

## Studies on Postharvest Diseases of Apple Fruits in El-Jabal Al-Akhder, Libya

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### ABSTRACT

A survey of apple fruits postharvest diseases in five locations of El-Jabal Al-Akhder, Libya, Between latitudes 32 and 33 North, and longitudes 20 and 23 East, at different periods during the growing season 2009 and 2010, revealed that the most prevalent postharvest apple pathogens were *Alternaria alternata* (Cke) Weber, *Aspergillus flavus* Linkx Gray, *Aspergillus niger*, *Penicillium expansum* (Link) Thom, *Botrytis cinerea* Press and *Rhizopus stolonifer*. *Alternaria alternata* proved to be the most prevalent and virulent; whereas, *Rhizopus stolonifer* and *Aspergillus niger* were the least virulent. Inoculation of the three apple varieties, i.e., Golden Delicious, Starkremson and Red Delicious, by the isolated fungi revealed that the highest infection percentages were obtained by *P. expansum*, *B. cinerea* and *A. alternata*, while differences in infection % among *R. stolonifer*, *A. flavus* and *A. niger* and control were insignificant. The antagonistic effect of two fungal (*Trichoderma harzianum* and *T. viride*) and two bacterial (*Pseudomonas fluorescens* and *Bacillus mycoides*) as biological control agents were tested against *P. expansum*, *B. cinerea* and *A. alternata* fruit rot pathogens. *T. harzianum* realized the highest inhibition%, compared with *T. viride*. Significant growth reduction was obtained by all the tested bacterial bioagents. Higher antagonistic effect was exerted by *B. mycoides* against *A. alternata* and *P. fluorescens* against *P. expansum*, whereas, *B. subtilis* had the lowest antagonistic values.

Keywords: Apple, Postharvest Diseases, El-Jabal Al-Akhder, biological control agents, A survey.

### INTRODUCTION

Apple trees are the most important fruit trees cultivated in El-Jabal Al-Akhder district, Libya, Between latitudes 32 and 33 North, and longitudes 20 and 23 East, characterized by cold to moderate climate suitable for cultivation of apple trees. Special attention was given by The Lybian Ministry of Agriculture to this region, where different varieties of apple were introduced from different countries since 1976, including France, Belgium, Turkey, Greece, Morocco and Yugoslavia. Cultivation was carried out in different regions of El-Jabal Al-Akhder, especially in Elguba, Elbiada and Elmarj. Two and half million trees of different apple varieties, i.e., Golden delicious, Red delicious, Star kremson, Stark delicious, Anna, Jonathan, Gold star, Ida red, Lorka, Gersy mac, Royle vala, Jona Good and Ain shimmer were cultivated until 2000 (FAO, 2004).

During period of growth, apple trees are subjected to the attack of many fungal, bacterial, nematodal and viral diseases. Many of these diseases attack apple fruits on mother trees or during storage (Fatima et

*al.*, 2009), causing great Losses in fruit yield (5-50%) and quality (Kader, 2005 and Agrios, 2005). Degradation forms include undesirable alterations in shape and pigmentation, and failure to ripen (Snowdon, 1992). Postharvest fungal pathogens cause the major losses in apple production (Spadaro *et al.*, 2004).

More than 90 fungal species have been described that cause decay of apples during growth, storage and transportation, including *A. alternata*, *R. stolonifer*, *Monilinia fructicola*, *Glomerella cingulata*, *Mucor piriformis*, *Botryosphaeria ribis*, *Botryosphaeria obtuse*, *Aspergillus sp.*, *Fusarium sp.*, *Pezizula malicorticis* and *B. cinerea* (Spotts, 1990). The relative importance of each pathogen depends on climatic and storage conditions.

Control of apple rot diseases had been accomplished primarily by the application of chemical fungicides. However, chemical control by fungicides is not economical and had met moderate success and their future use is in question due to increased regulatory restrictions. Biological control agents have been developed in recent years as successful and safe mean of controlling many diseases; however a few have actually been registered for use on fruit crops. The most commonly applied are *B. subtilis*, *P. syringae*, *B. pumilus* and *P. fluorescens* are common bacterial bioagents that found to control many postharvest diseases, i.e., peach brown rot, blue and gray mold of pome fruits and *B. cinerea* in field trials of strawberry (Janisiewicz and Marchi, 1992; Janisiewicz *et al.*, 2000; El-Ghaouth *et al.*, 2004; Demoz and Korsten, 2006 and Errampalli, 2007).

So, the main aims of this study were to isolate and identify the most important postharvest diseases of apple in El-Jabal Al-Akhder of Libya and the use of different biological agents *in vitro* and *in vivo* as an alternative to the use of chemical fungicides.

## **MATERIALS AND METHODS**

### **1. Pathogenicity experiments**

#### **1.1. Collection of samples**

Diseased apple fruits were collected from different fruit markets and orchards in Libya, particularly El-Jabal Al-Akhder regions at different periods during the growing season 2009 and 2010. Diseased fruits were sampled individually in separate clean labeled polyethylene bags and kept at low temperature for isolation purposes and further studies. Samples (50 fruits) with three replicates were taken at random for the test of frequency and occurrence of fruit-decaying fungi.

#### **1.2. Isolation of fungi from diseased fruit tissues**

Diseased apple fruits were rinsed several times in sterilized distilled

water, surface sterilized using 70% ethyl alcohol for 2 minutes, dried between sterilized filter paper and then cut with the help of a sharp razor into small parts each one containing diseased part with adjacent healthy tissues and transferred onto potato dextrose agar medium (PDA) plates containing streptomycin (0.2 g/L). Plates were then incubated for 5 days at  $25\pm 2^{\circ}\text{C}$  under 12 hours light and dark conditions.

### 1.3. Purification and identification

Developing fungal isolates were picked out, subcultured several times until growth looked homogenous. Subsequent purification was carried out using hyphal tip technique (Tuite, 1969). Purified isolates were maintained on PDA slants and kept in refrigerator at  $5^{\circ}\text{C}$ . Pure cultures of the obtained isolates were identified in laboratory on the basis of cultural and microscopic characteristics according to Talbot, (1971), Alexopoulos and Mims, (1979), Domsch *et al.* (1980), Webster (1991) and Barnett and Hunter, (1998). Isolates were checked and confirmed by Prof. El-Samra and Prof. M. Amer, Laboratory of Plant Pathology, Agricultural Botany Department, Faculty of Agriculture (Saba-Basha), Alexandria University, Egypt.

### 1.4. Inoculation trials

Apparently healthy and uniform mature apple fruits were carefully chosen during the season to be used for pathogenicity tests of the isolated fungi. Fruits were submerged about 3 minutes in solution of 0.5% sodium hypochlorite containing 0.15% Tween 20 and allowed to air-dry on a laboratory bench.

The tested fungal isolates were cultivated on PDA medium in petri dishes and incubated at  $25\pm 2^{\circ}\text{C}$  for 3-7 days. Inoculum of each isolate was prepared by flooding the surface of culture, rubbed using sterilized glass rods, blending the suspension for 1 min, then filtering through eight layers of cheesecloth. The inoculum concentration was measured with a haemocytometer and diluted to appropriate concentration  $1\times 10^6$ .

Under aseptic conditions, one wound (2 mm deep and 2 mm wide) was made on each fruit with a sterile needle. Twenty microliters of the fungal suspension was applied to the wound of each wounded fruit. The test was done twice (2009 and 2010 season) with three replicates per treatment and four fruits per replicate. After inoculation, fruits were kept in polyethylene bags for 24 hrs. to ensure about 12 hrs wetness on the inoculation sites and then kept at room temperature for 5 days (Hong *et al.*, 1998).

### 1.5. Disease assessment

Disease development data were recorded daily for 5 successive days after inoculation by measuring lesion diameter and degree of infection. Severity of infection was estimated according to the numerical rates as follows:

- 0= no decay development
- 1= decay less than 1.0 cm in diameter without sporulation
- 2= decay between 1.0 cm and 2.5 cm in diameter with sporulation
- 3= decay between 2.5 cm and 4.0 cm in diameter with sporulation
- 4= decay between 4.0 cm and 6.0 cm in diameter with sporulation
- 5= fruit completely rotted and heavily covered with mycelium or decayed up to 6.0 cm

Severity degrees were converted to infection severity percentages according to Horsfall and Heuberger (1942) by using the following equation

$$\text{Degree of infection (\%)} = \frac{\text{sum of individual rating}}{\text{No. of fruits assessed}} \times \frac{100}{5}$$

## 2. Biological control experiments

Four biological control agents, two fungal isolates of *T. harzianum* and *T. viride* and two bacterial isolates of *P. fluorescense* and *B. mycoides*, were applied throughout the present study. Cultures were obtained from laboratory of Plant Pathology, Dept. of Agric. Botany, Faculty of Agriculture, Saba-Basha, Alexandria University.

### 2.1. *In vitro* experiments

Petri-dishes containing 15 ml sterile PDA-Medium (Potato-Dextrose-Agar) were used for the Bio-assay experiment. Four wells were cut in the outer area of the plate by using a sterile cork-borer (No. 5). The wells were filled with mycelial disk of *T. harzianum* and *T. viride* and a mycelial disk of the tested pathogen was placed in the middle of the plate. All plates were held in an incubator at 28°C. Four replicates were observed for each pathogen. Wells filled with sterile PDA disks served as control. Observations were recorded daily for seven days by measuring the diameter of the pathogen mycelial growth between the wells (Ippolito, *et al.*, 2000).

In order to determine the antagonistic effect of the tested bacterial bioagents against the isolated pathogens, dual culture method recommended by Jamalizadeh *et al.* (2008) was applied. Accordingly, bacterial suspension (0.1 ml;  $1.0 \times 10^9$  CFU  $\text{m}^{-1}$  culture) was streaked on plates and mycelial disk of the pathogen was then placed in the middle of the plate. After incubation at  $27 \pm 2^\circ\text{C}$  in the dark for 24 hrs, PDA inoculated with the pathogen alone was used as control. Plates were incubated at  $20^\circ\text{C}$  for 20 days, at which time colony diameters and inhibition zones were measured. Percentage growth inhibition was calculated using the formula  $n = (a - b) \times 100 / a$ , where n is the percentage growth inhibition; a, is the colony area of uninhibited fungus species, and b, is the colony area of treated fungi (Etebarian *et al.*, 2006).

## 2.2. *In vivo* experiments

The postharvest pathogens *P. expansum*, *B. cinerea* and *A. alternata*, two fungal biological agents (*T. harzianum* and *T. viride*) and two bacterial isolates (*P. fluorescens* and *B. mycoides*) were grown on 9 cm diameter plates containing PDA media in case of fungi and nutrient agar for bacteria (9 cm diameter) for 7 to 14 days. Conidia were harvested by pouring few millilitres of sterile distilled water containing 0.05% Tween 20 in the plates. The conidial suspension was adjusted with a haemocytometer to  $1.0 \times 10^5$  for the tested pathogens,  $1.0 \times 10^7$  conidia / ml for *Trichoderma* spp. and  $1.0 \times 10^8$  for the tested bacterial isolates. Apple fruits (Golden Delicious) that had not been treated with late-season fungicide applications, were used for laboratory tests. Fruits were surface-sterilized by soaking in 70% ethanol for 3 min and then wounded twice on the opposite sides of the portion between the equator and peduncle with a nail-like pointer (5 mm × 5 mm). Aliquots of 20 µl of conidial suspension of biological agents or sterile distal water were applied to each wound, after 4 hrs, 20 µl conidial suspension of pathogens was applied in to each wound.

The fruits were sealed in polyethylene-lined plastic boxes, and they were incubated at 20°C, in 80% humidity under a photoperiod of 12 h light and 12 h dark. Four fruits arranged in a randomized block design were used per each treatment. All assays were designed in random blocks, with three replicates (Vero *et al.*, 2002 and Jamalizadeh *et al.*, 2008)

The percentage of disease severity reduction (DSR%) was calculated by the equation:

$$DSR\% = \frac{DSc - DS_t}{DSc} \times 100$$

Where DSc= lesion area on the positive control (pathogen alone) and DS<sub>t</sub>= lesion area on the treated fruit (Benbow and Sugar, 1999).

## 3. Statistical analysis

The obtained data were subjected to analysis of variance and a sample means tested for significant differences LSD 0.05 using NCSS PASS 2000 (Gomez and Gomez, 1984).

## RESULTS AND DISCUSSION

### 1. Occurrence frequencies (OF) and degree of infection (DI)

The obtained data (Table1) showed significant differences in occurrence frequency values among studied areas and among the isolated fruit-decaying fungi. *A. alternata* proved to be the most prevalent fruit-

decaying fungi with frequency of disease occurrence (OF). The obtained data (Table 1) showed significant differences in OF values among studied areas and among the isolated fruit-decaying fungi. *A. alternata* proved to be the most prevalent fruit-decaying fungi with (OF) values ranging from 12.4 to 22.8%, followed by *P. expansum* (14.6-18.7%), *B. cinerea* (11.8-16.3), *R. stolonifer* (5.6-7.8%) and *A. niger* (3.6-6.4%), whereas, *A. flavus* presented the lowest OF values (3.5-5.4%). On the other hand data of DI values showed significant variation. *A. alternata* was the most virulent on the collected apple fruits with (DI) values range (17.4-22.3%) followed by *P. expansum* (5.9-15.2%), *B. cinerea* (8.4-12.8), *A. niger* (2.4-3.4%) and *A. flavus* (1.8-6.6%), whereas, the lowest (DI) values detected with *R. stolonifer* (1.7-5.4%). These results indicated that the most frequent isolated fungi in the period between harvest and storage are *A. alternata*, *P. expansum* and *B. cinerea*. These fungi were pathogenic at different degrees, these results were also recorded by many investigator (Bennett, 2005; Beever and Weeds, 2004; Janisiewicz and Korsten, 2002; Rizzolli, 2006; Moshe, 2006). According to the obtained data, *A. alternata*, *P. expansum* and *B. cinerea* proved to be the most virulent fruit-decaying fungi and were selected for their pathogenic capabilities on mature apple fruits of Golden Delicious, Red delicious and Star kremson cultivars.

## 2. Pathogenicity studies

### 2.1. Identification

Six different species of fungi were isolated from diseased apple fruits during 2009 and 2010 season tissues. Identification procedures were carried out According to Talbot (1971); Ellis (1971); Alexopoulos and Mims (1979); Domsch *et al.* (1980); Webster (1991); Barnett and Hunter (1998) and Bennett (2005). Identification was verified by Prof. I. El-Samra and Prof. M. Amer, Laboratory of plant Pathology, Agricultural Botany Dept., Faculty of Agric. (Saba-Basha), Alexandria University. Accordingly, the isolated fungi were identified as *P. expansum* (Link) Thom, *Botrytis cinerea* Press, *A. alternata* (Cke) Weber, *A. flavus* Linkex Gray, *A. niger* and *R. stolonifer* (Table 1).

### 2.2. Inoculation and pathogenicity tests

Data presented in Table 2 indicated that all the isolated apple fruit-decaying fungi were pathogenic to all the applied apple varieties. Moreover, *P. expansum*, *B. cinerea* and *A. alternata* were proved to be the most pathogenic, exhibiting the highest DI values (16.6% to 58.3%). On the other hand, Differences in DI values between the other tested fungi (*R. stolonifer*, *A. flavus* and *A. niger*) and control were insignificant. Results also showed significant differences in the mean DI percentages among cultivars. *A. alternata* gave the highest DI values in Golden Delicious (58.3%) While the minimum significant DI was obtained by *B. cinerea* in Red Delicious

(16.6%). These results were in agreement with those obtained by Fatima *et al.* (2009) who mentioned that *P. expansum*, *B. cinerea* and *A. alternata* are the most dangerous postharvest pathogens on pome fruits and she also noted a variation in susceptibility between the tested cultivars. Jorgensen *et al.* (2011) mentioned that *A. niger* is a weak parasite, whereas, it was reported that *P. expansum*, *A. alternata*, *B. cinerea* and *P. expansum* were the most prevalent postharvest pathogens on apple under storage conditions (Spadaro *et al.*, 2004; Reuveni, 2006 and Welke *et al.*, 2010).

## 1. Biological control

### 1.1. *In vitro*

Two fungal isolates (*T. harzianum* and *T. viride*) and three bacterial isolates; i.e., *P. fluorescens*, *B. mycoides* and *B. subtilis* were tested as biological control agents to determine their efficiency in reducing growth of the tested apple fruit rot decaying agents (Table 3 and 4). Table (3) showed that *B. mycoides* had significant effect on all the tested fruit-decaying fungi. Moreover, the highest antagonistic effect was obtained by *B. subtilis* on *A. alternata*, where the average radial growth was 1.62 cm. *P. fluorescens* had also significant effect on growth of all the tested fruit-decaying fungi; however, the highest antagonistic effect was noted on *P. expansum* (1.6 cm). *B. subtilis* showed little but significant antagonistic effect on the studied fruit-decaying fungi with average radial growth of *B. cinerea*, *A. alternata* and *P. expansum* 2.2, 2.1 and 4.1 cm, respectively.

Results in Table (4) indicated that the highest growth inhibition rates were obtained by *T. harzianum* biocontrol agent, where reduction rates in *B. cinerea*, *A. alternata* and *P. expansum* were 65.2, 65.7 and 71.87 %, respectively. *T. viride* was less antagonistic than *T. harzianum*, where reduction rates were 40.57, 20.9 and 21.4 %, respectively. It was reported that microbial antagonists produce lytic enzymes such as gluconase, chitinase, and proteinases that help in the cell wall degradation of the pathogenic fungi (Castoria *et al.*, 2001). Production of antibiotics is the second important mechanism by which microbial antagonists suppress the pathogens of harvested fruits and vegetables. For instance, bacterial antagonists like *B. subtilis* and *Pseudomonas cepacia* Burkh are known to kill pathogens by producing the antibiotic iturin (Spadaro *et al.*, 2004 and Vinas, 2004). The antagonism so produced by *B. subtilis* was effective in controlling fungal rot in avocado (Demoz and Korsten, 2006) and *M. fructicola* winter honey in peaches and cherries (Pusey, 1989). Furthermore, *P. fluorescens* inhibited the growth of postharvest pathogens like *B. cinerea* and *P. expansum* in apple by producing an antibiotic, pyrrolnitrin (Etebarian *et al.*, 2006).

### 1.2. *In vivo*

According to data in Table 5 and 6, reductions in disease incidence *in vivo* differed greatly according the applied biological control agent, pathogen,

cultivar and storage period. Treatment with *P. fluorescens* resulted in the least infection percentages in all treatments, ranging from 6.6 to 36.6% in Red Delicious and Golden Delicious inoculated with *A. alternata*. Significant reductions in disease incidence were also obtained by *B. mycooides* and *B. subtilis*; however, reduction rates were less than those of *P. fluorescens*.

These results were in agreement with those obtained by (Etebarian *et al.*, 2006), who reported that *P. fluorescens* was effective in controlling green mold (*P. digitatum*) in lemon (*Citrus limon* L.) due to production of specific antibiotics. It was reported that antibiosis might be an effective tool for controlling postharvest diseases in a few fruits and vegetables; however, at present emphasis is being given for the development of antibiotic producing microbial antagonists for the control of postharvest diseases of fruits and vegetables (El-Ghaouth *et al.*, 2004 and Singh and Sumbali, 2007). Researchers are aiming to isolate, evaluate or to develop those antagonistic microorganisms that control postharvest diseases of harvested commodities by the mechanism of competition for space and nutrient, direct parasitism or induced resistance (Droby, 2006).

Treatment with the fungal biological control agents *T. harzianum* and *T. viride* resulted in significant reductions in infection % in all the tested fruit-decaying fungi and cultivars (Table 6). *T. harzianum* gave the highest degree of reduction, where degree of infection range from 7.3% in case of infection of Red Delicious with *P. expansum* to 27.6% in the infection of Golden Delicious with *P. expansum*, while the infection of the same varieties with the same fungi was 31.6% and 13 % compared with control 55% and 36%, respectively. On the other hand Strains of *Trichoderma* sp. have been tested under field conditions for control of Grey mold rot caused by *B. cinerea*, *A. alternata* causing core rot of pome fruits and *P. expansum*, the causative fungus of blue mold on apples in previous studied. Batta (2003) showed that the application of formulated *T. harzianum* conidia inhibited *Botrytis* sporulation on the surface of typical *Botrytis* lesions.

Batta (2004) have been demonstrated the effect of *T. harzianum* on postharvest diseases which cause fruit rot, for example, significant curative and preventive effect was provided by the antagonistic strain of *T. harzianum* against *A. alternata* causing core rot of pome fruits (Reuveni, 2006). Another significant effect was obtained in controlling *P. expansum*, the causative fungus of blue mold on apples, through studying the effect of treatment with *T. harzianum* Rifai formulated in invert emulsion on postharvest decay of apple blue mold.

A significant amount of research on the use of the microbial antagonists has been reviewed by several workers (Droby, 2006; Janisiewicz *et al.*, 2000; El-Ghaouth *et al.*, 2004, Kota *et al.*, 2006.).



However, the mechanism(s) by which microbial antagonists exert their influence on the pathogens has not yet been fully understood. (Wilson and Pusey, 1985; Vinas *et al.*, 1998). Several modes of action have been suggested to explain the biocontrol activity of microbial antagonists). Still, competition for nutrient and space between the pathogen and the antagonist is considered as the major modes of action by which microbial agents control pathogens causing postharvest decay (Wilson *et al.*, 1987; Ippolito *et al.*, 2000; Jijakli *et al.*, 2001; Elmer *et al.* 2005). In addition, production of antibiotics (antibiosis), direct parasitism, and possibly induced resistance are other modes of action of the microbial antagonists by which they suppress the activity of postharvest pathogens on fruits and vegetables (Janisiewicz *et al.*, 2000; El-Ghaouth *et al.*, 2004).

## REFERENCES

- Agrios, G.N. (2005).** Plant Pathology, Academic Press, New York.
- Alexopoulos, C. J. and Mims, C. W. (1979).** Introductory Mycology. John Wiley and Sons, New York, Manchester, Brisbane, Toronto, 632 pp.
- Barnett, H. L. and Hunter, B. B. (1998).** Illustrated Genera of Imperfect Fungi. 4th ed. APS Press, St. Paul, Minnesota, pp. 218
- Batta, Y. A. (2003).** Postharvest biological control of apple gray mold by *Trichoderma harzianum* Rifai formulated in an invert emulsion. Crop Protection, 23: 19 – 26.
- Batta, Y. A. (2004).** Effect of treatment with *Trichoderma harzianum* Rifai formulated in invert emulsion on postharvest decay of apple bluemold. International Journal of Food Microbiology, 96: 281 – 288.
- Benbow, J. M. and Sugar, D. (1999).** Fruit surface colonization and biological control of postharvest diseases of pear by preharvest yeast applications. Plant Dis., 83: 839-844.
- Bennett, M., Mehta, M. and Grant, M. 2005.** Biophoton imaging: a nondestructive method for assaying R gene responses. Mol. Plant Microbe Interact., 18: 95–102.
- Beever, R. E. and Weeds, P. L. (2004).** Taxonomy and genetic variation of *Botrytis* and *Botryotinia*. Pages 29-52 in: *Botrytis: Biology, Pathology and Control*. Y. Elad, B. Williamson, P. Tudzynski, and N. Delen, eds. Kluwer Academic Publ., Dordrecht, The Netherlands

- Castoria, R., deCurtis, F., Lima, G., Caputo, L., Pacifico, S. and Cicco, V. (2001).** *Aureobasidium pullulans* (LS-30), an antagonist of postharvest pathogens of fruits: study on its mode of action. *Postharvest Biology and Technology*, 32: 717–724.
- Demoz, B. T. and Korsten, L. (2006).** *Bacillus subtilis* attachment, colonization and survival on avocado flowers and its mode of action on stem-end rot pathogens. *Biol. Contr.*, 37: 68-74.
- Domsch, K., Gams, W. and Anderson, T. (1980).** *Compendium of Soil Fungi*, Vol. 1, Academic Press, New York. pp. 859.
- Droby, S. (2006).** Improving quality and safety of fresh fruit and vegetables after harvest by the use of biocontrol agents and natural materials. *Acta Horticulturae*, 709:45–51.
- EI-Ghaouth, A., Wilson, C. L. and Wisniewski, M. (2004).** Biologically based alternative to synthetic fungicides for the postharvest diseases of fruit and vegetables. In: Naqvi, S.A.M.H. (Ed.), *Diseases of Fruit and Vegetables.Vol.2.*, Kluwer Academic Publishers, The Netherlands, pp. 511–535.
- Elmer, P. A., Hoyte, S. M., Vanneste, J. L., Reglinski, T., Wood, P. N. and Parry, F. J. (2005).** Biological control of fruit pathogens. *New Zealand Plant Protection*, 58:47-54.
- Ellis, B. (1971).** *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England. pp. 608.
- Errampalli, D. (2007).** Effect of a biological control fungicide agent (*Pseudomonas syringae*) and a chemical fungicide SCALA (pyrimethanil) on postharvest blue and gray mold of apple. *Agriculture and Agri-Food Canada*, 25:5-15
- Etebarian, H. R., Sholberg, E. L., Eastwell, K. C. and Saylor, R. J. (2006).** Biological control of apple blue mold with *Pseudomonas fluorescens*. *Can. J. Microbiol.*, 51: 591-598.
- Fatima, N., Humaira, B., Viqar S., Jehan, A. and Syed, E. (2009).** Prevalence of postharvest rot of vegetables and fruits in Karachi, Pakistan. *Pak. J. Bot.*, 41(6): 3185-3190.
- FAO. (2004).** Food and Agriculture Organization of The United Nations. <http://www.fao.org/>

<http://web.entomology.cornell.edu/shelton/cornell-biocontrol-conf/talks/harman.html>

- Gomez, K. A., and Gomez, A. A. (1984).** Statistical procedures for agricultural research. John Wiley and Sons, New York, USA perspective. FEMS Microbiology Letters, 171: 1-9.
- Hong, C. X., Michailides, T. J. and Holtz, B. A. (1998).** Effects of wounding, inoculum density and biological control agents on postharvest brown rot of stone fruits. Plant Disease, 82: 1210 – 1216.
- Horsfall, J. G. and Heuberger, J. W. (1942).** Measuring of a defoliation disease of tomatoes. Phytopathology, 32: 226-232.
- Ippolito, A., El Ghaouth, A., Wilson, C.L. and Wisniewski, M., (2000).** Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. Postharvest Biol. Technol. 19: 265–272.
- Jamalizadeh, M., Etebarian, H., Alizadeh, A. and Aminian, H. (2008).** Biological Control of Gray Mold on Apple Fruits by *Bacillus licheniformis* (EN74-1). Phytoparasitica, 36 (1): 23-29.
- Janisiewicz, W.J. and Korsten, L. (2002).** Biological control of postharvest diseases of fruits. Annual Review of Phytopathology, 40: 411- 441.
- Janisiewicz, W.J., and Marchi, A. (1992).** Control of storage rots on various pear cultivars with a saprophytic strain of *Pseudomonas syringae*. Plant Dis., 76: 555-560.
- Janisiewicz, W. J. Tworkoski, T. J. and Sharer, C. (2000).** Characterizing the mechanism of biological control of postharvest diseases on fruit with a simple method to study competition for nutrients. Phytopathology, 90 (11): 1196–1200.
- Jijakli, M. H., Grevesse, C. and Lepoivre, P. (2001).** Modes of action of biocontrol agents of postharvest diseases: challenges and difficulties. Bulletin-OILB/SROP., 24 (3): 317–318.
- Jorgensen, T., Joohae, P., Mark, A., Anne Marie, W., Gerda, L., Patricia, A., Robbert, A., Cees, A., Kristian, F., Jens, C. and Arthur, F. (2011).** The molecular and genetic basis of conidial pigmentation in *Aspergillus niger*. Fungal Genet. Biol: 10:1016.

- Kader, A. A. (2005).** Increasing food availability by reducing postharvest losses of fresh products. *Acta Hort.*, 682: 2169-2175.
- Kota, V. R. Kulkarni, S. and Hegde, Y. R. (2006).** Postharvest diseases of mango and their biological management. *Journal of Plant Disease Science*, 1(2): 186-188.
- Monte, E. (2001).** Understanding *Trichoderma*: between biotechnology and microbial ecology. *International Micobiology*, 4: 1 – 4.
- Moshe, R., (2006).** Inhibition of germination and growth of *Alternaria alternata* and mouldy-core development in Red Delicious apple fruit by Bromuconazole and Sygnum. *Crop Protection*, 25 : 253-258
- Pusey, P. L. (1989).** Use of *Bacillus subtilis* and related organisms as biofungicides. *Pesticide Sci.*, 27: 133-140.
- Rizzolli, W. G. (2006).** Efficacy of some fungicides against *Alternaria alternata* on apple. *Fitopatologiche*, 2: 97-102.
- Reuveni, M. (2006).** Inhibition of germination and growth of *Alternaria alternata* and mouldy-core development in Red Delicious apple fruit by Bromuconazole and Sygnum *Crop Protection*, 25: 253-258.
- Singh, Y. P. and Sumbali, G. (2007).** Efficacy of leaf extracts and essential oils of some plant species against *Penicillium expansum* rot of apples. *Annals of Plant Protection Sciences*: 15: 0971-3573.
- Snowdon, A.L. (1992).** Color Atlas of Post-Harvest Diseases and Disorders of Fruits and Vegetables, Vol. 2, Vegetables. pp. 18, 51.
- Spadaro, D., Garibaldi, A. and Gullino, M. L. (2004).** Control of *Penicillium expansum* and *Botrytis cinerea* on apple combining a biocontrol agent with hot water dipping and acibenzolar-S-methyl, baking soda, or ethanol application. *Postharvest Biol. Technol.*, 33: 141-151.
- Spotts, R. A. (1990).** Bull's eye rot. pp: 56. In: Jones, A. L., and Aldwinckle, H. S., (eds). *Compendium of Apple and Pear Diseases*. APS Press. St. Paul MN, St – German, G., and Summerbell, R. (1996). *Identifying Filamentous Fungi: A clinical Laboratory Handbook*. 1st ed. Star Publishing Company, Belmont. California. USA.

- Talbot, P. H. (1971).** Principles of fungal taxonomy. Macmilan Press LTD. Hong Kong, 274 pp.
- Tuite, J. (1969).** Plant pathological methods, fungi and bacteria. Minneapolis: Burgess, 239 pp.
- Vero, S., Mondino, P., Burgueno, J., Souhes, M. and Wisniewski, M. (2002).** Characterization of biocontrol activity of two yeast strains from Uruguay against blue mould of apple. *Postharvest Biol. Technol.*, 26: 91-98.
- Vinās, I. (2004).** Development of biocontrol agents for commercial application against postharvest diseases of perishable foods. Universitate Leida. <http://www.biopostharvest.com/wp2.htm>
- Vinas, I., Usall, J., Texido, N. and Sanchis, V. (1998).** Biological control of major postharvest pathogens in apple with *Candida sake*. *Int. J. Food Micro.*, 40: 9-16.
- Webster, J. (1991).** Introduction to fungi. Cambridge University Press, 669 pp.
- Welke, J. E., Michele H., Horacio, A. and Isa, B. (2010).** Patulin accumulation in apples during storage by *Penicillium expansum* and *Penicillium griseofulvum* strains. *Braz. J. Microbiol.*, 42: 165-173.
- Wilson, C. L. and Pusey, P. L. (1985).** Potential for biological control of postharvest plant diseases. *Plant Disease*, 69: 375 – 378.
- Wilson, C. L., Franklin, J. D. and Pusey, P. L. (1987).** Biological Control of *Rhizopus* rot of peach with *Enterobacter cloacae*. *Phytopathology*, 77: 303 – 305.

## الملخص العربي

دراسات على أمراض ما بعد الحصاد لثمار التفاح في منطقة الجبل الأخضر،

ليبيا

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أظهرت نتائج حصر أعفان ثمار التفاح في خمسة مناطق مختلفة من منطقة الجبل الأخضر بالدولة الليبية عن وجود خمسة مسببات مرضية لأعفان ما بعد الحصاد في التفاح وهي: ألترناريا ألترناتا، أسبرجلس فلافس، أسبرجلس نيجر، بنسليوم إكسبوسوم، بوتريتس سينيريا و ريزوبس ستولونييفر وكان أكثر المسببات شراسة مرضية هو فطر ألترناريا ألترناتا، بينما كان الفطر ريزوبس ستولونييفر و أسبرجلس نيجر أقل الفطريات من ناحية شراستها المرضية.

أثبتت الدراسات المرضية على ثلاثة أصناف من التفاح (جولدن ديليشس، ستاركريمسون و ريد ديليشس) أن الممسبب المرضي بنسليوم إكسبوسوم، بوتريتس سينيريا و ألترناريا ألترناتا هما الأكثر في القدرة الإراضية، بينما لم تثبت فروق معنوية بين كل من الفطر ريزوبس ستولونييفر و أسبرجلس فلافس و أسبرجلس نيجر مقارنة بالكنترول (المقارنة).

أثبت اختبار استخدام عناصر المكافحة البيولوجية وجود درجات مختلفة من التأثير على شدة الإصابة ضد الفطريات الممرضة على أصناف التفاح التي تم اختبارها. تم اختبار خمسة من عوامل المكافحة البيولوجية منها عزلتين من الفطريات (تريكوثيرما فيريدي و تريكوثيرما هارزيانم) وثلاث عزلات بكتيرية (سيدوموناس فلوروسينس، باسيلس ميكويدز و باسيلس ستلس) لتحديد فعاليتها في خفض حدوث أمراض أعفان ثمار التفاح المتسببة عن كل من الفطر بنسليوم إكسبوسوم، بوتريتس سينيريا و ألترناريا ألترناتا. وكانت عزلة الفطر تريكوثيرما هارزيانم هي الأكثر تثبيطاً لنمو فطريات أعفان التفاح على أطباق بتري، في حين كانت عزلة الفطر تريكوثيرما فيريدي هي الأقل تأثيراً. وكان للبكتيريا باسيلس ميكويدز تأثيراً معنوياً على أعفان ثمار التفاح المتسببة عن الفطريات الثلاثة المختبرة. وكان تأثيرها ملحوظاً بشكل كبير على الفطر ألترناريا. وكان للبكتيريا سيدوموناس فلوروسينس كبيراً على الفطريات المحللة لثمار التفاح ولوحظ تأثيرها العالي على المسبب المرضي بنسليوم إكسبوسوم. وقد أظهرت البكتيريا باسيلس ستلس أقل تأثير معنوياً كبير على الفطريات المحللة لثمار التفاح والتي شملتها الدراسة.

**Table (1): Degree of infection (DI%) and frequency of disease occurrence (OF %) of postharvest apple fruits rots diseases in different locations in Al-Jabal Al-Akhdar region(Libya)**

Location	DI and OF (%)											
	<i>B. cinerea</i>		<i>A. alternata</i>		<i>P. expansum</i>		<i>A. niger</i>		<i>A. flavus</i>		<i>R. stolonifer</i>	
	DI	OF	DI	OF	DI	OF	DI	OF	DI	OF	DI	OF
Al-wasiata	8.4*	11.8**	23.4	15.2	5.21	17.2	3.4	3.6	3.4	3.5	5.4	7.8
Shahat	10.2	14.6	17.4	12.4	10.5	16.3	2.4	5.7	6.6	4.7	2.6	5.6
Al-gubba	10.9	16.3	22.3	22.8	5.9	18.7	3.6	6.4	1.8	5.1	3.7	6.9
Massa	12.8	14.9	8.71	21.3	3.8	14.6	2.5	4.6	4.4	5.4	1.9	7.5
Al-Biada	10.7	12.7	15.9	19.1	1.7	18.7	1.9	5.3	4.5	3.6	1.7	6.9
LSD ( $\alpha=0.05$ )	1.8		2.5		1.8		1.8		1.8		1.8	

\* Degree of infection (DI%) for (50 fruits) with three replicates

\*\* Frequency of disease occurrence (O.F. %).

Table (2): Pathogenicity of the tested fruit-decaying fungi on different apple tested cultivars

Pathogen	Degree of infection (%)												LSD ( $\alpha=0.05$ )
	Golden Delicious cv.				Starkrenson cv.				Red Delicious cv.				
	Incubation days			Average	Incubation days			Average	Incubation days			Average	
	7	10	14		7	10	14		7	10	14		
<i>B. cinerea</i>	38	45	55	46.0	18	25	30	24.3	13	17	20	16.6	12.6
<i>A. alternata</i>	40	60	75	58.3	30	35	40	35.0	20	28	40	29.3	23.9
<i>P. expansum</i>	45	55	60	53.3	22	28	38	29.3	18	22	30	23.3	14.5
<i>R. stolonifer</i>	5.0	7.0	9.0	7.00	9.0	12	13	11.3	12	14	15	13.6	10.0
<i>A. flavus</i>	2.0	4.0	4.0	3.33	2.0	3.0	3.0	2.66	2.0	3.0	3.0	2.66	1.61
<i>A. niger</i>	1.0	2.0	2.0	1.66	2.0	2.0	3.0	2.33	1.0	3.0	4.0	2.66	1.99
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-----
LSD ( $\alpha=0.05$ )				15.2				8.26				9.03	

\* Degree of infection (%) for three replicates with four fruits per replicate.



Table (3): Antagonistic effect of the applied bacterial isolates against the tested fruit-decaying fungi

Bioccontrol agent	Storage period days)	<i>B. cinerea</i>		<i>A. alternata</i>		<i>P. expansum</i>		LSD ( $\alpha=0.05$ )
		Linear growth (cm)	% of reduction	Linear growth (cm)	% of reduction	Linear growth (cm)	% of reduction	
<i>B. mycoides</i>	4	1.63*	23.4**	1.23	63.3	3.36	30.8	0.73
	7	1.96	46.0	1.56	70.1	3.83	36.4	
	10	2.3	59.2	2.06	71.6	4.33	37.7	
	Average	1.96		1.62		3.8		
<i>P. fluorescens</i>	4	1.43	32.8	1.36	59.5	1.33	72.6	0.43
	7	1.73	52.3	1.96	62.5	1.6	73.4	
	10	2.16	61.7	2.66	63.6	1.9	73.6	
	Average	1.7		2		1.63		
<i>B. subtilis</i>	4	1.53	28.6	1.03	69.3	3.46	28.8	0.76
	7	2.2	39.3	2.2	57.9	4.23	29.8	
	10	2.86	49.3	3.16	56.4	4.66	33	
	Average	2.2		2.1		4.1		
Control	4	2.13	0.00	3.36	0.00	4.86	0.00	
	7	3.63	0.00	5.23	0.00	6.03	0.00	
	10	5.65	0.00	7.26	0.00	6.96	0.00	
	Average	3.7		5.2		5.9		
LSD ( $\alpha=0.05$ )		0.82		1.04		0.63		

\* Linear growth (cm) (mean of three replicate).

\*\* Reduction of growth= growth in control - growth in treatment / growth in control × 100.

Table (4): Antagonistic effect of the applied fungal biological control agents against the tested fruit-decaying fungi

Biocontrol agent	Storage period (days)	<i>B. cinerea</i>		<i>A. alternata</i>		<i>P. expansum</i>		LSD ( $\alpha=0.05$ )
		Linear growth	% of reduction	Linear growth (cm)	% of reduction	Linear growth (cm)	% of reduction	
<i>T. harzianum</i>	4	1.13 *	60.4 **	1.56	62.5	1.5	69.1	0.9
	7	1.63	64.2	2	65.6	1.6	73.3	
	10	2.10	66.2	2.2	69.0	1.83	73.5	
	Average	1.62	65.2	1.92	65.7	1.64	71.97	
<i>T. viride</i>	4	2.06	27.9	3.26	21.6	4.03	20.5	0.87
	7	2.63	42	4.56	21.7	4.73	21.1	
	10	3.00	51.8	5.5	22.5	5.33	22.7	
	Average	2.56	40.57	4.4	21.9	4.7	21.43	
Control	4	2.86	0.00	4.16	0.00	4.86	0.00	
	7	4.56	0.00	5.83	0.00	6.00	0.00	
	10	6.23	0.00	7.1	0.00	6.9	0.00	
	Average	4.55	0.0	5.7	0.0	6.02	0.0	
LSD ( $\alpha=0.05$ )		0.98		1.05		0.73		

\* Linear growth (cm) (mean of three replicate).

\*\* Reduction of growth= growth in control - growth in treatment / growth in control × 100.

Table (5): Effect of treatment of different apple cultivars with the tested bacterial biocontrol agents on % infection with fruit rot decaying fungi

Biocontrol agent	Storage period (days)	Degree of infection (%)											
		<i>B. cinerea</i>				<i>A. alternata</i>				<i>P. expansum</i>			
		Golden Delicious	Starkrenson	Red Delicious	LSD ( $\alpha=0.05$ )	Golden Delicious	Starkrenson	Red Delicious	LSD ( $\alpha=0.05$ )	Golden Delicious	Starkrenson	Red Delicious	LSD ( $\alpha=0.05$ )
<i>B. mycolidae</i>	7	32	27	12		22	12	10		30	12	16	
	10	45	35	13.5		45	17	15		38	25	15	18.9
	14	50	40	15	13.1	65	27	25	27.6	45	37	25	
	Average	42.3	34	13.5		44	18.6	16		37.8	24.6	16.6	
<i>P. fluorescens</i>	7	12	8	5		25	5	5		15	10	8	
	10	17	10	7		40	8	7		25	12	10	14.8
	14	22	15	9	4.88	45	10	8	12.4	30	25	20	
	Average	17	11	7		36.6	7.6	6.6		23.3	15.6	12.6	
<i>B. subtilis</i>	7	25	15	10		35	15	10		35	15	10	
	10	30	18	15		55	20	18		55	27	18	30.8
	14	65	22	20	25.9	80	30	25	28.6	80	38	28	
	Average	40	18.3	15		56.6	21.6	17.6		56.6	26.6	18.6	
Control	7	65	3	15		40	35	30		35	28	2	
	10	70	55	20		55	55	40		65	40	37	
	14	80	60	25		85	60	48		80	55	45	
	Average	71	50	20		60	50	39.3		60	41	36	
LSD ( $\alpha=0.05$ )			20.4	14.6	7.02		37.6	16.2	13.2		31.8	21.7	15.4

Table (6): Effect of treatment of different apple cultivars with the tested fungal biocontrol agents on % infection with fruit rot decaying fungi

Biocontrol agents	Storage period (days)	Degree of infection (%)											
		<i>B. cinerea</i>				<i>A. alternata</i>				<i>P. expansum</i>			
		Golden Delicious	Starkrimson	Red Delicious	LSD ( $\alpha=0.05$ )	Golden Delicious	Starkrimson	Red Delicious	LSD ( $\alpha=0.05$ )	Golden Delicious	Starkrimson	Red Delicious	LSD ( $\alpha=0.05$ )
<i>T. herzenum</i>	7	20	18	18		10	20	10		20	7	5	
	10	25	20	18		18	25	15		28	10	7	
	14	35	25	20	11.4	20	30	25	12.2	35	15	10	10.2
	Average	26.8	21	18		16	25	16.6		27.6	10.6	7.3	
<i>T. viride</i>	7	20	20	18		20	18	15		25	15	7	
	10	28	25	22		28	22	18		30	20	12	
	14	40	35	37	18.5	35	27	30	13.5	40	35	20	16.6
	Average	29.3	26.6	25.6		27.6	22.3	21		31.6%	23.3	13	
Control	7	40	28	20		20	40	20		35	28	26	
	10	55	35	35		35	55	35		55	40	37	
	14	60	60	55		55	75	55		75	55	45	
	Average	51.6	41	36.6		36.6	56.6	36.6		55	41	36	
LSD ( $\alpha=0.05$ )		18.8	21.6	40		22.7	21.5	23.8		26	20.1	13.6	