978).

RESULTS

Biocontrol activity

Two isolates of *T. virens* were effective in controlling decay of apple fruit caused by *P. expansum*. They prevented the disease spread by 4.1 to 4.8 cm^2 compared with 9.8 cm^2 in control 8 days after inoculation with the pathogenat 20°C.

Lesion size was from 1.3 to 2.2 cm^2 for treatment with antagonistic and 4.1 cm^2 for the control treatment 4 after days (Table 1).

Peroxidase activity

POD activity remained on the same level in the apple fruits treated with water (healthy control). POD activity in apple fruits treated with antagonist and pathogen alone or in combination showed the increase, reaching maximum level 6 days after storage at 20°C, and then decreased 8 days after treatment, although there was no significant difference between POD activities in treated fruit with *T. virens* T6 in combination with pathogen 6 and 8 days after inoculation.

Table 1. Decay area (cm^2) of Golden delicious apples inoculated with *P. expansum* after treatment with *T. virens* and incubated 4 and 8 days at 20°C

Treatments	Decay area after 4 days	Decay area after 8 days
P. expansum	4.1 a	9. 8 a
T. virens (T8) + *P. expansum*	1.3 c	4.1 c
T. virens (T6) + *P. expansum*	2.2 b	4.8 b

Each treatment was replicated 4 times. Means followed by the same letter within a column do not differ significantly at $p < 0.05$ according to Duncan's multiple range tests

Six days after treatment, POD activity increased by more than three-fold in apple fruits treated in combination of antagonist with pathogen in comparison with in wounded fruits, non-inoculated control tissue (Fig. 1A, B).

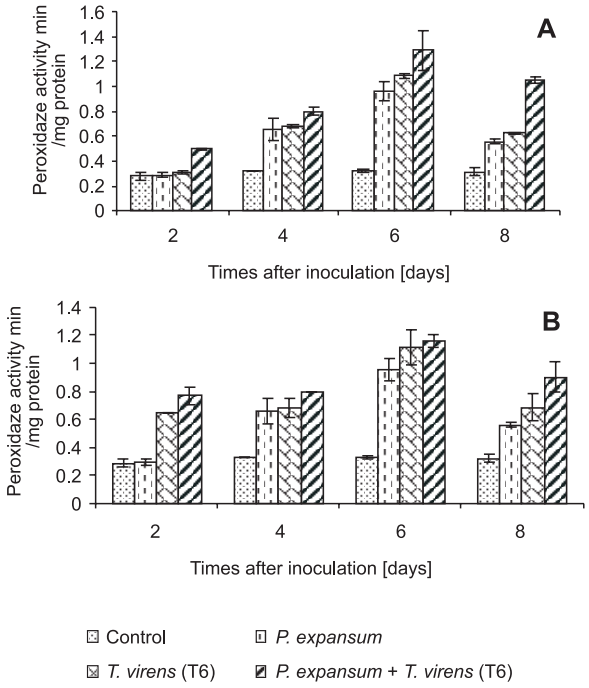

Fig. 1. Peroxidase activity in apple tissue in the presence or absence of *T. virens* or *P. expansum*. Peroxidase activity in apple fruit treated with sterile water, *P. expansum*, *T. virens*, *T. virens* + *P. expansum*. Isolates of *T. virens* are: T6 (A) and T8 (B)
The activity of peroxidase was presented as Δ $OD_{485\ nm}$/min/mg protein, values are averages of 4 replicates. Error bars indicate ±SE.

Catalase activity

CAT activity remained on the same level in the apple fruits treated with water (healthy control).

CAT activity increased slightly in fruits treated with antagonist and pathogen alone or in combination 6 days after storage and declined rapidly after 8th days. At the local wound site, the combination of antagonist with pathogen induced an approximately two fold increase in CAT activity 6 days after storage (Fig. 2).

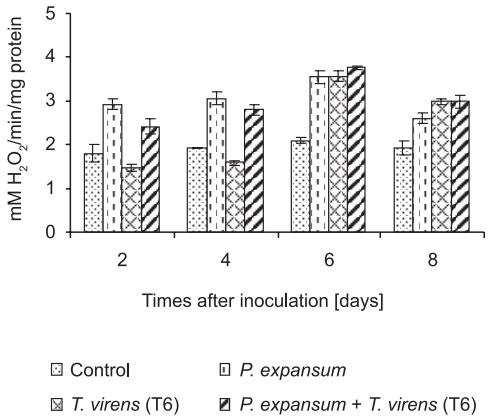

Fig. 2. Catalase activity in apple tissue in the presence or absence of *T. virens* or *P. expansum*. Peroxidase activity in apple fruit treated with sterile water, *P. expansum*, *T. virens* T6, *T. virens* T6 + *P. expansum*.
The activity of catalase was presented as mM H_2O_2/min/mg protein, values are averages of 4 replicates. Error bars indicate ±SE

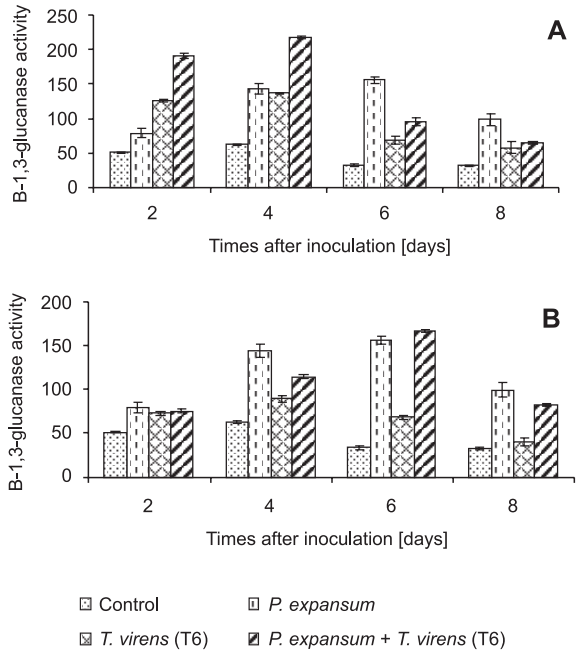

Fig. 3. β-1,3-glucanase activity in apple tissue in presence or absence of *T. virens* or *P. expansum*
β-1,3-glucanase activity in apple fruit treated with sterile water, *P. expansum*, *T. virens*, *T. virens* + *P. expansum*. Isolates of *T. virens* are: T6 (A) and T8 (B).
The activity of β-1,3-glucanase was expressed as nm glucose/min/μg protein, values are averages of 4 replicates. Error bars indicate ±SE.

β-1,3-glucanase activity

Six days after treatment, β-1,3-glucanase activity increased by more than three-fold in apple fruits treated with combination of T6 and pathogen in comparison with wounded, non-inoculated control tissue and then decreased rapidly at 6th day while at the local wound site, the combination of T8 with pathogen induced an approximately 5-fold increase in β-1,3-glucanase activity after 6 days and decline at 8th day of storage. However, in the fruits treated with T6 or T8 alone it induced an approximately 2.2 and 1.4 fold increase in β-1,3-glucanase activities 6 days after inoculation although there was no significant difference between β-1,3-glucanase activities in treated fruit with T6, 2 and 4 days after inoculation. The treatment with pathogen alone stimulated the increase in β-1,3-glucanase activity of approximately 2.3 and 4.8 higher than of control with 4 and 6 days after of inoculation, respectively.

The increase in β-1,3-glucanase activity 4 days after was observed also in wounded control fruits, but the level of increase was markedly lower than that detected in treated fruits (Fig. 3A, B).

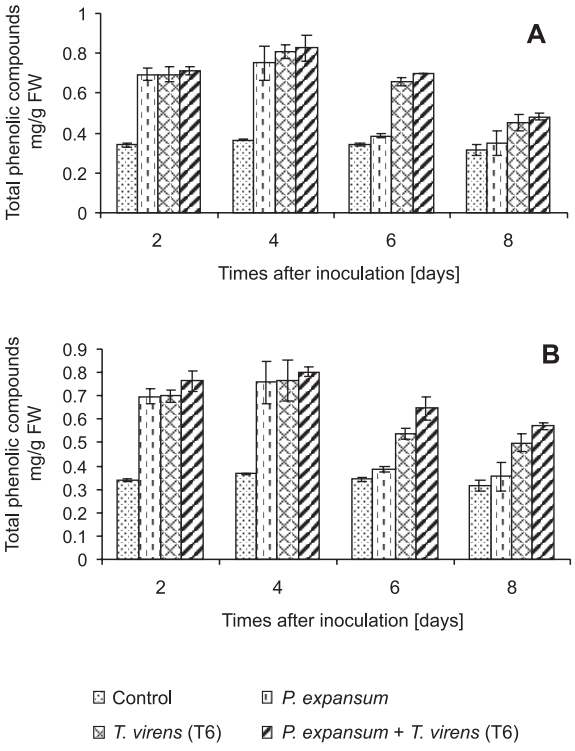

Fig. 4. Total phenolic compounds in apple tissue in the presence or absence of *T. virens* or *P. expansum*. Total phenol content of apple fruit treated with sterile water, *P. expansum*, *T. virens*, *T. virens* + *P. expansum*. Isolates of *T. virens* are: T6 (A) and T8 (B).
Total phenolic compounds of the roots were expressed as mg caffeic acid g^{-1} of fruit weight and values are averages of 4 replicates. Error bars indicate ± SE

Total phenolic compounds

Total phenolic compounds remained on the same level in the apple fruit treated with water (healthy control).

At a local wound site, the combination of antagonist with pathogen caused an approximately 2.2–2.24-fold increase in total phenolic compounds 4 days after inoculation. Total phenolic compounds in apple fruits treated with antagonist and pathogen alone or in combination decreased rapidly 6 days after inoculation. There was no difference between phenolic compound content in treated fruits with antagonist in combination with pathogen or fruits treated with pathogen alone 2 and 4 days after inoculation (Fig. 4A, B).

DISCUSSION

The presented data show that isolates of *T. virens* applied as a wound treatment were effective in controlling postharvest decay of apple fruit caused by *P. expansum*.

They also induced the accumulation of peroxidase, β-1, 3-glucanase and phenol compounds in apple fruits.

Comparable levels of decay control were reported for other microbial antagonists (Chand-Goyal and Spott 1997; El-Ghaouth *et al*. 1998; Nigro *et al*. 1999; Ippolito *et al*. 2000; Wang *et al*. 2004; Chan and Tian 2006) and it was attributed to a complex mechanism that may involve nutrient competition, site exclusion, direct parasitism, production of lytic enzymes and possibly of induced resistance (Droby and Chalutz 1994).

In harvested commodities, the induction of disease resistance by microbial antagonists was suggested (Ippolito *et al*. 2000; Droby *et al*. 2002). Here we used two isolates of *T. virens* and studied the changes of peroxidase, catalase, β-1,3-glucanase and total phenolic compounds which contribute to enhance the ability against infection with the pathogen. Chitinase, β-1,3-glucanase, PAL and POD were suggested as being involved in plant defense responses against fungal infection (Schlumbaum *et al*. 1986; Collinge *et al*. 1993; Dixon and Paiva 1995; Huckelhoven *et al*. 1999). Chitinase and β-1,3-glucanase is capable of hydrolyzing fungal cells, and in combination they were shown to inhibit the growth of several pathogenic fungi *in vitro* (Schlumbaum *et al*. 1986; Sela-Buurlage *et al*. 1993). Induction and accumulation of glucanohydrolases and peroxidases is often correlated with the onset of induced resistance, and suggest an active role for these enzymes in defense against pathogenic fungi (Kuc 1990; Sticher *et al*. 1997; Van Loon *et al*. 1998).

Peroxidase and antioxidant enzymes (superoxide dismutase and catalase) convert potentially dangerous O_2 and H_2O_2 to water through their combined action (Gong *et al*. 2001). The balance between SOD and POD or CAT activities in cells is crucial for determining the steady-state level of O_2− and H_2O_2 (Chan and Tian 2006).

Ippolito *et al.* (2000) indicated that *Aureobasidium pullulans* caused a transient increase in chitinase, β-1,3-glucanase, and peroxidase activity in apple fruits.

Similarly in this study two isolates of *T. virens* caused increases in peroxidase and β-1,3-glucanase activities in apple fruit at 20°C. T8 isolate had the most peroxidase activities 6 days after inoculation with pathogen.

It is not possible however, to determine the extent of the role played by host defense responses in the observed protection because of the antagonistic activity of *T. virens* at the wound site. The accumulation of peroxidase and β-1,3-glucanase can be expected by retarding fungal growth (Ippolito *et al*. 2000).

In this study, CAT activity was increased in inoculated fruits with combination of antagonist- pathogen in comparison with control at all days.

Cao *et al*. (2005) indicated that acibenzolar-S–methyl (ASM) treatment significantly enhanced activities of the main defense enzymes including peroxidase, phenylalanine ammonia-lyase and chitinase, and activities of antioxidant enzymes including superoxide dismutase and catalase in fruits during infection. Plants mobilize anti oxidative defense mechanisms, in order to eliminate the effect of free radicals, the causal agents of most stresses. The components of these defense mechanisms are several stress-enzymes (superoxide dismutase, catalase, peroxidase and polyphenol oxidase) and other components like phenols (Honty *et al*. 2005). Although H_2O_2 in fruit tissue could contribute to enhancement of disease resistance; it could be scavenged off by CAT to prevent harmful effects of H_2O_2 excess to fruit (Cao *et al*. 2005). Maybe, in coordination with SOD, CAT acts as one of enhanced self- protective mechanisms to alleviate oxidative stress in apple fruit tissue after *T. virens* inoculation.

Our results showed that phenolics accumulation was increased in apple fruits treated with two isolates of *T. virens* and inoculated with *P. expansum.*

Similarly, Guleria and Kumar (2006) reported higher activity of phenylalanine ammonia lyase (PAL) and peroxidase and the increase in level of phenolic compounds in sesame (*Sesamum indicum*) leaves treated with leaf extract of neem (*Azadirachta indica*) and inoculated with Alternaria leaf spot pathogen (*Alternaria sesame*). It is suggested that neem leaf extract induced activity of PAL and peroxidase enzymes in sesame leaves, which resulted in increased biosynthesis and metabolism of phenol which might have protected the sesame plant from *A. sesame.*

Phenolic compounds and flavonoids are two kinds of metabolites of phenylpropanoid pathway in plant (Cao *et al*. 2005). To direct effects of phenols on fungal pathogen, phenolic compounds are oxidized to form more toxic, quinones, by peroxidases (Gogoi *et al*. 2001).

Induction of these defense-related reactions might indicate the recognition by the host tissue of *T. virens* isolates which might facilitate the formation of the appropriate defense mechanism in the fruit against a potential attacking pathogen because the success in the defense response depends on speed at which the plant recognizes the attacking pathogen and on the intensity with which an appropriate defense mechanism is activated. If the plant fails to respond in time, the appropriate defense will be activated too late so that the pathogen can colonize the plant tissue (Kuc 2001; Conrath *et al*. 2002; Ton *et al*. 2005).

Pathogenesis in fruit inoculated with *P. expansum* at 20°C may mainly be associated with fruit senescence, as fruit resistance to postharvest diseases is closely linked to the ripening process, and drops markedly with the onset of tissue senescence (Mari and Guizzardi 1998).

In conclusion, the present results show that two isolates of *T. virens* have potential as biocontrol agents for the control of postharvest decay of apple caused by *P. expansum* and are capable of inducing the accumulation of peroxidase, β-1, 3-glucanase, catalase and increase the level of phenolic compounds which suggests that postharvest treatment with *T. virens* hold promise as a new technology, substituting for fungicidal control of postharvest diseases in apple fruits.

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REFERENCES

Abeles F.B., Forrence L.E. 1979. Temporal and hormonal control β-1,3-glucanase in *Phaseolus vulgaris* L. Plant Physiol. 45: 395–400.

Brennan T., Frenkel C. 1977. Involvement of hydrogen peroxide in the regulation of senecence in pear. Plant Physiol. 59: 411–416.

Barbosa M.A.G., Rehn K.G., Menezes M., Mariano R.D.L.R. 2001. Antagonism of *Trichoderma* species on *Cladosporium herbarum* and their enzamatic characterization. Braz. J. Microbiol. 32: 98–104.

Benitez T., Rincon A.M., Limon M.C., Condon A.C. 2004. Biocontrol mechanisms of *Trichoderma* strains. Int. Microbiol. 7: 249–260.

Bradford M.M. 1976. A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

Cao J., Jiang W., He H. 2005. Induced resistance in Yali Pear (*Pyrus bretschneideri* Rehd.) fruit against infection by *Penicillium expansum* by postharvest infiltration of Acibenzolar-S–methyl. J. Phytopathol. 153: 640–646.

Chan Z., Tian S. 2006. Induction of H_2O_2-metabolizing enzymes and total protein synthesis by antagonistic yeast and salicylic acid in harvested sweet cherry fruit. Postharvest Biol. Technol. 39: 314–320.

Chand-Goyal T., Spotts R.A. 1997. Biological control of postharvest diseases of apple and pear under semi-commercial and commercial conditions using three saprophytic yeasts. Biol. Control 10: 199–206.

Collinge B., Kragh K.M., Mikkelsen J.D., Nielsen K.K., Rasmussen U., Vad K. 1993. Plant chitinases. Plant J. 3: 31–40.

Conrath U., Pieterse C.M.J., Mauch–Mani B. 2002. Priming in plant–pathogen interactions. Trends Plant Sci. 7: 210–216.

Dixon R.A., Paiva N.L. 1995. Stress-induced phenylpropanoid metabolism. Plant Cell 7: 1085–1097.

Droby S., Chalutz E. 1994. Mode of action of biocontrol agents for postharvest diseases. p. 63–75 In: "Biological Control of Postharvest Diseases of Fruits and Vegetables – Theory and Practice" (C.L. Wilson, M.E. Wisniewski, eds.). CRC Press, Boca Raton, FL.

Droby S., Vinokur V., Weiss B., Cohen L., Daus A., Goldschmidt E.E., Porat R. 2002. Induction of resistance to *Penicillium digitatum* in grapefruit by the yeast biocontrol agent *Candida oleophila*. Phytopathology 92: 393–399.

Droby S., Wisniewski M., Ei-Ghaouth A., Wilson C. 2003. Biological control of postharvest diseases of fruit and vegetables: current achievements and future challenges. Acta Hort. 628: 703–713.

Du Z., Bramlage W.J. 1994. Superoxide dismutase activities in senescing apple fruit (*Malus domestica* Borkh.). J. Food Sci. 59: 581–584.

El-Ghaouth A., Wilson C., Wisniewski M. 1998. Ultrastructural and cytochemical aspect of the biocontrol activity of *Candida saitoana* in apple fruit. Phytopathology 88: 282–291.

Friendrich L., Lawton K., Ruess W., Masner P., Specker N., Gut Rella M., Meier B., Dincher S., Staub T., Uknes S., Métraux J-P., Kessmann H., Ryals J. 1996. A benzothiadiazole derivative induces systemic acquired resistance in tobacco. The Plant J. 10: 61–70.

Gogoi R., Singh D.V., Sivastava K.D. 2001. Phenolic as a biochemical basis of resistance in wheat against karnal bunt. Plant Pathol. 50: 470–476.

Gorin N., Heidema F.T. 1976. Peroxidase activity in Golden Delicious apples as a possible parameter of ripening and senescence. J. Agric. Food Chem. 24: 200–201.

Gong Y., Toivonen P., Lau O.L., Wiersma P.A. 2001. Antioxidant system level in Braeburn`apple is related to its browning disorder. Bot. Bull. Acad. Sin. 42: 259–264.

Guleria S., Kumar A. 2006. *Azadirachta indica* leaf extract induces resistance in sesame against *Alternaria* leaf spot diseases. J. Cell Mol. Biol. 5: 81–86.

Hancock J.T., Desikan R., Charke A., Hurst R.D., Neill S.J. 2002. Cell signaling following plant/pathogen interactions involves the generation of reactive oxygen and reactive nitrogen species. Plant Physiol. Biochem. 40: 611–617.

Honty K., Hevesi M., Toth M., Stefanovits-Banyai E. 2005. Some biochemical changes in pear fruit tissue induced by *Erwinia amylovora*. Proc. of the 8th Hungarian Congress on Plant Physiology and the 6th Hungarian Conference on Photosynthesis. Acta Biol. Szegediensis 49: 127–129.

Huckelhoven 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 Physiol. 19: 1251–1260.

Ippolito A., El-Ghaouth A., Wilson C.L., Wisniewski M. 2000. Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. Postharvest Biol. Technol. 19: 265–272.

Janisiewicz W.J., Korsten L. 2002. Biological control of postharvest diseases of fruits. Annu. Rev. Phytopathol. 40: 411–441.

Kogel K., Gregor L. 2005. Induced disease resistance and gene expression in cereals. Cell Microbiol. 7: 1555–1564.

Kuc J. 1990. Immunization for the control of plant disease. p. 355–366. In: "Biological Control of Soil-borne Plant Pathogens" (D. Hornby, ed.). CAB International, 496 pp.

Kuc J. 2001. Concepts and direction of induced systemic resistance in plants and its application. Eur. J. Plant Pathol. 107: 7–12.

Little T.M., Hills F.J. 1978. Agricultural experimentation design and analysis. John Wiley and Sons, New York, USA, 368 pp.

Low P.S., Merida J.R., 1996. The oxidative burst in plant defense: function and signal transduction. Physiol. Plant. 96: 533–542.

Ryalls J., Neuenschwander U., Willits M., Molina A., Steiner H.Y., Hunt M. 1996. Systemic acquired resistance. Plant Cell 8: 1809–1819.

Mari M., Guizzardi M. 1998. The postharvest phase: emerging technologies for the control of fungal diseases. Phytoparasitica 26: 59–66.

Nigro F., Finetti Sialer M.M., Gallitelli D. 1999. Transformation of *Metschnikowia pulcherrima* 320, biocontrol agent of storage rot, with the green fluorescent protein gene. J. Plant Pathol. 81: 205–208.

Schlumbaum A., Mauch F., Vogeli U., Boller T. 1986. Plant chitinases are potent inhibitors of fungal growth. Nature 324: 365–367.

Sela-Buurlage M.B., Ponstein A.S., Bres-Vloemans B., Melchers L.O., Van Den Lzen P., Cornelissen B.J.C. 1993. Only specific tobacco chitinases and β-1,3-glucanase exhibit antifungal activity. Plant Physiol. 101: 857–863.

Sivakumar D., Wilson Wijeratnam R.S., Wijesundera R.L.C., Abeyeskere M. 2000. Antagonistic effect of *Trichoderma harzianum* on postharvest pathogens of rambutan (*Nephelium lappaceum*). Phytoparasitica 28 (3): 240–247.

Sticher L., Mauch-Mani B., Metraux J.P. 1997. Systemic acquired resistance. Annu. Rev. Phytopathol. 35: 235–270.

Ton J., Jakab G., Toquin V., Flors V., Iavicoli A., Maeder M.M., Métraux J-P., Mauch-Mani B. 2005. Dissecting the B-aminobutyric acid-induced priming phenomenon in arabidopsis. Plant Cell 17: 987–999.

Van Loon L.C., Bakker P.A., Pieterse C.M. 1998. Systemic resistance induced by rhizophere bacteria. Annu. Rev. Phytopathol. 36: 453–483.

Vetter J.L., Steinberg M.P., Nelson A.I. 1958. Quantitative determination of peroxidase in sweet corn. J. Agric. Food Chem. 6: 39–41.

Wang Y., Tian S. 2004. Changes in the activities of pro- and antioxidant enzyme in peach fruit inoculated with *Cryptococcus laurentii* or *Penicillium expansum* at 0 or 20°C. Postharvest Biol. Technol. 34: 21–28.

Wantoch-Rekowski R. 2004. Pflanzenschutz-antagonisten zu bodenbürtigen krankheiten (2). Deutscher. Gartenbau 50, p. 38.

Wilson G.L., Ei-Ghaouth A., Chalutz E., Droby S., Stevens G., Lu J.Y., Khan V., Arul J. 1994. Potential of induced resistance to control postharvest diseases of fruits and vegetables. Plant Dis.78: 837–844.

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

POLISH SUMMARY

ZWALCZANIE POZBIOROWEGO GNICIA JABŁEK IZOLATAMI *TRICHODERMA VIRENS* I INDUKOWANIE REAKCJI OBRONNYCH

Badano aktywność biologicznego zwalczania dwóch izolatów *Trichoderma virens* w ograniczaniu niebieskiej pleśni wywoływanej przez *Penicillium expansum* oraz ich zdolność indukowania biochemicznych reakcji obronnych w tkance jabłek. Rany na jabłkach (*Malus dimestica*) inokulowano z 20 µl zawiesiny konidiów (10^7 konidiów/ml) *T. virens*, a po następnych 4 godzinach z 20 µl zawiesiny konidiów *P. expansum* (10^5 konidiów/ml), po czym jabłka inokulowano w 20°C w ciągu 8 dni. Po 4 i 8 dniach, po inokulacji patogenem, mierzono średnicę ran. Dwa izobaty *T. virens* wykazywały efektywność w zwalczaniu gnicia jabłek wywoływanego przez *P. expansum*. Sześć dni po tym zabiegu aktywność peroksydazy wzrastała ponad trzykrotnie w jabłkach traktowanych w kombinacji z antagonistą i patogenem, w porównaniu do nieuszkodzonej, nie inokulowanej tkanki owoców w kombinacji kontrolnej. Aktywność katalazy (CAT) wzrastała w inokulowanych owocach w kombinacji z *T. virens* i *P. expansum* we wszystkich odmianach w porównaniu do zdrowej kontroli, a maksymalny poziom aktywności stwierdzono 6 dni po inokulacji. Uzyskane wyniki wykazały, że izobaty T6 i T8 *T. virens* powodowały wzrost aktywności β-1,3- glukazyny, która osiągała odpowiednio maksymalne poziomy po 4 i 6 dniach po inokulacji patogenem. Zranienie stymulowało wzrost aktywności β-1,3-glukazyny, chociaż poziom wzrostu był znacznie niższy niż wykryty w owocach traktowanych. Akumulacja związków fenolowych była na najwyższym poziomie 2–4 dni po inokulacji i później zmniejszała się. Zdolność *T. virens* do zwiększania aktywności peroksydazy, katalazy, β-1,3-glukazyny oraz poziomu związków fenolowych może być jednym z mechanizmów odpowiedzialnych za procesy biologicznego zwalczania.