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Evaluation of Antimicrobial Activity of Some Enzymes of *Trichoderma harzianum* Immobilized on Polyester Cloth Films on The Disease Incidence of Postharvest Black Mold Disease of Tomatoes

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THE CRUDE extract activity was tested in the present study for some enzymes of *Trichoderma harzianum*, immobilized on polyester cloth films against the postharvest black mold disease of tomato fruit caused by *Alternaria alternata*. Firstly, the antifungal activity of *T. harzianum* against *A. alternata* was examined by the dual culture method using PDA plates, as it clearly inhibited the linear growth of *A. alternata* where the inhibition percentage was 66.5%. The hyphal interactions between both fungi were explored microscopically where hyphae of *T. harzianum* penetrated inside the hyphae of *A. alternata* then lysed them. The lysis of pathogenic cells was referred to the presence of cellulase, chitinase and glucose oxidase enzymes of *T. harzianum*. These enzymes were assayed quantitatively; then immobilized separately on sterile polyester films where their activity and stability were estimated in both crude cell free filtrates and saturated films. The immobilization of the tested enzymes on the polyester films showed promising findings in raising the enzyme persistence. In the meantime, these enzymatic polyester films were tested as tomato fruit coverage against the postharvest black mold disease at room temperature for 15 days. The cellulase films showed promising results, where they completely lowered the disease incidence to 0% after 4 and 7 days while it recorded 33.3 and 100% after 10 and 15 days, respectively.

Keywords: Polyester film, *Trichoderma harzianum*, *Alternaria alternata*, Tomatoes, Black mold, Cellulase, Chitinase and Glucose oxidase.

Introduction

Tomato (*Lycospersicon esculentum* Mill) is considered one of the most important vegetable crops which is highly distributed all over the world. Egypt is the fifth largest producer of tomatoes in the world where its production of tomatoes exceeds 9 million tons annually (Moussa et al., 2013). These fleshy fruits are rich of several nutritive materials such as carotenoids (e.g. lycopene), phenolics, vitamin C, carbohydrates, proteins, fats and potassium (Causse et al., 2003 and Talvas et al., 2010). Ripe tomato fruits contain approximately 94% of water, 4.3% carbohydrates, 1% protein, 0.1% fat, 0.6% fiber and vitamins (Asan & Ekmekçi, 2002 and Samuel & Orji, 2015). This indicated that they are suitable to mechanical injury at postharvest handling and practices of storage, transportation and marketing which all affecting the quality of tomato fruit (Sajad & Jamaluddin Abid, 2017). Moreover, these physical damages coupled with the large water content of tomatoes make them more susceptible to spoilage by fungi (Asan & Ekmekçi, 2002 and Samuel & Orji, 2015). Several fungal species are responsible of tomato fruit rots such as *Geotrichum candidum*, *Rhizopus stolonifer*, black mold rot caused by *Alternaria* sp., *Fusarium* rot by *Fusarium* sp. (Sajad & Jamaluddin Abid, 2017). The black mold (*Alternaria* rot) is caused by the dematiaceous fungus, *A. alternata* and causing huge postharvest losses in tomato fruit at marketing period (Troncoso-Rojas & Tiznado-Hernández, 2014).

Chemical control using fungicides is effective in reduction of many postharvest diseases in various fruit and vegetables (Spotts & Cervantes, 1986). But, the use of these synthetic chemicals becomes restricted, due to their dangerous effects where lots of them are carcinogenic and highly toxic to human. Also, they have long degradation periods and causing environmental pollution

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(Lingk, 1991). Because of the problems caused by chemical fungicides, many alternative controlling methods of postharvest diseases are developed. In the last decades, many researchers interested in production of antimicrobial films which are used for packaging of food products and saving them from microbial contamination (Cha & Chinnan, 2004).

Microbial degradation of insoluble macromolecules lignin, cellulose and chitin depends on the production of extracellular enzymes. Cellulases are produced by several microorganisms such as bacteria, yeast and fungi. However, the most extensively studied cellulases are those produced by efficient lignocellulose degrading fungi, particularly *Trichoderma* (Henrissat et al., 1985). Also, chitinases can hydrolyze the cell walls of many fungi. The microorganisms that can produce these enzymes are able to destroy the cell wall of many pathogenic fungi for nutrition purpose. Some antagonistic fungi such as *Trichoderma* can attack several plant pathogenic fungi by mycoparasitism as a result of chitinase production (Budi et al., 2000 and Papavizas, 1985).

Glucose oxidase appears to be an important source of H_2O_2 in ligninolytic cultures. Glucose supports the highest level of H_2O_2 production in cell extracts. Both glucose oxidase and ligninolytic activities were shown to be secondary metabolic events, and both are important in a combination for lignin degradation as a mean of attack by antagonistic fungi. The ability of fungal glucose oxidase to utilize monosaccharides as a substrate may allow the organism to utilize sugars derived not only from cellulose but also from hemicelluloses found in woody material, its natural habitat, to produce H_2O_2, which is known to be important to the ligninolytic system and act as an efficient strategy for antagonistic microbial interactions (Kelley & Reddy, 1986).

The present study was performed due to the great importance of tomato fruits for human consumption in Egypt, and the need for safe preservative methods for them. Accordingly, the present study aimed firstly to produce an antimicrobial film from the polyester cloth amended with some enzymes (cellulase, chitinase and glucose oxidase) of the common antagonist *T. harzianum.* Secondly, to test the activity of these antimicrobial polyester films to protect the tomato fruit against *Alternaria* black rot postharvest disease.

Materials and Methods

Tested fungi

Culture of *T. harzianum* was purchased from the mycological center, Assiut University, Egypt. While the postharvest pathogenic fungus, *A. alternata* was isolated from spoiled tomatoes. All the cultures were sub-cultured and maintained on potato dextrose agar medium (PDA) at 4°C for further work.

Used media

Potato dextrose agar medium (PDA)

This medium was prepared using the method found in Moubasher (1993) using the following components (g L^{-1}), potato tubers, 200; D-Glucose, 20. Potatoes were cut after peeling to small cubes and boiled in 1L distilled water for 1 h. The filtrate was collected after filtration of potatoes through a cloth piece and thereafter glucose was added. The mixture volume was adjusted to one liter with distilled water. Chloramphenicol antibiotic (0.05%) was added to prevent bacterial contamination and agar was added for solidification (2%). Then, the medium was sterilized in autoclave and stored for further work.

Czapek's dox agar medium (CZA)

Czapek's dox agar medium (CZA) was used in the enzymes tests for the growth of *T. harzianum*. The medium has the following composition (g L^{-1}); sucrose, 30; sodium Nitrate ($NaNO_3$), 2; potassium dihydrogen orthophosphate (KH_2PO_4), 1; magnesium sulphate ($MgSO_4$), 0.5; potassium chloride (KCl), 0.5; ferrous sulphate ($FeSO_4$), 0.01 and agar, 20 (Moubasher, 1993).

Antifungal activity of T. harzianum against A. alternata in vitro

The activity of *T. harzianum* in inhibiting the postharvest pathogen, *A. alternata* was studied using a modified dual culture method adopted by Jamdar et al. (2013). PDA plate was inoculated with 8mm disc of *A. alternata*, 10mm from the edge of the plate. Then, 8mm disc of *T. harzianum* was cut by sterile cork borer, and then placed in the same plate 60mm far from *A. alternata* disc. Three replicates were performed. The inoculated plates were incubated in dark at 27±2°C for 4 days in a static incubator. Control plates were inoculated with *A. alternata* only. After the incubation period,

the mean diameter of the pathogen growth in dual culture plates was calculated and compared to the control plates. The percentage growth inhibition (%) was calculated (Vincent, 1947 and Jayasinghe & Wijesundera, 1995):

$$I = (C - T)/C \times 100$$

where: I= inhibition percentage, C= control growth diameter and T= treatment growth diameter.

The antagonistic mechanisms and hyphal interactions between *T. harzianum* against *A. alternata* were investigated microscopically and photographed.

Quantitative colorimetric assay of offensive enzymes of T. harzianum

Preparation of T. harzianum pure liquid culture

Pure liquid cultures of *T. harzianum* was prepared in flasks containing sterilized liquid Czapek's dox agar medium, modified by changing the carbon source to induce the desired hydrolytic enzyme. Carboxymethyl cellulose was used as a sole carbon source for cellulase production; while, colloidal chitin was used for chitinase and glucose for glucose oxidase. All the flasks were incubated at 27±2°C for 4 days on a shaking incubator (80 rpm) to get the maximum enzyme production during the exponential growth phase.

Extraction of intracellular glucose oxidase enzyme

The cultivated fungal mycelium of *T. harzianum* was filtered and then blended in an ice box to extract intracellular enzymes, and the filtrate was pre-cooled and centrifuged at 6000 rpm for 15 min (Kim et al., 1990). The supernatant was separated from the mycelium pellet through Whatman no.1 filter paper. The clear supernatant was considered as the crude enzyme and stored at 4°C for both polyester cloth saturation and for glucose oxidase assay.

Stability test for all enzymes in the collected filtrates

To ensure the stability of the tested enzymes at room temperature in their suspensions, the collected crude enzyme filtrates (both extra and intra cellular) were stored at room temperature (≈25°C) and their activities were assayed after regular intervals (0, 6, 12 and 24 h.).

Cellulase activity: Total extracellular cellulase activity was determined by a modified method adopted by Mandels et al. (1976). An aliquot of 0.5 ml of cell-free culture supernatant was transferred to a clean test tube and 1ml of sodium citrate buffer (pH 4.8) was added. 0.5ml of 1% (w/v) carboxy methyl cellulose (CMC) was added to each tube as a substrate. Tubes were incubated in a water bath at 50°C for 1h followed by an addition of 3ml of di nitro salicylic acid reagent (DNSA). Tubes were then placed in a boiling water bath for 5 min and then in an ice-bath, followed by the addition of 15ml distilled water to each tube. Contents of the tube were mixed, and absorbance was noted at 550 nm and compared with a standard curve of known glucose concentrations. Enzyme activity was determined in terms of International Unit (Tanaka et al., 1988) which is defined as an amount of enzyme that produces 1 μM of glucose per minute under standard assay conditions.

Chitinase activity: Total extracellular chitinase activity was determined by estimating the amount of free reducing groups formed after colloidal chitin hydrolysis (Joshi et al., 1988). The reaction mixture was composed of 0.5ml of 1% colloidal chitin suspended in 0.02 M phosphate buffer (pH=7) and 0.5ml of the enzyme solution (cell-free culture filtrate). The mixture was incubated for 30 min at 40°C, 0.75ml of DNSA was added, the suspension was heated for 10 min at 100°C and then centrifuged at 5000 rpm for 10 min. The absorbance of supernatant was measured at 530nm. A standard curve was obtained using N-acetylglucosamine with known concentrations. One unit of chitinase was defined as the amount of enzyme which yields 1 μmol of reducing sugar as N-acetylglucosamine equivalent per minute.

Glucose oxidase activity: Glucose oxidase intracellular activity in the crude enzyme extract was determined by spectrophotometer method at 460nm using glucose as a substrate (Reese & Mandels, 1959). 0.5ml of 1% glucose dissolved in 0.02 M phosphate buffer (pH=7) and 0.5ml of the enzyme solution (cell extract supernatant), then the reaction was allowed for 10 min. The absorbance of the mixture was recorded before and after the reaction to determine the amount of remnant reducing sugar released in the supernatant, compared with a standard curve of N-acetylglucosamine..

Preparation of antimicrobial polyester films saturated with crude enzymes extracts of T. harzianum:

Pieces (15 x 15 cm) of white unstained clean 100% polyester cloth were cut, washed twice by distilled water and soaked in distilled water in conical flasks to be sterilized in autoclave at 121°C, 1.5 atm. for 15 min then cooled at room temperature. The sterile polyester cloth pieces were soaked separately in the previously stored flasks of crude enzymes extracts (extracellular cellulase, chitinase cell-free culture filtrates and the supernatant of intracellular glucose oxidase) overnight at 4°C. Then representative enzyme-saturated polyester films were assayed for different enzyme activities to ensure their stability; while other pieces were involved in antimicrobial tests against tomato black mold incidence as follows.

Antimicrobial activity of enzyme-saturated polyester films on tomato fruit and black mold incidence

Intact tomato fruits (55±10 g) were selected, cleaned and washed by tap water then by distilled water. The tomato fruits were distributed separately in petri dishes. The experiment was designated as follow where each treatment was performed in triplicate:

General control

The fruits were stored without any treatments.

Infected control

A small hole (1 x1 cm) was done in each fruit by sterilized scalpel, then inoculated by fungal disc (8mm) of *A. alternata*.

Polyester films

The fruits were infected as previous then each fruit covered completely by the polyester film amended by glucose oxidase, chitinase and cellulase enzymes separately. (The steps of infection and covering the tomato fruits by polyester films were shown in Photo 1)

Control of polyester films

This was designated to test the effect of the polyester films amended by crude enzymes extracts on the tomato fruits themselves. Three straight wounds (about 10mm) were done on each fruit by sterilized scalpel then covered by polyester films amended with distilled water and crude enzyme extracts separately.

All the fruits were stored up to 15 days in dark at room temperatures (Max: 26°C, Min: 14°C). The disease incidence was calculated as mentioned in Aborisade & Olusola (2016) after 4, 7, 10 and 15 days as follow:

$$\text{Disease incidence} = N/3 \times 100$$

where; N= number of fruits with spoiled lesions surrounded the inoculated fungal disc and 3 is the total number of inoculated fruits.

Also, the activity of the prepared films in reduction of fungal growth of *A. alternata* and spoiled lesions area on tomato fruits was calculated using the percentage growth inhibition (I %) according to the infected control.

Photo.1. Steps of infection and covering the tomato fruit by polyester films.

Statistical analysis

Statistical analysis of the present study was conducted, using the mean, standard deviation and analysis of variance "ANOVA" using Microsoft Excel 2016 programme and the online free source; Free Statistics Calculators version 4.0.

Results

Antifungal activity of T. harzianum against A. alternata in vitro

In vitro investigations of the dual culture of *T. harzianum* against *A. alternata* revealed that, the growth of *A. alternata* was inhibited (I = 66.5%) as a result of the growth of *T.harzianum.* Photo 2 shows the over growth of *T. harzianum* mycelia on the mycelial growth of *A. alternata*. Moreover, Photo 3 illustrated the hyphal interaction mechanisms between *T. harzianum* and *A. alternata.* The hyphae of *T. harzianum* penetrated inside the hyphae of *A. alternata* leading to their lysis (Photo 3A), also, the conidial shape of *A. alternata* was malformed (Photo 3B). This lysis of hyphal cells of *A. alternata* might be due to the secretion of lytic enzymes of *T. harzianum*. Accordingly, the presence and activity of some offensive lytic enzymes (cellulase, chitinase and glucose oxidase) were assayed for the tested antagonist, *T. harzianum*.

Stability test for all enzymes in the collected filtrates and polyester films

The antagonistic fungus, *T. harzianum* possesses an observable enzymatic activity as a tool for attacking other pathogenic fungi, that was evaluated in the present study. Cellulase activity was recorded in Table 1 as it started with 14.14 u ml^{-1} in the crude culture filtrate, which was significantly decreased by time to reach 3.79 u ml^{-1} after stored for 24 h. This indicating the loss of enzyme activity from 100% to 27% during storage at room temperature. Also, the activity of chitinase enzyme of *T. harzianum* was recorded in Table 2 where it started with 4.72 u ml^{-1} in the crude culture filtrate, and significantly decreased by time to reach 1.65 u ml^{-1} after 24 h. This means that, the enzyme activity was decreased from 100% to 35% during storage at room temperature. Similar observations were considered for glucose oxidaseas an important tool in fungal cell metabolism and ligninolytic systems of *T. harzianum*. The activity of glucose oxidase was recorded in Table 3 as it started with 8.35 u ml^{-1} in the crude culture filtrate, which was significantly decreased by time to reach 2.13 u ml^{-1} after 24 h. This revealed the loss of enzyme activity from 100% to 35% during storage period at room temperature. A promising observation was recorded for all the tested enzymes where their stability was increased with immobilization of each enzyme separately within polyester cloth pieces at room temperature. The enzyme activity was maintained up to 93%, 91% and 90% for cellulase, chitinase and glucose oxidase after stored to 24 h, respectively.

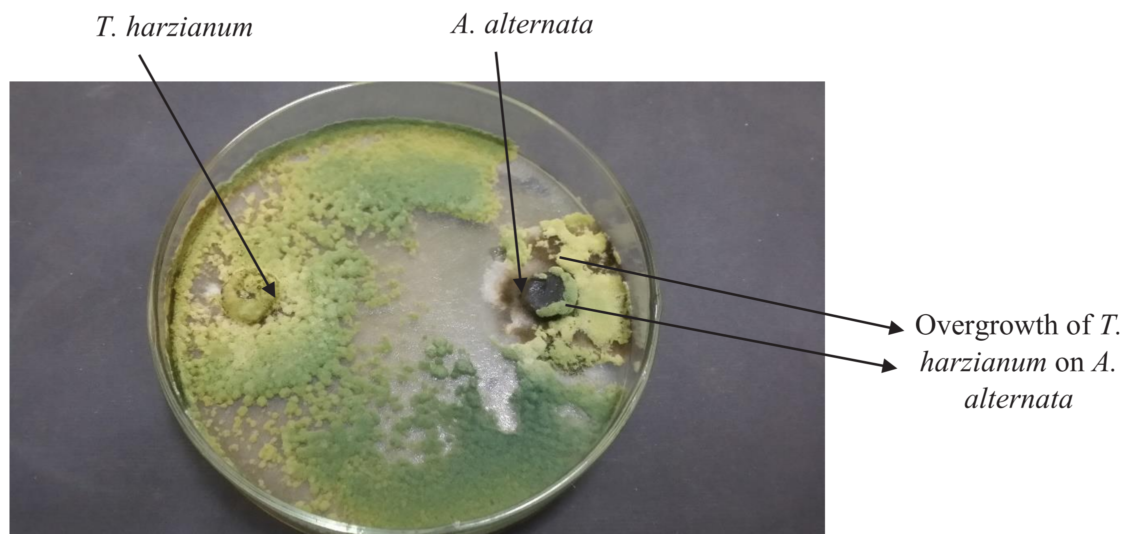

Photo 2. Dual cultute between *T. harzianum* and *A. alternata*.

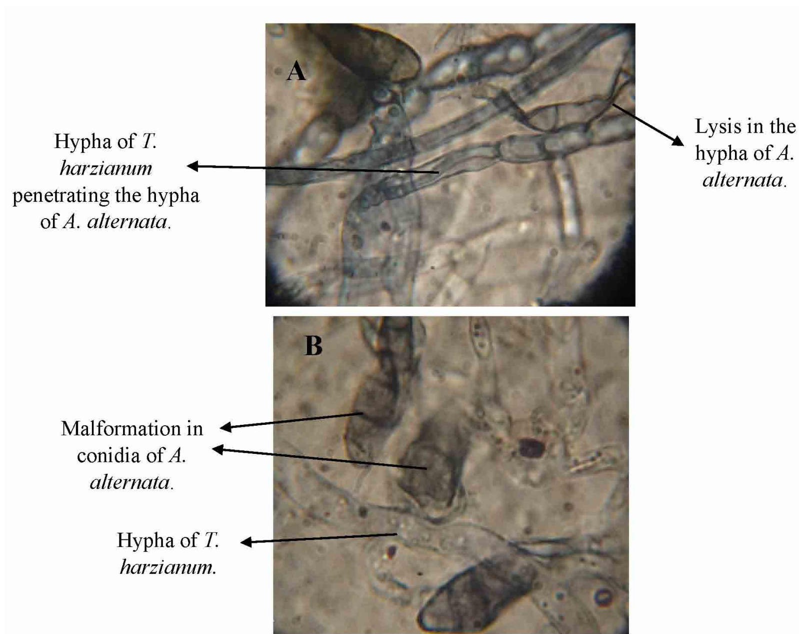

Photo 3. Hyphal interactions between *T. harzianum* and *A. alternata.*

TABLE 1. Extracellular cellulase activity of *T. harzianum* and its stability in crude filtrates and saturated polyester films.

Treatment	Value	Cellulase activity (u ml^{-1}± SD) after regular storage intervals (h)				ANOVA	
		0	6	12	24	F-value	P
Crude cell-free filtrate	Enzyme activity	14.14±0.31	11.88±0.27	8.31±0.22	3.79±0.11	1066.851	0.000*
	% Remaining activity	100	84	59	27		
Immobilized enzyme-saturated polyester films	Enzyme activity	3.75±0.12	3.63±0.13	3.60±0.12	3.48±0.09	2.743	0.113
	% Remaining activity	100	97	96	93		

TABLE 2. Extracellular chitinase activity of *T. harzianum* and its stability in crude filtrates and saturated polyester films.

Treatment	Value	Chitinase activity (u ml^{-1}± SD) after regular storage intervals (h)				ANOVA	
		0.0	6	12	24	F-value	P
Crude cell-free filtrate	Enzyme activity	4.72±0.26	3.55±0.23	2.28±0.18	1.65±0.12	133.695	0.000*
	% Remaining activity	100	75	48	35		
Immobilized enzyme-saturated polyester films	Enzyme activity	1.62±0.09	1.58±0.09	1.53±0.08	1.47±0.08	1.738	0.236
	% Remaining activity	100	98	94	91		

SD: Standard deviation & P: probability
*: Significant at P ≤ 0.05

TABLE 3. Extracellular glucose oxidase activity of *T. harzianum* and its stability in crude filtrates and saturated polyester films.

Treatment	Value	Glucose oxidase activity (u ml^{-1}± SD) after regular storage intervals (h)				ANOVA	
		0.0	6	12	24	F-value	P
Crude cell-free filtrate	Enzyme activity	8.53±0.24	6.64±0.22	3.24±0.16	2.13±0.12	723.619	0.000*
	% Remaining activity	100	78	38	25		
Immobilized enzyme-saturated polyester films	Enzyme activity	2.13±0.17	2.04±0.12	1.99±0.11	1.91±0.11	1.510	0.285
	% Remaining activity	100	96	93	90		

SD: Standard deviation & P: probability
*: Highly significant at P ≤ 0.05

Antimicrobial activity of enzyme-saturated polyester films on tomato fruit and black mold incidence

All the general control fruits were intact all over the experiment time but some of them began to normally spoiled and attacked by normal postharvest infections after 10 and 15 days where the postharvest infections incidence was 33.33 and 66.66%, respectively (Photo 5 A & B). The results in Table 4 and Photos 4 & 5 illustrated the activity of the polyester films immobilized with crude enzymes extract of (glucose oxidase, chitinase and cellulase) of *T. harzianum* on the growth of *A. alternata* and the development of black rot disease on tomato fruit after 4, 7, 10 and 15 days. Films with crude extract of glucose oxidase enzyme revealed negative results in inhibition of fungal growth and disease incidence related to the infected control. The disease incidence was 100% and the inhibition percentage of *A. alternata* was -6.16 after 4 days. This means that the presence of glucose oxidase film increases the fungal growth and spoilage of tomato fruit tissues more than the infected control.

The results also revealed that, the polyester film immobilized with crude extract of cellulase enzyme recorded the best results in protecting the fruit from black mold incidence. The growth of *A. alternata* was limited only inside the holes made on tomato fruit without spoiling of the surrounded tissues after stored for 4 and 7 days (Photo 4 A & B). This result indicated that the immobilized cellulase enzyme affected only the pathogen and in the same time did not degrade the tomato tissues. Therefore, the inhibition percentage was 45.72 and 46.06% after 4 and 7 days while, the disease incidence was 0% after the two periods. The above results were illustrated according to the fact revealed that, cellulose and hemicellulose of normal tomatoes decreased during ripening (Huber, 1983 and Lunn et al., 2013). Consequently, by increasing storage period, the disease incidence began to appear where it became 33.3 and 100% after 10 and 15 days, respectively (Photo 5: A & B). In the meantime, film of chitinase enzyme gave small values of inhibition percentage (2.22 and 1.34%) after 4 and 7 days, respectively with disease incidence equal 66.66%. Then the disease incidence became 100% after 10 days with negative inhibition percentage (-30%) where the fungal growth and spoiled area on tomato fruit was increased more than the infected control.

The effect of polyester films immobilized by enzymes of *T. harzianum* on wounded tomato fruit was shown in Photo.6. Control films were immobilized by distilled water. The obtained results indicated that, the used films did not affect the intact part of each tomato fruit while the wounded parts were more suitable to spoiling and infections by increasing storage period. Cellulase enzyme films protected the wounded fruit from infections for long period than the other films where the fruit remain intact, but somewhat shrinkage, to 10 days storage.

TABLE 4. Effect of enzyme polyester films on the development of black rot on tomato fruit inoculated with *A. alternata.*

Enzyme-polyester Film	I (%) after period (days)				Disease incidence (%) after period (days)			
	4	7	10	15	4	7	10	15
Glucose oxidase	- 6.16	- 3.6	- 9.7	- 40.42	100	100	100	100
Chitinase	2.22	1.34	- 30	- 4.3	66.66	66.66	100	100
Cellulase	45.72	46.06	56.96	18.19	0	0	33.33	100

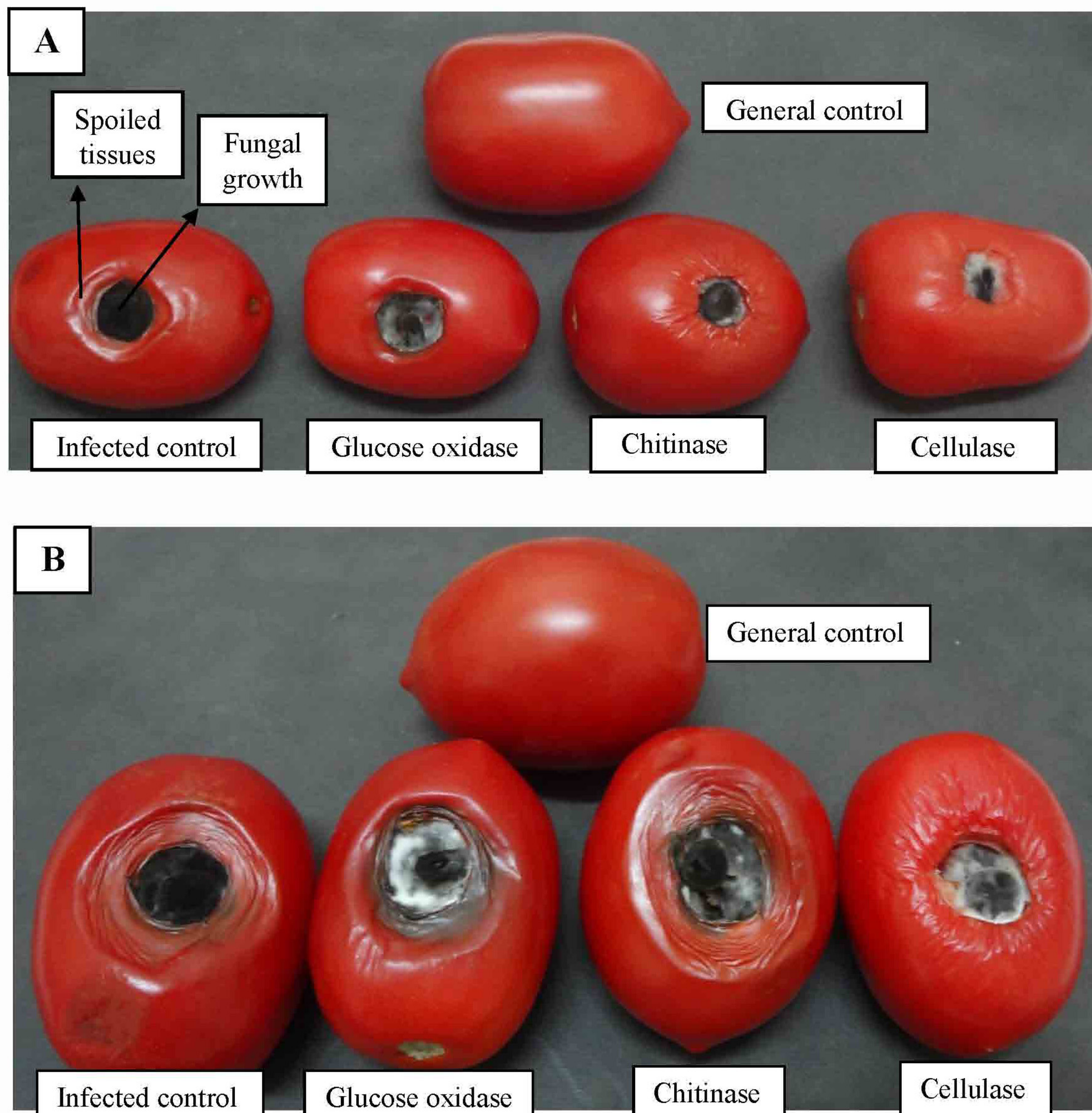

Photo 4.Effect of polyester films of enzymes of *T. harzianum* on the growth of *A. alternata* and black rot incidence on tomato fruit after 4 days (A) and 7 days (B).

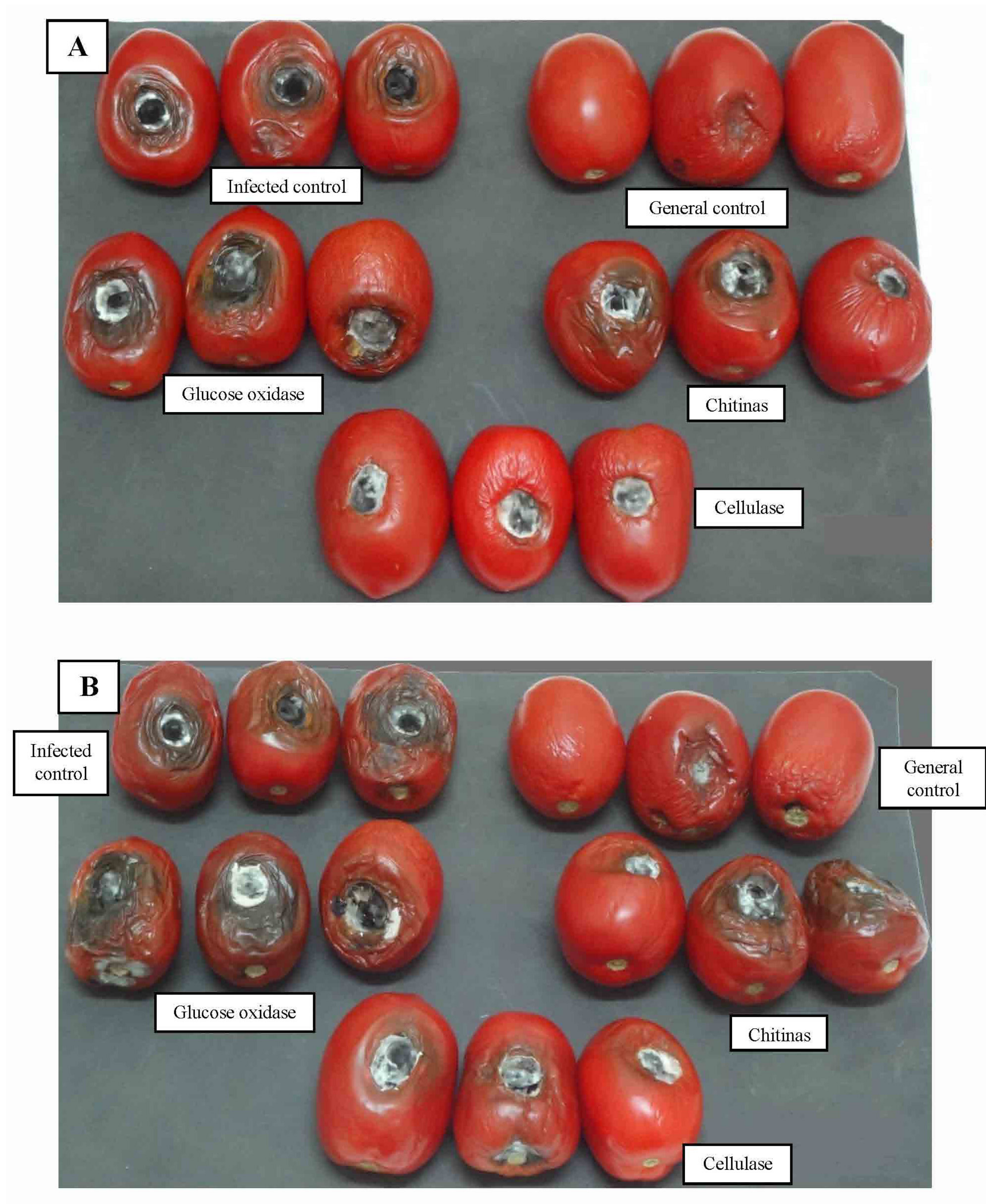

Photo 5. Effect of polyester films of enzymes of *T. harzianum* on the growth of *A. alternata* and black rot incidence on tomato fruit after 10 days (A) and 15 days (B).

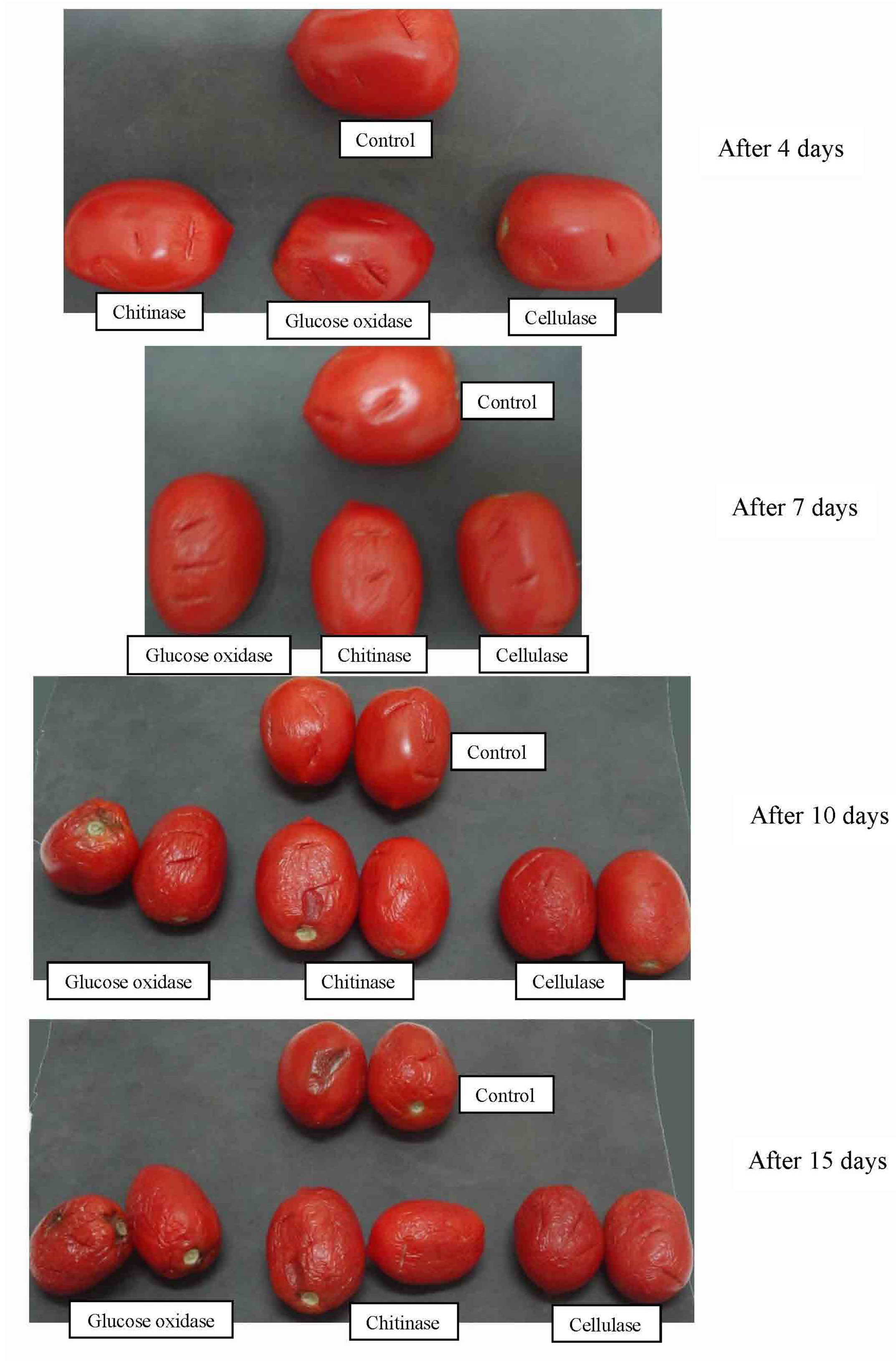

Photo 6. Effect of polyester films of enzymes of *T. harzianum* on wounded tomato fruit.

Discussion

The present findings represent that *T. harzianum* recorded an antifungal activity against *A. alternata* in dual culture using mycoparasitism and antibiosis mechanisms. This is in agreement with a previous study of the second author (El-Debaiky, 2017) who examined the activity of *T. harzianum* on the growth of other isolate of *A. alternata* in paired plates and found that the inhibition percentage was 61.58%. In addition, she reported that *T. harzianum* attacked the hyphae of *A. alternata* by coiling around them leading to their lysis.

The immobilized enzymes of *T. harzianum* on polyester films in this study show promising stability than in free-cell filtrate. These findings agree with other studies which revealed that, immobilization of the enzyme to a matrix prevents excessive loss of enzyme activity after immobilization, increases enzyme stability in microenvironment of matrix and protects enzyme from microbial contamination (Cabral & Kennedy, 1993). Also, the immobilization of amylases on different activated fabrics increases the enzymatic stability than the free enzyme (Rani, 2012). On the other hand, in the present work the enzymes immobilized films were tested against black mold incidence of tomato fruit and the results revealed that glucose oxidase enzyme recorded lowest results followed by chitinase then cellulase which was the best. These findings may be due to the formation of H_2O_2 in the oxidation process of glucose using glucose oxidase. Then, the formed H_2O_2 was used with the ligninolytic enzyme of the pathogen in degradation of lignin found in tomato fruit tissues (Zhao & Janse, 1996 and Eichlerová et al., 2006).

Also, the results of chitinase and cellulase showed an unexpected behavior of the enzymatic activity of *T. harzianum* against the hyphae of *A. alternata*. Where the cell walls of deuteromycetes did not contain cellulose but, the pathogen; *A. alternata* was markedly inhibited by cellulase enzyme of *T. harzianum* immobilized on the polyester cloth pieces after 4 and 7 days. These interesting findings may be illustrated according to other literatures. The cell walls of *Alternaria* sp. consists mainly from glucuronic acid which is characteristic to plant cell wall hemicelluloses (Gancedo et al., 1966). The glucuronic acid is closely related to cellulose where it contains β (1-4) linkage (Tavernier et al., 2008) also, poly-glucuronic acid can be produced by selective oxidation of cellulose using nitrogen oxides (Yackel & Kenyon, 1942 and Maurer & Reiff, 1943). While, the production and antifungal activity of chitinase enzyme of *T. harzianum* may be suppressed or activated by many compounds produced during the relationship among the pathogen, the antagonist and the host plant (Ulhoa & Peberdy, 1991). Accordingly, the chitinolytic antifungal activity of *T. harzianum* against *A. alternata* was found to be unexpectedly moderate than the cellulolytic activity.

Conclusion

The antagonist, *T. harzianum* was tested for its antifungal activity against *A. alternata*, the causal agent of postharvest black mold of tomato fruit. It inhibited the mycelial growth of *A. alternata* by attacking its mycelia using the mycoparasitism, antibiosis and lytic enzymes mechanisms. Polyester film immobilization of *T. harzianum* enzymes (cellulase, chitinase and glucose oxidase) raised their stability and persistence at room temperature. These enzymes of *T. harzianum* immobilized on polyester cloth films were tested for their activity in preserving tomato fruit against spoilage by black mold. Cellulase followed by chitinase showed observable antifungal activity against *A. alternata* and protecting the tomato fruit from black mold incidence for prolonged time whenever they were contact without wounds. This study is considered a base for further studies in food protecting technology filed using the immobilized enzymes on polyester cloth films in packing fruit and other foods in storage.

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تقييم النشاط ضد الميكروبى لبعض إنزيمات الترايكوديرما هارزيانم المحملة على قطع من نسيج البولى إيستر، على مدى إنتشار مرض العفن الأسود للطماطم ما بعد الحصاد

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فى الدراسة الحالية تم إختبار نشاط بعض إنزيمات الترايكوديرما هارزيانم فى مستخلصها الخام والمحملة على قطع من نسيج البولي إيستر لمقاومة العفن الأسود الذى يسببه فطرالألترناريا ألترناتا لثمار الطماطم ما بعد حصادها. بدايةً تم إختبار نشاط الترايكوديرما هارزيانم ضد الألترناريا ألترناتا بطريقة المزارع المزدوجة على أطباق الوسط الغذائى المحضر من مستخلص البطاطس، حيث تم تثبيط نمو الألترناريا ألترناتا بنسبة 66.5%. باستكشاف التداخل الخيطى لكلا الفطرين مجهرياً وجد أن خيوط الترايكوديرما هارزيانم قد إخترقت خيوط الألترناريا ألترناتا وحللتها. ويعود تحلل الخلايا الممرضة لوجود إنزيمات التحلل المائى للسليلوز و الكايتين وإنزيمات أكسدة الجلوكوز لدى فطر الترايكوديرما هارزيانم. حيث تم تقدير هذه الإنزيمات كمياً وتثبيتها بشكل منفرد على قطع مستقله من نسيج البولى إيستر، ثم تحدد نشاطها ومدى ثبوتها فى كل من قطع النسيج المشبعة ومستخلص المزارع الخام الخالى من الخلايا. بشكل واعد لوحظ أن تثبيت الإنزيمات على قطع نسيج البولى إيستر قد زاد من معدل بقائها، كما زاد من فعاليتها ضد مرض العفن الأسود لثمار الطماطم ما بعد الحصاد وذلك عند تخزينها مغلفة فى درجة حرارة الغرفة لمدة تصل إلى 15 يوماً. وبصفة خاصة أظهرت القطع المشبعة بإنزيم التحلل المائى للسليلوز كفاءة واعدة فى منع ظهور المرض نهائياً لمدة من 4 إلى 7 أيام، بينما ظهرت العدوى فقط فى 33.3% من الثمار بعد 10 أيام، و أصيبت 100% من الثمار بعد 15 يوم.