An–Najah National University
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Biological Control of Gray Mold, Blue Mold & Rhizopus Soft Rot on Grape, Pear, Kiwi, Strawberry *by Trichoderma harzianum.*

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Submitted in Partial Fulfillment of the Requirements for the Degree of Master in Environmental Sciences, Faculty of Graduate Studies, at An–Najah National University, Nablus, Palestine.

2006

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Dedication

To my parents and brothers with love.

Acknowledgements

All praise to Allah for this accomplishment. Thanks for Dr. Yacoub Batta for this guidance, encouragements and supervision during the study and dissertation preparation.

I would like to record my special thanks to my parents, my sister in law for their efforts in all steps of my life and combine harvesting. Thanks to my brothers.

At the end, my thanks to the many other people who helped in this work.

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List of Abbreviations

CA: controlled atmosphere

CDP: Cazpec Dox Broth

CRD: completely randomized design

CWDEs: cell wall- degrading enzymes

DPA: diphenylamine

ED: effective dose

EPA: Environmental Protection Agency

FID: flame ionization detector

GC: gas chromatograph

GFP: green fluorescent protein

PDA: Potato Dextrose Agar

PR: pathogenesis- related protein

RH: relative humidty

SOPP: Sodium O- Phenyl Phenate

TBZ: Thiabendazole

USDA: United States Department of Agriculture

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Abstract

This research aimed at evaluation of biological effectiveness of *Trichoderma harzianum* against the gray mold, blue mold and rhizopus soft rot caused by *B. cinerea*, *P. expansum* and *R. stolonifer,* respectively, on four types of fruits (Grape, Pear, Kiwi, and strawberry). Also, it aimed at determination of the minimum protection period from infection with gray mold, blue mold and Rhizopus soft rot on the same types of fruits. *T. harzianum* was applied in form of invert emulsion (water- in- oil formulation) after being introduced into the emulsion in form of conidia, in addition to using non-formulated form of the fungus. The experiments of evaluation of efficacy was carried out under laboratory conditions (18± 2 °C) and 16 hours of illumination per day under humid conditions (100٪). Results obtained have demonstrated that the treatment with the fungus (*T. harzianum*) formulated in invert emulsion reduced significantly gray mold, blue mold and Rhizopus soft rot lesion diameter compared to other treatments. Significant differences ($P< 0.05$) were obtained in reducing the lesion diameters of gray mold, blue mold and Rhizopus soft rot treated with *Trichoderma* in invert emulsion in comparison with the control treatment. Results have also indicated that *T. harzianum* formulated in invert emulsion on unwounded grape fruits gave the longest minimum protection period against the gray mold infections. This demonstrated the biological effectiveness of *Trichoderma harzianum* in protecting

unwounded grape berries from gray mold infection for long time (up to 25 days). Moreover, it is recommended to confirm the efficacy of the fungus against *B.cinerea*, *P. expansum* and *R. stolonifer* especially in the formulated form under a wide range of temperatures and relative humidity's, in addition to the controlled atmosphere conditions.

Chapter one

Introduction

Introduction

World trends are moving towards reduced postharvest pesticide use on fresh fruits and vegetables. In response to this trend, physical and biological approaches have been evaluated as safer alternative to the use of chemical fungicides (Droby et al., 2002). Pre- and postharvest diseases of fruit crops are responsible for devastating financial losses to growers and are difficult to manage with current technologies. Biological approaches, including the use of antagonistic organisms, natural compounds, cultural practices, and biotechnology, will be used to develop new methods of managing diseases of fruit crops (Wisniewski et al., 2003). The application of fungicides to fruits after harvest to reduce decay has been increasingly curtailed by the development of pathogen resistance to many key fungicides, the lack of replacement fungicides, negative public perception regarding the safety of pesticides and consequent restrictions on fungicide use. Biological control of postharvest diseases has emerged as an effective alternative. Because wound-invading necrotrophic pathogens are vulnerable to biocontrol, antagonists can be applied directly to the targeted area (fruit wounds), and a single application using existing delivery systems (drenches, line sprayers, on-line dips) can significantly reduce fruit decays. The pioneering biocontrol products “BioSave” and “Aspire” were registered by EPA in 1995 for control of postharvest rots of pome and citrus fruit, respectively, and are commercially available. The limitations of these biocontrol products can be addressed by enhancing biocontrol through manipulation of the environment, using mixtures of beneficial organisms, physiological and genetic enhancement of the biocontrol mechanisms, manipulation of formulations, and integration of biocontrol

with other alternative methods that alone do not provide adequate protection but in combination with biocontrol provide additive or synergistic effects (Janisiewicz and Korsten, 2002). In the Palestinian territories, fruit trees constitute the largest percentage compared to the total planted area, it constitutes approximately 63.8%, and this equals to 1,158,000 dunums in west bank and Gaza strip (Palestinian Central Bureau of Statistics, 2004).

The objective of the present study are:

1- To asses the biological effectiveness of *Trichoderma harzianum* against the gray mold, blue mold and Rhizopus soft rot caused by *B. cinerea*, *P. expansum* and *R. stolonifer,* respectively, on four types of fruits (Grape, Pear, Kiwi, and strawberry) at 18±2°C, 16 hours of illumination per day under humid conditions.

2. To determine the protection period from infection with the three diseases on the four types of fruits mentioned – above.

Chapter two

Literature Review

1. Gray mold

Description

1.1 Identification and classification

Botrytis cinerea can be classified as cosmopolitan filamentous higher fungi. It belongs to the kingdom Mycetae and division Eumycota. Under the high power of the microscope, the fungus looks like bunches of grapes. *B.cinerea* belong to the class Hyphomycetes and family Monliacacea. *Botrytis cinerea* is not nearly a spoilage mould of soft fruit, but is a very common saprotroph and pathogen on all kinds of damp plant material. It can be seen in the wild on, for example, dead flower petals, particularly in damp weather. It commonly forms resting structures, sclerotia, in the remains of the substrate. In the wild hosts, these can sometimes be seen on dead, standing stems of tougher herbaceous plants. Normally these germinate to produce new mycelium and a continuation of the asexual or conidial stage. Only rarely is the sexual fruit body (teleomorph) seen; this is a small cup-fungus, *Sclerotinia fuckeliana* (named after the distinguished mycologist). Since it reproduces almost entirely asexually, any chance mutations, if successful, will be perpetuated as individual genotypes. This means that over the course of time, *B. cinerea* has become represented by many variants. Some of these have become more specialised and in many cases can be regarded as distinct species. Under the microscope, the conidia are colourless (or nearly so), as are the tips of the conidiophores. The vegetative mycelium of the fungus is also colorless, appearing white to the naked eye in its "fluffy" stage. But mature fungal colonies are a dingy grey.

The colour is in the lower parts of the conidiophores, which are distinctly brown and thick-walled when seen under the microscope.(Agrios, 1988).

1.2 Distribution

Botrytis diseases are very common and widely distributed on vegetables, ornamentals, fruits, and field crops throughout the world. They commonly appear as blossom blights and fruit rots. Other diseases caused by this fungus are damping off, stem cankers and rots, leaf spots, and tuber, corm, bulb, and root rots (Gonsalves and Ferreira, 1994).

1.3 Host Range

The most serious diseases caused by this fungus are: gray mold of strawberry, gray mold rot of vegetables (artichoke, bean, beet, cabbage, carrot, cucumber, eggplant), tip-end rot (bananas, lettuce, pepper, squash, tomato), onion blast and neck rot, calyx end rot of apples, blossom and twig blight of blueberries, blight or gray mold of ornamentals (African violet, begonia, cyclamen, chrysanthemum, dahlia, geranium, hyacinth, lily, peony, rose, snapdragon, tulip), bulb rot of amaryllis, corm rot, leaf spot, and stem rot of gladiolus. As a postharvest disease, this fungus can cause gray mold of many fruits and vegetables (Gonsalves and Ferreira, 1994).

1.4 Symptoms of Gray molud on Fruits

In early spring young stalks may suddenly wilt and fall over. Young buds turn black and dry up. Larger buds that become infected later in the spring turn brown and become covered with a brown or gray mass of fungal spores. Flowers may fail to open. Usually the stalks below infected

buds and flowers are rotted for short distances below the necks. Large, irregular, dark brown blotches may also occur on the leaves. In severe cases, crown and root rot may occur; however, these symptoms are not as common as above ground symptoms. In wet weather the diseased plant parts soon become covered with a grayish, felty growth of fungus spores. Small, black sclerotia may form on the base of infected stalks or in other invaded portions of plants that have fallen to the ground. The causal fungus overwinters in this sclerotial stage. Bud and flower symptoms are often confused with injury from the sucking insects, thrips. If thrips are the cause of bud or flower symptoms, these insects can usually be shaken from among the petals onto a piece of paper. The presence of tiny, orange, scurrying insects barely visible to the naked eye is evidence of thrips injury. Thrips do not cause the leaf blotches or stem rot associated with Botrytis blight (Hansen et al., 2000).

1.5 Biology and Life Cycle

Botrytis survives as dormant mycelium or small, hard, black sclerotia over the winter. The life cycle of *B. cinerea* is illustrated in Figure 1 (Agrios, 1997).

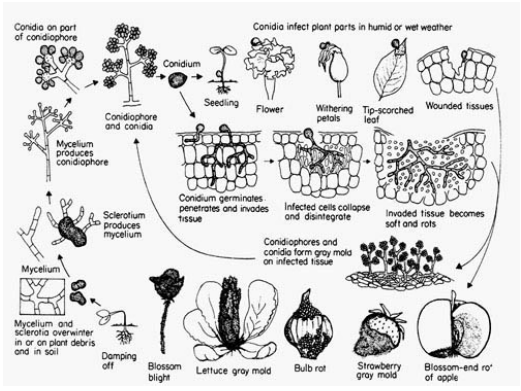


Figure (1) Life cycle of *Botrytis cinerea* on fruits and vegetables

In the spring, germination takes place to produce conidia asexual spores which are dispersed by wind. Early in the growing season, most of the spores are produced from mulched pruning on the ground or from *Botrytis* sources outside the block (e.g. glasshouse vegetables). By petal fall, *Botrytis* populations can increase dramatically up to 9600 million spores which coincides with abundant dead and dying flower parts. Midway through the growing season *Botrytis* is found on senescent petals attached to fruit and blowouts. By harvest, the green leaves with necrosis (dead patches) and dead leaves are the primary source of *Botrytis* inoculums. Research has shown that the hairy kiwifruit acts as a natural spore trap and thousands of *Botrytis* spores have been measured on the fruit surface. At the time of harvest, spores on the skin surface contaminate the picking scar which leads to stem end rot in cool storage. (Elmer, 1997). Conidia are easily released in humid weather and are disseminated by wind. This fungus over winters in the soil either as mycelium on decaying plant debris or as sclerotia. At this stage, the fungus can be spread by the movement of contaminated soil and plant debris. Cool (18-23 °C) and damp weather are ideal for growth, sporulation, spore release and germination, and infection. Conidia can germinate and penetrate tissue primarily through wounds. Conidia seldom penetrate tissue directly. However, once the spore germinates and the mycelium is actively growing, the fungus can penetrate the tissue of old flower petals, dying foliage, and dead bulb scales (Gonsalves and Ferreira, 1994). Botrytis is a common fungus which infects many plant hosts. It survives the winter months as sclerotia (small, black lesions which can survive tough conditions) or as mycelia (fine, threadlike growth within plant tissues). Both these forms can be found on dead and decaying plant material or in dormant living tissues.

They produce spores on structures called conidia when suitable environmental conditions occur. The warm, moist conditions of spring are ideal for spore development. The spores are carried to plants mostly by wind, but also by rain splash, machinery and people. The most important infections are from spores which germinate on flowers. These are called latent infections because the fungus grows very slowly and symptoms of the disease are not yet visible. When the fruit begins to ripen, sugar levels increase, providing more food for the fungus and stimulating its growth. When environmental conditions become moist, the fungus again forms conidia and spores, resulting in the typical grey, fuzzy fruit rot most commonly associated with the disease. Spores from the rotting fruit will spread to other tissues, entering through wounds, and grow rapidly if conditions remain suitable. At the end of the season the fungus will again go into a resting state within infected tissues such as dried up fruit, dead leaves and canes (Stanley et al., 1996).

1.6 Control of *B.cinerea*

1.6.1Chemical Control

Chemical fungicides will further make the environment unsuitable for Botrytis infection. Several materials are effective against the fungus, None of these materials offer complete protection on its own. They must be used as one part of an overall management plan in order to achieve maximum effectiveness. Different fungicides should be alternated or combined to prevent the development of resistance by the fungus. Timing of fungicide applications is critical. Because the most damaging infections occur during the flowering period, this is when sprays should be

concentrated. Any time, there is high humidity and temperatures in the range of 18-30°C, *Botrytis* spores will be in the air. Flowers should be protected throughout the bloom period to prevent infection. Most fungicides are protective, meaning they are most effective if they are on the flower prior to an infection. Therefore, sprays should start when the flowers begin to open, not delayed until after the conditions have become favorable for spores to be active. Thus, it is important to spray for Botrytis before rainfall, following rainfall of more than 2 mm, and before periods likely to have heavy dew (cool, still nights with high relative humidity)(Stanley et al, 1996). Several fungicides are available for control of Botrytis blight. Mancozeb-containing fungicides for Botrytis blight control include Dithane T/O®, Fore®, and Junction®. These can be applied as a soil and foliar drench in early spring and early fall. Cleary 3336®, a thiophanate methyl-containing fungicide, should be applied when the disease first appears and at 10-14 day intervals (Hansen, 2000).

1.6.2 Cultural Control

Removal of contaminated tissue from the field and from storage rooms can help to manage Botrytis infection. Proper aeration to allow quick drying of plants and plant products, and other means of keeping greenhouses and storage rooms at low humdity levels can also aid in managing this fungus (Gonsalves and Ferreira,1994). For fresh market fruit, pickers must be trained to harvest fruit at the appropriate stage of maturity, and to avoid harvesting over ripe and infected fruit. The fruit should be handled carefully to avoid damage or wounds and placed in shallow containers to prevent crushing fruit on the bottom layers. Fruit should be cooled as rapidly as possible to a temperature of 0-2°C to inhibit

fungal growth. Fruit destined for freezing should also be cooled as soon as possible after harvest. Machine harvesters can cause considerable damage to plants and fruit and pave the way for Botrytis to infect fruit to be harvested later (Stanley et al., 1996)

1.6.3 Biological Control Using Bacteria

1.6.3.1 L-*form bacteria*

Plants respond to environmental stimuli and pathogen attack via induction of different defense mechanisms such as the synthesis of phytoalexins, the deposition of callose/lignin in plant cell walls and the accumulation of pathogenesis-related proteins (PR proteins). In particular, studies have been focused on the antifungal activity of the chitinase (PR-3) and b-1,3-glucanase (PR-2), because of the abundance of chitin and b-1,3-glucan in the cell walls of many pathogenic fungi. L-form bacteria have been shown to associate with plants and can be used for disease control and to determine: the induction of chitinases and b-1,3-glucanases in *Pseudomonas syringae* pv. *phaseolicola* L-form associated Chinese cabbage (*Brassica campestris* var. *pekinensis*) seedlings and the potential of these hydrolytic enzymes in controlling grey mould disease caused by *Botrytis cinerea*. Plant L-form association was confirmed in Chinese cabbage, using a slide agglutination test, ELISA and re-isolation of the symbiont. The accumulation of chitinase was analysed using 4-methylumbelliferyl substrates (fluorometric assay) and dye substrates (dye-labeled assay). The development of grey mould in L-form associated plants was delayed as assessed by standard bioassays on detached leaves and on whole seedlings (Allan et al., 2000).

1.6.3.2*Bacillus subtilis*

Botrytis decay is the principal phytosanitary problem of table grape for export in Chilean exported market. The chemical control of this pathogen becomes difficult due to the development of benzimidazole and dicarboximide resistant strains. The possibility of an alternative product of different mode of action, without negative impact on the environment and with effective control, is really important for the export industry. For these reasons, in two field studies conducted at a Thompson Seedless table grape vineyard, we assesed the efficacy level of *Bacillus subtilis* QST-713 strain on *Botrytis cinerea* control. In both seasons (1998-1999), the assays were conducted totally randomized, consisting of six treatments and four replications (Esterio, 2000). In the first study was evaluated on two rates of product (7.5 and 15 kg /ha) applied in two period in preharvest time (15 and 2 days before harvest); and in the second study, three rates of a new formulation of *Bacillus subtilis* QST-713 strain (4.5, 4.8 and 11 kg/ha) were applied in four cases: full bloom; fruit set; version and two days before harvest. All the treatments were inoculated with *B. cinerea* (10^5 conidia/mL) before the first application at full bloom stage. The effectiveness of this treatment was compared with traditional treatment used to control the disease. BC-1000 in full bloom; Forced air plus Captan in fruit set and version, and Iprodione in preharvest; and with the control without fungicides. The parameters assessed were the degree of infection on flowers and berries during each period and the rotting rate during post harvest on table grape stored for 35 days at 0C°.The results of the study indicated that *Bacillus subtilis* QST-713 strain is a good alternative to control *B. cinerea*. In both seasons, *Bacillus subtilis* QST-713 had higher

rate obtained and lowest rotting level and it was similar to the traditional Botrytis chemical control program (Esterio, 2000).

1.6.3.3 Using naturally occurring fungal antagonists

Microbial isolations were made from the phyllo/fructoplane of grapevines. A total of 125 microbial isolations were obtained representing 8 genera (*Alternaria, Aureobasidium, Cladosporium, Cryptococcus, Epicoccum, Gliocladium, Trichoderma* and *Ulocladium*). These organisms were evaluated for antagonism towards *Botrytis cinerea* in a succession of laboratory bioassays using necrotic grape leaf tissue. The assays were designated to evaluate the test isolates ability to suppress sporulation of the pathogen under different levels of water deficit and inoculums pressure. Based on a criterion of >90% suppression of *Botrytis* sporulation, six fungi (isolates of *Epicoccum* E21, E26, *Trichoderma* T13, T16 and *Ulocladium* U13, U16) were selected for further evaluation under field conditions. Test isolates were applied as a spore suspension to necrotic grape leaf tissue 24 h after application of *Botrytis* conidia. Leaf discs were exposed to natural conditions in a grape canopy for periods of 10, 20 and 30 days. At all sites and throughout all experiments, the six test fungi significantly reduced *Botrytis* sporulation compared to the control and performed equal to or better than the fungicide (iprodione) treatment. One *Ulocladium* isolate (U13) consistently suppressed *Botrytis* sporulation more effectively than the others (Stewart et al., 2000).

1.6.3.4 The yeast *pichia guillermondii*

Botrytis cinerea infects stem wounds of greenhouse tomatoes and can cause considerable losses of yield and quality. The pathogen spreads

rapidly and is difficult to control due to the limited availability of effective fungicides and the ease with which fungicide resistance may develop. A bioassay using stem segments and atomized aqueous pathogen suspension was used to study wound infection and to screen potential yeast antagonists for activity against *B. cinerea*. It was demonstrated that stem infection was related to the pathogen inoculums concentration and 0.25 spores mm^{-2} was enough to cause 70% infection (Saligkarias et al., 2000). It was observed that stem susceptibility to infection was reduced by 50% as wound age increased to 48 h. The strains US-7 and 101 of *Pichia guilliermondii* reduced stem infection by 70% and 80%, respectively, when applied simultaneously with the pathogen at a concentration of $2.5x10^5$ cfu mm^2. When yeasts were applied 24 h before the inoculation with the pathogen, in all cases, the biocontrol activity significantly improved (Saligkarias et al., 2000).

1.6.3.5 Using *Pseudomonas antimicrobica* against the grey mould

Antagonism against the grey mould pathogen *Botrytis cinerea* by *Pseudomonas antimicrobica* was demonstrated in vitro and in vivo. Cell-free filtrates showed activity against *B. cinerea* growing on Potato Dextrose Agar (PDA) in a media-dependent manner with the most distinct antagonism being produced in Czapek Dox Broth (CDB). Cell-free filtrates of CDB-grown cultures also significantly reduced conidial germination of *B. cinerea*. An assay based on the inhibition of conidial germination was compared with two assays measuring the antagonism of mycelial growth on PDA. The conidial germination bioassay was more sensitive in the detection of this antifungal activity than the Petri dish bioassay while a bioassay using Microdetection plates did not detect antagonism due to the

small loading capacity of the latter. The conidial germination bioassay was modified for detection of antibiosis on the surface of strawberry leaves. Significant reductions in percentage conidial germination were recorded on the surface of leaves of both micropropagated and glasshouse grown strawberry plants when the antifungal compounds of *Ps. antimicrobica* were applied to the leaf tissue with the conidia. In addition, antifungal compounds were also detectable when conidia were applied to leaf tissue which had previously been sprayed with cells of *Ps. antimicrobica*. These tests indicate that *Ps. antimicrobica* would be a suitable biocontrol agent for the control of *B. cinerea* (Walker et al., 1996).

1.6.3.6 Using modified atmosphere in combination with *Erwinia* sp.

The combined effect of modified-atmosphere packaging and the application of a bacterial antagonist (*Erwinia sp*.) on *Botrytis cinerea* growth on apples was investigated. Inoculated apples were stored in polyethylene bags at 5 °C. The initial gas composition in each bag was set according to a central composite experimental design involving five levels of O2 (1 to 15%) and CO2 (0 to 15%). Control samples under ambient conditions were also included. Without the antagonist, measurements of mold colony diameter over time showed that O2 had no effect on the growth of *B. cinerea*, while increased CO2 levels delayed its growth by about 4 days. Application of the antagonist resulted in a significant interaction between O2 and CO2. At low O2 levels, CO2 had no effect on mold growth, but at high O2, CO2 enhanced mold growth. O2 and the antagonist worked synergistically to reduce mold growth by about 6 days at low levels of CO2. However, at high CO2 levels, O2 had no effect. The strongest antagonistic effect was observed under ambient conditions.

Overall, results showed that high CO2 atmospheres can slow the growth of *B. cinerea* and that *Erwinia sp*. was an effective antagonist against *B. cinerea* growth on apples, particularly under ambient conditions (Dock et al., 1998).

1.6.3.7 Using Postharvest ethanoland hot water treatments

Complete inhibition of the germination of spores of *Botrytis cinerea* occurred after a 10 s exposure to 30% ethanol or more at 24 °C. Mortality of spores in heated 10% ethanol was higher than in water at the same temperatures. Immersion of naturally infected, freshly harvested table grapes for 30 s in 30% ethanol at 24 °C reduced decay approximately 50% after 35 days of storage at 1 °C. The addition of ethanol significantly improved the efficacy of a hot water treatment applied to grapes that were inoculated with *B. cinerea* two hours prior to immersion in heated solutions. Immersion of inoculated, freshly harvested table grapes for 3 min at 30, 40, or 50 °C in 10% ethanol reduced decay to 20.7, 6.7, and 0.1 berries/kg after 30 days of storage at 1 °C, while decay after immersion in water at these temperatures was 35.9, 17.6, and 1.7 berries/kg, respectively. Immersion for 30 or 60 s at 50, 55, or 60 °C in water or 10% ethanol also significantly reduced the number of decayed berries that developed after storage for 30 days at 1 °C. The appearance of the rachis and berries, incidence of cracked berries, flesh browning, flavor, weight loss, and berry color were examined and most treatments did not adversely affect these quality parameters. Prompt drying of the fruit after treatment was important to prevent berry cracking (Karabulut et al., 2004).

1.6.4.8 **Using T*ricoderma harzianum* formulated in invert emulation**

An invert emulsion (water-in-oil formulation) based on coconut and soyabean oils provided the most stable emulsion layer (93 % V/V) and lowest viscosity (27 ± 0. 81 cps), suitable for a formulation of *Trichderma harzianum* conidia. Conidia remained viable for 36 months (shelf life) with 50% reduction in viability (half life) after 5.3 months at 20±1 ºC compared with 2.7 and 0.7 months, respectively, for dry non formulated conidia. Stability and viscosity of the formulation remained constant during the time period of violability study. Bio assay tests on wounded apple fruit indicated the presence of significant preventive effect of formulated *Trichoderma* conidia against the causative organism of apple gray mold *Botrytis cinerea*. The diameter of *Botrytis* lesions was significantly reduced (P< 0.05) 5 days after inoculation and treatment with formulated *Trichoderma* conidia. Botrytis sporulation on the lesion surface was also inhibited 10 days after inoculation. These results were confirmed under simulated natural conditions by spraying a *Botrytis* conidial suspension on microwounded apple fruit following treatment by fruit dipping. Treatment of healthy unwounded apple fruits resulted in protection from Botrytis infections for >2 months following inoculation and treatment (Batta, 2004).

2. Rhizopus soft rot

Description:

2.1. Identification and Classification:

Rhizopus stolonifer, causal organism of soft rot of fruits and vegetables, can be classified as a cosmopolitan filamentous lower fungus

living in the soil, decaying fruit and vegetables, animal feces, and old bread. It belongs to the class zygomycetes which contains two other genera: Choanephora and Musor known to cause diseases in plants (Agrios, 1997). The spores of zygomycetes are often floating around in the air, they are either saprophytes or weak parasites of plants and plant products on which they cause soft rots or molds (Agrios, 1997). *R. stolonifer* belongs to Mucoraceae family and order: Mucorales. It is named as *Rhizopus stolonifer* because it produces a mycelium with long sporangiophores connected by an aerial stolon. The stolons connect sporangiophores along various points of host contact; a root-like structure called a "rhizoid" extends beneath the sporangiophores and fastens them with the host tissues (Agrios, 1997).

2.2. Distribution:

Rhizopus soft rot of fruits and vegetables occurs thoughout the world on harvested fleshy organs of vegetables, fruits and flower crops during storage, transit, and marketing of these products (Agrios, 1997). The disease, when occurs on wet or wounded fruits packed in card board boxes, can be an unsightly mess due to the watery leakage from fruits causing the boxes collapse (Alvarez & Nshijima, 1987).

2.3. Host range:

Several fruits and vegetables are susceptible to infection and include the following genera: Alium, Ananas, Brassica, Cucumis, Cucurbita, Fragaria, Lycopersica, Phaseolus, Pisum, Solanum (Nishiijima et al., 1990) in addition to sweet potatoes, strawberries, peaches, cherries, and peanuts, corn and some other cereals are effected under fairly high conditions of

moisture. Bulbs, corms, and rhizomes of flower crops, for example, gladilus and tulips, are also susceptible to this disease (Agrios, 1997).

According to the USDA fungus- host distributions, rhizopus stolonifer has a very broad host range (over 240 species in many countries around the world).

2.4. Symptoms of Rhizopus soft rot on fruits:

Symptoms of *R. stolonifer* on infected arease of fleshy fruits appear water soaked at first, and are very soft. If the skin of the infected organ remains intact, the tissue loses moisture gradually until it shrivels into a mummy; otherwise they break down and rupture softened skin during handling or under pressure. Fungal hyphae then grow outward through the wounds and cover the effected portions by producing tufts of whisker – like gray sporangiophores which carry sporangium. The bushy growth of the fungus often extends to the surface of the healthy portions of affected fruits and even to the surface of the containers within a few days when they become wet with the exuding whitish _ yellow liquid, the infected fruit is often covered by coarse, gray, hairy mycelia that from a mass of black sporangia at their tips (Nishijima et al., 1990). Affected tissues at first give off mildly pleasant smell, but soon yeasts and bacteria move in and a sour odor develops (Agrios, 1997).

2.5. Biology and life cylce:

Rhizopus exists everwhere, usually as a saprophyte and sometimes as a weak parasite on stored organs of plants. The mycelium of the fungus produces long, aerial sporangiophores at the tips of which black spherical

sporangia develop (Agrios, 1997). The sporangia contain thousands of spherical gray sporangiospores. When the mycelium grows on a surface, it produces stolons or superficial hyhyae that arch over the surface and at the next point of contact with the surface produce both root – like hyphae or rhizoids which grow outward the surface piercing the softened epidermis and then go through the oraganic material, secreting the enzymes, absorbing water, and digested sugars and starches (Agrios, 1997). The aerial sporangiophores bearing sporangia, and from each poing of contact more stolons are produced in all directions. Adjacent hyphae produce short branches called progametangia, which grow toward one another, when they come in contact, the tip of each high face is separated from the progamtangium by a cross wall. The terminal cells are the gametangia. These gametangia fuse together and their nuclei pair. The cell formed by fusion enlarges and develops a thichk, black, and watery cell wall (Barness, 1979). This sexually produced spore is called zygospore, it is used by the fungus in the overwentering or as a resting stage. When it germinates, it produces a sporangiophore bearing sporangium full of sporangiospores. Throughout the year, sporangiospores float about and if they land on wounds of fleshy fruits, roots, corms, or pulps, rodents enhance the infection (Barnes, 1979) the produced hyphae secrete pectionlytic enzymes, which break down and dissolve the pectic subastances of the middle lamella the hold the plant cells in place in the tissues, this result in loss of cohestion among the cells and development of "soft rot" the pectinoltic enzymes secreted by the fungus advance ahead of amycelium and separate the plant cells, which are then attacked by the celluloytic anzymes of the fungus. The cellulases break down the cellulose of the cell wall, and the cells disintegrate, mycelium does not seem to invade cells but it is

surrounded by dead cells and non living organic substances, it is living more likely as a saprophyte than a parasite. The fungus continues to grow inside the tissues. When the epidermis breaks, the fungus emerges through the wounds and produces serial sporangiosphores, sporangia, stolons, and rhizoids. In extremely fleshy fruits the mycelium can penetrate even healthy fruit. Unfavorable temperature and humidity or insufficient maturity of the fruit slow down the growth and activity of the fungus, so it reproduces asexually (Moniz de Sa, 2003).

2.6. Control of *R. stolonifer*

2.6.1. Chemical control:

Fungicides used for postharvest decay control should only be used after the following critical poing are considered: type of pathogen involved in the decay; location of the pathogen in the produce; best time for application of the treatment; maturity of the host; and environmental conditions during storage, transportation and marketing of produce (Ogawa and Manji, 1984). Preventive field fungicide sprays control Rhizopus soft rot reducing field inoculum levels, fungicide sprays also reduce the incidence of fruit lesions, caused by other fungi since rhizopus can act as court of entry into the papaya fruit (Alvares and Nishijima, 1987). Irodione has been used for several years as a preharvest spray in combination with wax and/ or oil. Its decay control spectrum is increased and will also control postsharvest fungi such as *rhizopus*, and *alternania* (Ogawa et al., 1992) many of the former products that were used postharvest are no longer permitted to be used or discontinued because of concerns with residues and possible toxiv effects. The most notable fungicides that

contained benomyl, thiabendazole, dichloron, and imazalil are examples of postharvest chemical treatments that are presently used, however, resistance to thiabendazole and imazalil is widespread (Holmes and Eckert, 1999; Conway et al., 1999) and their use as effective materials is declining. Preservative or antimicrobial food additive are not generally thought of as postharvest treatments but they do control decay, these products include sodium benzoate, sorbic acid, propionic acid, so2, acetc acid, nitrites and nitrates, and some antibiotics such as nisin (Chichester and Tanner, 1972). The demand for new postharvest fungicide treatments is strong, especially since the discontinuation of iprodione in 1996, Fludioxinil was granted an emergency registration in 1998 to curb potential losses in nectarines, peaches, and plums that would have resulted (Foster and Adaskaveg, 1999). Sanitation is the cornerstone of any effective postharvest decay reduction program. It must be a parthnership between grown and packer and it must begin in the orchard, storge containers and warehouses must be disinfeted with a copper sulfat solution, formaldehyde, sulfur fumes, chloropicrin (Agios, 1997).

2.6.2. Cultural control:

As *Rhizopus* soft rot acts as a saprophyte which exists everywhere, it can affect the fleshy organs when it reaches the maturity through wounds and bruises made by harvesting and handling (Agrios, 1997). At this point, disease may begin at the field if the previous conditions are available. Host eradication (rouging) is one of the cultural control methods carried out routinely in many nurseries, greenhouses, and fields to prevent the spread of numerous diseases by elimination of infected plants that provide a ready source of inoculum within the crop, this elimination prevents greater losses

from the spread of the pathogen to additional plants. Crop rotation can reduce population of the pathogen in the soil, and appreciable yields from the susceptible crop can be obtained every tird or fourth year of the rotation. Plowing under infected plants after harvest, such as left over infected fruit, stems, tubers, or leaves, hegps cover the inoculum with soil and speeds up its disintegration (roting) and concurrent destruction of most pathogens carried in on them, pruning infected or dead branches, and removing infected fruit and any other plant debris that may harbor the pathogen to grow into still healthy parts of the tree, spacing plants properly in the field or greenhouse prevents the creation of high humidity conditions on plant surfaces and inhibits infection (Agios, 1997). Also appropriate choice of fertilizer, such as low nitrogen and high calcium in the fruit reduced severity of postharvest decay. Handling fruit properly at harvest, as fungi often enter through wounds so never include fruit for storage that has fallen on the ground or has been in contact with grass or soil, use wood chips where bins are hegd to minimize contact with soil (Kupferman, 1990).

2.6.3 Biological control using bacteria:

So far, only three strains of bacteria have been registered and are commercially available for use as antagonistic microoroganisms for biological control of plant diseases, they are: *Agrobacterium radiobacter K-84*, sold as Gallex® or Galltrol® for being used against crown gall; *Pseudomonas fluorescens*, sold as Dagger® g for being used against *Rhizoctonia* and *pythium* damping- off of cotton; and *Bacillus subtilis*, sold as Kodiak® used as a seed treatment and postharvest biological control agent of stone fruit brown rot (Agrios, 1997; Pusey and Wilson, 1984).

Then other studies have been finally appeared that increased the information on anagonistic microorganisms such as *Enterobactor cloacae* (Wilson et al., 1987; Qing and Shiping, 2000). Also Pseudomonas species had a biological effect against postharvest rot of nectarines and peaches (Smilanick et al., 1993).

2.6.3.1. Pantoea agglomerans Eps 125:

Treatment of stone fruits (Apricot, Peach and Nectarine) with *Pantoea agglomerans* strain EPS125 decreased the incidence and diameter of lesions of brown rot caused by *Monilinia laxa* and soft rot caused by *Rhizopus stolonifer*. Rot control was achieved on fruits either wounded and subsequently inoculated with the pathogens or non- wounded and naturally infected from orchards. The efficacy of biocontrol was dependent on the concentration of the biocontrol agent and pathogen. At medium to low pathogen dose, optimal concentrations of *P. aggolmerans* EPS 125 were above 10^7CFU/ml.The medium effective dose of EPS 125 was 2.2×10^5 CFU/ml in case of controlling *R. stolonifer*. Significant inhibition of conidial germination and hyphal growth of *R. stolonifer a*nd *M.laxa* was achieved when the fungal and EPS cells were cocultivated on appel leachate on nectarine juice.

However, no effect was observed when the antagonist and the pathogen cells were physically separated by a membrane filter which permits nutrient and metabolite interchange. Therefore, wound colonization and direct interaction between the strain and the pathogen cells is necessary for antagonism, which propsed as the mechanism of biocontrol, without a significant contribution of the production of antibiotic substances or nutrient competition (Bonaterra et al., 2003).

2.6.3.2. Pantoea agglomerans CPA-2*:*

Two hundreds fourty seven apiphytic microorganisms isolated from the fruits and leaf surfaces of apples and pears were tested for antagonistic properties against *Penicilluim expansum*, *Botrytis cinerer*a and *Rhizopus stolonife*r. A bacterium strain identified as *Pantoea agglomerans* (CPA-2) was selected (Nunes et al., 2001). Complete control at the three tested concentrations (2×10^7,8×10^7 and 1×10^8CFU/ml) was obtained on wounded pears inoculated with 10^3, 10^4, and 10^5 condia /ml of each *P. expansum* and *R.. stolonifer*, respectively. In over 3 years of experiments in semicommerical trials, *P. agglomerans* CPA-2 provided excellent control against the pervious pathogens, it grew well inside wounded of pears at both room and cold temperatures and under modified atmospheres. In contrast, it grew poorly on the surface of intact fruit (Nunes et al., 2001).

2.6.3.3 Pseudomonas syringae:

This starin of bacteria acts as an active ingredient in Bio-save 11 LP,a biological – based decay control product. It was recently registered by the U.S envirommental protection agency (EPA) for aiding in control of *Rhizopus soft rot* on sweet potatoes. Bio-save 11 LP is marketed as a frozen powdered formulation (Holmes, 2005). Efficacy data against *Rhizopus soft rot* is limited but very encouraging. Two small trials in 2004 in which sweet potato roots were impact- wounded and inoculated with spores of *Rhizopus stolonifer*. Inoculated roots were submerged for thirty seconds in a bio- save 11 LP solution (799 grams of Bio- save 11 LP per 40 gallons of water). This treatment resulted in an average of 95 percent control of *Rhizopus soft rot* compared to no control in the untreated check and

average 58 percent control by Botran® (dicloran) treatment (0.25 pound or 113 grams per 40 gallons). Bio- save 11 LP should not be added directly to waxes, soaps, sanitizers or chlorinated water. The product should be applied to freshly washed sweet potatoes and recycled suspension need to be recharged periodically throughout the day. It is a natural product that provides an alternative control method for decay control for packers shipping to market which do not accept Botran®-treated sweet potatoes (Holmes, 2005).

2.6.4. Biolobical control using fungi and yeasts:

So far, only three strains of fungi have been registered and are commercially available for use as antagonistic fungi, they are: *Gliocladuim virens*, sold as Glio G® for control of seeding diseases of ornamental bedding plants, *Trichoderma harzianum*, sold as F- stop® and others, for control of seedling diseases of ornamental bedding plants, *Trichoderma harzianum*, sold as F-stop® and others, for control of several soil borne plant pathogenic fungi, and *T. harzianum* / *T. polysporum*, sold as Binab T® for control of wood decays (Agrios, 1997). Most postharvest rots of several fruits could be reduced considerably by spraying with spores of antagonistic fungi and saprophytic yeasts to different stages of fruit development, or by dipping the harvested fruit in their suspensions. Several antagonistic yeasts (as a unicellular fungi) protected grapes and tomatoes from *Botrytis cinerea*, *Penicillium expansum, Monilinia fructicola, and Rhizoctonia rots* (Agrios, 1997; Karabulut and Baykal, 2003). The yeast *Candida oleophil*a was approved for postharvest decay control in citrus apples under the trade name Aspire® (Agrios, 1997), Also, *K. apiculata* partially controlled postharvest *Rhizopus* rot of peaches (Mc Laughlin et

al., 1992; Qing and Shiping, 2000). Roberts (1990) discovered that *Cryptococcus laurentii* has antagonistic activity against many postharvest pathogens, *Rhodotorula glutinis* also has limited effect on *Rhizopus* rot in apple, table grapes and strawberries (Lima et al., 1998; Qing and shiping, 2000;Lima et al., 1997) mentioned that treated strawberries with *Aureobasidium Pullulans* yeast before storage reduced 70% of decay caused by *Rhizopus* spp.

2.6.4.1.Candida guilliermondii:

Postharvest rot of peach fruits was studied in vitro and in vivo under different storage temperatures using *Candida gulliermondii*. To show if the presence of *C. guilliermondii* had any antagonistic effect against *Rhizopus stolonifer*, and what is the mode of action that *C. guilliermondii* may use its biocontrol efficacy against *R. stolonifer*. *C. guilliermondii* at 5.0×10^{8}CFU/ ml of washed cells provided complete control of 5×10^{4} spores /ml of *R. stolonifer* during storage at 25C°for 4 days, 15C° for 7 days and 3C° for 30 days. Temperature had no significant effect on the biocontrol efficacy. Cell free culture filtrate of *C. guilliermondii* was not effective in preventing decay and resulted in even greater lesion diameter than those of sterile distilled water at 3C°. These results showed that competition for nutrient, but not antibiotic production plays a major role in the biocontrol capability of *C. guilliermondii* against *Rhizopus* rot of peach fruits. As the interval between wounding and inoculation with the pathogen increased from 0 to 72h, susceptibility of wounds to decay by *R. stolonifer* decreased from 100% of 48h and 40 % of 72h (Fan et al., 2000).

2.6.4.2 Pichia membranefaciens:

A new yeast antagonist, *Pichia memberanefaciens*, isolated from wounds of peach fruit, was evaluated for its biocontrol capability against *Rhizopus stolonifer* on nectarine fruits at different temperatures and with other treatments. *P. membranefaciens* at 5×10^8 CFU/ml of washed cell suspension completely inhibited *Rhizopus* rot in nectarine wounds artificially inoculated with 5×10^4 spores per ml at 25, 15, and 3°C. A culture filtrate of the yeast antagonist failed to provide any protection against *Rhizopus* rot in nectarine fruits compared with the washed cells, which supported the premise that competition for nutrients may play a major role in the biocontrol capability of *P. membranefaciens* against *R. stolonifer.* the importance of nutrient competition has been previously demonstrated with other antagonistic yeasts (Droby and Chalutz, 1994; Janisiewicz and Roitman, 1988). The yeast mixed with iprodione at 100 mg a.i./ ml gave better control of *R. stolonifer* than either yeast or iprodione alone. A solution of 20g CaCl2 per litter enhanced the efficacy of *P.membranefaciens* (10^7 to 10^8 CFU/ml) as an aqueous suspension. This is due mainly to the role of calcium in amelioratin of physiological disorders and thus indirectly reducing pathogen activity (Conway et al., 1992). The role of calcium in resistance may be in interfering with the activity of pectinolytic enzymes (Conway, 1984). Rapid colonization of the Yeast in wound was observed during the first 48h at 25 and 15 C°and then stabilized for the remaining time. As previously ovserved for other antagoistic yeasts (Piano et al., 1997; Mercier and Wilson, 1995). *P.membranefaciens* at 5×10^8& CFU/ml was effective when applied O2 to 72h before the pathogen, while at 1×10^8 CFU/ml, its efficacy was best when applicaction

was significantly reduced when the yeast was applied simultaneously with the pathogen, with disease incidence of 60% and lesion diameter of 37mm (Qing & Shiping, 2000). Some reports have demonstrated that a direct relationship exists between the population density of an antagonist and the efficacy of postharvest biological control treatment (Hong et al., 1998; Janisiewicz, 1988).

3. Blue mold

Description

3.1 Identification and classification

Penicillium expansum can be classified as a cosmoplitan filamentous higher fungi. It belongs to the kingdom Eucaryota and division Deuteromycetes, or Fungi imperfect. The name *Penicillium expansum* comes from the word "brush"; this refers to the appearance of spores in the fungus. *Penicillium expansum* have branched conidiospores. Conidia are round and unicellular. Glucans are common in the cell walls of *Penicillium expansum* tend to have small hyphae. This makes protoplasmic movement difficult to detect. The small hyphae also lead to smaller peripheral growth zones. Penicillium spores have a hydrophobic surface. However, they are capable of being wetted; this is necessary for germination to occur. Penicillium are osmotolerant, meaning that although they grow better with high water levels, they are able to tolerate low water potential. *Penicillium expansum* are heterotrophic. The pathogenic species feed off of the fruit they destroy. It produces asexually, and are unable to sporulate when submerged. However, they begin reproduction easily when hyphae emerge into a gas phase. No species has the exact same method of reproduction.

Each species is classified based on the way it reproduces. For example, in some species, conidia are borne on phial dies, which group out of the conidiophore. In others the conidiophore bears metullae, where phialidies are borne. Still in others the conidiophore may branch out before bearing metullae. Branching may or may not be symmetrical, depending on species. Sporulation is not stimulated by changes in oxygen, carbon dioxide, or water loss. Instead, it is associated with change in physical environment at the hyphal surface. There is no specific method for ascospore dispersal (Talyor et al, 2005).

3.2. Distribution

Blue mold is a common postharvest disease on apples and pears worldwide. This disease is an economic concern not only to the fresh-fruit industry but also to the fruit-processing industry because some strains of *Penicillium expansum* produce the mycotoxin patulin, which can rise to unacceptable levels and thus affect the quality of apple juice. (Rosenberger et al, 1990)

3.3 symptoms

Blue mold originates primarily from infection of wounds such as punctures, bruises and limb rubs on the fruit. Blue mold can also originate from infection at the stem of fruit. Stem-end blue mold is commonly seen on d'Anjou pears, but it also occurs on apples such as Red Delicious. Calyx-end blue mold occurs on Red Delicious apples but is usually associated with fruit that are drenched prior to storage. The decayed area appears light tan to dark brown. The decayed tissue is soft and watery and the lesion has a very sharp margin between diseased and healthy tissues.

Decayed tissue can be readily separated from the healthy tissue, leaving it like a “bowl”. Blue or blue-green spore masses may appear on the decayed area, starting at the infection site. Decayed fruit has an earthy, musty odor. Under cold storage conditions, blue mold lesions (from wound infections) caused by the *P. expansum* may be expected to be one to one and a quarter inches in diameter eight to ten weeks after infection. In rots caused by other *Penicillium* species, the decayed tissues are firmer, may lack surface growth under cold storage conditions, and are slow-growing compared with *P. expansum*. The importance of these species should not be ignored, since, it is probable that under certain conditions, they may assume considerable importance (Janisiewcz, 1999).

3.4 Biology and life cycle

In the orchard, *Penicillium* spp. survive in organic debris on the orchard floor, in the soil, and perhaps on dead bark on the trees. Conidia are also present in the air and on the surface of fruit. In the packinghouse facility, diphenyl amine- or fungicide-drench solutions, flume water and dump-tank water are common sources of *Penicillium* spores for fruit infection during the handling and packing processes. Spores of *P. expansum* are also commonly present in the air and on the walls of storage rooms. *P. expansum* is essentially a wound pathogen. Wounds on the fruit skin such as punctures and bruises that are created at harvest or during the postharvest handling process are the major avenue of invasion by the fungus. Fruit with wounds can be inoculated with spores of thiabendazole-resistant isolates of *P. expansum* during postharvest drenching with diphenylamine and Mertect (thiabendazole or TBZ). Fruit may also be inoculated with *Penicillium* during the packing process. *P. expansum* can

also cause decay through infection at lenticels, but this type of infection usually occurs on over-mature fruit or when lenticels have been injured. More than 50% of the *P. expansum* isolates recovered from decayed fruit collected from packinghouses in Washington State are resistant to TBZ, whereas only approximately 3% of the *P. expansum* isolates from apple orchards are resistant to TBZ. A prestorage application of TBZ is likely the major source of TBZ-resistant strains (Rosenberger et al, 1990).

The *Penicillium expansum* type of blue mold has been the form most frequently reported, but a number of other less common species, which are also usually less aggressive, have been encountered. The positive identification of the different species that cause blue mold is only possible by means of laboratory cultures and microscopic examination, and even then positive determination is difficult because of the very slight differences encountered among species. All of the blue molds are primarily wound parasites, most frequently gaining entrance through fresh mechanical injuries such as stem punctures, bruises and insect injuries, finger-nail scratches by pickers, necrotic tissues of diverse origin or through normal stems or open calyx canals. Sometimes infections may occur through lenticels, especially when they are damaged by cracking after a sudden abundant supply of water following a period of dryness, or after bruising late in the storage season when fruit have been weakened by ripening and aging. The blue mold spores are long-lived and may easily survive from season to season on contaminated bins, where the fungus can grow and produce copious amounts of spores. Contamination with these spores may come from various other sources including orchard soil carried on bins from the orchard, decaying fruit or air. Inoculation of the fruit

going into storage is believed to occur mainly from the diphenylamine (DPA) drenching solution used for protection against superficial scald, where the spore concentrations increase with each successively drenched bin and may reach high levels if solutions are not changed regularly. Inoculation can also occur during fruit handling in water contaminated with the fungus in packinghouses. A single decayed fruit may contain enough spores to contaminate water on the entire packing line (Janisiewcz, 1999).

3.5. Control of *P.expansum*

3.5.1. Chemical control

Thiabendazole (TBZ) is commonly used as either a prestorage drench treatment or a line spray to control gray mold and blue mold. TBZ is effective to control gray mold but is not effective to control TBZ-resistant *Penicillium*. Two new postharvest fungicides, fludioxonil (Scholar) and pyrimethanil (Penbotec), can be used as drenches, dips or line sprays and have been reported to be effective to control blue mold originating from wound infections (Rosenberger et al, 1990) Chemical control kills the blue mold spores in dump tanks, on bins, or in flume water with chlorine (100 ppm) or sodium O- phenylphenate (SOPP) (0.3 to 0.5%) has been effective in reducing the spore load and the resulting amount of decay. Chlorine and diphenylamine (DPA) are not compatible, so for fruit requiring DPA treatment (for superficial scald), chlorine must be allowed to dissipate before treatment with DPA. Ozone treatment, although not yet frequently used, can also be effective. However, as with chlorine, ozone has no eradicate or residual effect. Both of these treatments are most effective in conjunction with other sanitary measures that prevent exposure

of fruit to reinoculation with fungal spores. The thiabendazoles (e.g. Mertect 16 fl oz/100gal) are the main fungicides available to combat decays in storage, and Captan 50W (2.5 lbs/100gal) is used to a lesser extent. Captan has been used mainly in combination with other fungicides because it acts as a protectant and has limited effectiveness. In areas where fungicide resistant strains of the fungus have developed, treatments with these fungicides may be unsuccessful (Janisiewcz. 1999)

Postharvest fungicidal treatment on apples is considered the main control measure that can be applied to decrease or to prevent the losses caused by Postharvest pathogens. For example, imazalil (Freshguard® and fungaflor®) is one of the most common systemic fugicides that can be effectively used in control of Posthavest fungal decay especially apples (Nunes et al., 2001)

3.5.2 Cultural control

Orchard sanitation to remove decayed fruit and organic debris on the orchard floor helps reduce inoculums levels of *Penicillium* spp. in the orchard. Good harvest and handling management to minimize punctures and bruises on the fruit, helps prevent the fruit from infection at wounds by *P. expansum* and other *Penicillium* species (Rosenberger et al, 1990). General sanitation and avoidance of conditions favorable to infection are very helpful. The general aim of sanitary practices is to reduce the available supply of fungal spores to the lowest possible point for any given environment. This includes reducing contamination of bins with orchard soil, which is a reservoir for the spores, sterilization of contaminated bins and packing machinery, and frequent changes of solutions and water used

for drenching and handling fruit. Fruit should be picked at the proper maturity (not over-mature) and placed in cold storage as soon as possible. Picking wet fruit should be avoided. Bins containing harvested fruit in an orchard should be protected from rain so fruit will not become wet. The avoidance of fruit injuries, gentle handling of fruit by pickers during harvesting and care during the transportation of fruit from the orchard to the packinghouse may prevent many injuries. Attention should be given to mechanical features of the handling machinery in packinghouses to eliminate sources of injury from rough corners, unnecessary drops or gravity runs, or hard or unprotected receiving surfaces (Janisiewcz, 1999).

3.6. Biological control

3.6.1 Using biofumigant fungus *Muscodor albus*

The potential of the volatile-producing fungus *Muscodor albus* for controlling postharvest diseases of fresh fruit by biological fumigation was investigated. In vitro tests showed that *M. albus* volatiles inhibited and killed a wide range of storage pathogens belonging to species of *Botrytis*, *Colletotrichum*, *Geotrichum*, *Monilinia*, *Penicillium* and on autoclaved grain gave complete control of blue mold (*Penicillium expansum*) and gray mold (*Botrytis cinerea*) in wound-inoculated fruits. There was no direct contact between the fruit and the *M. albus* culture. Shorter fumigation times ranging between 24 and 72 h, applied immediately or 24 h after inoculation, also controlled blue mold and gray mold. In wound-inoculated peaches, 24–72 h fumigation with *M. albus* provided complete control of brown rot (*Monilinia fructicola*). The volatile profile *Rhizopus*. Fumigation of apples for 7 days with culture of *M. albus* grown of *M. albus*-colonized

grain was measured by gas chromatograph connected to a flame ionization detector (GC-FID) and showed that 2-methyl-1-butanol and isobutyric acid were the major volatile compounds found in the headspace. Since *M. albus* is a sterile mycelium and does not require direct contact with the crops to be treated, it could be an attractive biological fumigant for controlling postharvest diseases (Mercier and Jimenez, 2004).

3.6.2 Using *Candida sake* under several controlled atmosphere conditions.

The biocontrol potential of the yeast *Candida sake* (CPA-1) against *Penicillium expansum* decay of apples under several controlled atmosphere conditions was investigated. In a laboratory trial under different commercial cold storage conditions, increasing concentrations of *C. sake* improved decay control. A maximum reduction of decay was achieved at 3% O2-3% CO2 atmosphere. It amounted to a 97% lesion reduction after treatment with a suspension containing 2.4 x 10^6CFU/ml of *C. sake* (CPA-1). In a semi-commercial trial at 1 degree C with wounded fruits, the reduction in decay diameter caused by C. sake exceeded 80% after 60 days at 21% O2 and 60% after 120 days of storage under controlled atmosphere conditions. For seven controlled atmosphere conditions studied, a significant influence by *C. sake* on the *P. expansum* decay was observed, and the lesion size was reduced more than 70% by *C. sake* at 10^7 CFU/ml. The populations of *C. sake* (CPA-1) on the apple surface followed the same pattern under all controlled atmosphere conditions studied. They decreased 4-10-fold during the first 2 weeks, followed by an increase to the initial level after 45 days, and thereafter the count remained constant for the period of 90 days examined. This indicated the capacity of *C. sake* (CPA-1)

to colonize the surface of apples under various storage conditions. The ability to colonize was even higher in apple wounds (Usall et al, 2001).

3.6.3 Using a combination of *Candida sake* and *Pantoea agglomerans*

The effectiveness of *Candida sake* (CPA-1) in combination with *Pantoea agglomerans* (CPA-2) for controlling *Penicillium expansum* and *Botrytis cinerea* on pears and apples was determined. The concentrations tested were 2 x $10^6$and 2 x 10^7 CFU/ml for C. sake and 2 x 10^7 and 8 x 10^7CFU/ml for *P. agglomerans*. At room temperature, the two antagonists were combined in proportions of 0 to 100% in 25% increments. At the proportion of 50:50, no rot development was observed in pears, and the greatest control of blue mold in apples was observed at this proportion for all the tested concentrations. Under cold temperatures on pears, the highest effectiveness of the mixture was observed when *C. sake* at 2 x 10^7 CFU/ml was combined with *P. agglomerans* at 2 x 10^7 or at 8 x 10^7 CFU/ml at the proportion 50:50. Under these conditions, no rot development of blue mold was reported, and gray mold lesion size was reduced by more than 95%. On apples, the mixture of *C. sake* at 2 x 10^7 CFU/ml and *P. agglomerans* at 8 x 10^7 CFU/ml at the proportion 50:50 reduced blue and gray mold incidence by 90%. Populations of the two antagonists had the same growth pattern at 20 °C when they were applied individually or in combination, but the population level was always higher when they grew alone. In contrast, at 1 degrees C, the population of both antagonists in combination formed a stable community with the same levels as individual application during the first 30 days; after that, *C. sake* dominated, and *P. agglomerans* decreased on apples and pears. At both temperatures, the maximum population level

of *C. sake* was observed in apples, and the maximum population level of *P. agglomerans* was observed in pears (Nunes et al., 2002).

3.6.4 Combining heat treatment, calcium infiltration, and biological control

The viability of *Penicillium expansum* Link conidia in sporulating culture declined rapidly when exposed to 38 °C, and when conidia were exposed to 38 °C prior to inoculation of apple fruits, the resulting lesions were smaller than those on fruit inoculated with nonheated conidia. Apples were heated after harvest (38 °C for 4 days), pressure infiltrated with a 2% solution of $CaCl_2$, or treated with the antagonist *Pseudomonas syringae* van Hall, alone or in combination to reduce postharvest decay caused by *Penicillium expansum*. After up to 6 months in storage at 1 °C, no decay lesions developed on fruit that were heated after inoculation with *P. expansum*, or any combination of *P. expansum*, antagonist, or Ca. Parallel lots of heat-treated and nonheated fruit that were either infiltrated or not infiltrated with Ca were stored up to 6 months. They were then inoculated with *P. expansum* alone, or with the antagonist followed by *P. expansum*. Prior heat treatment did not influence lesion size. Calcium alone, the antagonist alone, and heat plus Ca all reduced the incidence of decay by ≈25%, whereas heat plus the antagonist reduced it by 70%. Calcium plus the antagonist or Ca plus the antagonist and heat reduced decay incidence by 89% and 91%, respectively. The integrated strategy of heat-treating fruit, followed by Ca infiltration and then treatment with an antagonist, may be a useful alternative to controlling postharvest decay with fungicides (Conway et al., 1999).

3.6.5 Integrating heat and antagonist treatments on 1-MCP treated fruit stored under controlled atmosphere conditions

To maximize control of fruit decay by alternatives to synthetic fungicides after harvest, various control strategies could be integrated. Treatment of fruit with antagonists is one of the most promising alternatives. This treatment, however, has little or no eradicative activity, which limits its use. Fruit treatment with hot air (at 38 °C) for 4 d has eradicative effect but no residual activity against blue mold (caused by *Penicillium expansum*) on apple, and 1-methylcyclopropene (1-MCP) is an ethylene receptor inhibitor which slows apple maturation and, presumably, extends action of natural defense mechanisms. An antagonist, *Metchnikowia pulcherrima* T5-A2, was used in combination with heat and 1-MCP treatments to control bitter rot (caused by *Colletotrichum acutatum*) and blue mold (caused by *P. expansum*) on 'Golden Delicious' apples under controlled atmosphere (CA) conditions. 1-MCP treatment increased bitter rot and blue mold decays, but both of these decays were effectively controlled on 1-MCP treated apples by a combination of the antagonist and heat treatments. *C. acutatum* is a weaker pathogen than *P. expansum*, and bitter rot, even on the control treatments, developed only after 4 months in CA storage followed by 2 weeks incubation at 24 °C. In contrast, non-treated fruit inoculated with *P. expansum* were completely decayed after 2 months in CA. The antagonist controlled bitter rot more effectively than blue mold, while blue mold was more effectively controlled by heat treatment. The use of 1-MCP on harvested fruit to inhibit maturation can predispose fruit to decay, but the alternatives to synthetic fungicides are capable of preventing this increase in decay (Janisiewicz et al., 2003).

3.6.6 Using *Pseudomonas fluorescens*

Pseudomonas fluorescens isolate 1100-6 was evaluated as a potential biological control agent for apple blue mold caused by *Penicillium expansum*. Both the wild-type isolate 1100-6 and a genetically modified derivative labeled with the gene encoding the green fluorescent protein (GFP) were compared. The *P. fluorescens* isolates with or without GFP equally reduced the growth of *Penicillium expansum* and produced large zones of inhibition in dual culture plate assays. Cell-free metabolites produced by the bacterial antagonists reduced the colony area of Penicillium isolates by 17.3% to 78.5%. The effect of iron chelate on the antagonistic potential of *P. fluorescens* was also studied. The use of iron chelate did not have a major effect on the antagonistic activity of P. *fluorescens*. With or without GFP, *P. fluorescens* significantly reduced the severity and incidence of apple decay by *P. expansum* isolates after 11 d at 20 degrees C and by *P. expansum* after 25 days at 5 degrees C when the biocontrol agents were applied in wounds 24 or 48 h before challenging with Penicillium spp. Populations of *P. fluorescens* labeled with the GFP were determined 1, 9, 14, and 20 d after inoculation at 5 degrees C. The log CFU/mL per wound increased from 6.95 at the time of inoculation to 9.12 CFU/mL ($P < 0.05$) 25 d after inoculation at 5 degrees C. The GFP strain did not appear to penetrate deeply into wounds based on digital photographs taken with an inverted fluorescence microscope. These results indicate that *P. fluorescens* isolate 1100-6 could be an important new biological control agent for apple blue mold (Etebarian et al., 2005).

3.6.7 Involvement of hydrogen peroxide in the development of resistance mechanisms in apple fruit

Apples (*Malus domestica* L. cv Golden Delicious) were picked 7 days before (harvest 1), or after (harvest 2) the commercial harvest time. Changes in H_2O_2 levels and in the activity of the H_2O_2-generating enzyme superoxide dismutase (SOD) and H_2O_2-scavenging enzymes catalase (CAT) and unspecific peroxidase (POX) were estimated immediately after harvest and during the first 24 h following wounding and inoculation with *Penicillium expansum*. Fruit from harvest 1 had lower decay incidence and severity of infection than those from harvest 2. Immediately after wounding or inoculation, the less susceptible fruit (harvest 1) showed a significant increase in H_2O_2 levels concomitantly with higher activity of SOD. No significant changes in CAT and POX were found. In susceptible fruit (harvest 2), both H_2O_2 levels and SOD activity in wounded/inoculated fruit remained similar to those in control fruit. In contrast, CAT and POX activity significantly increased as a consequence of wounding and inoculation. Collectively, these results provide evidence that harvest date is important in determining the susceptibility of 'Golden Delicious' fruit to *P. expansum*, and that H_2O_2 and associated metabolism induced by wounding could play a role in defence mechanisms in this fruit(Torres et al., 2003).

3.6.8 Effect of hexanal vapor on Spore Viability of *Penicillium expansum*

The effects of hexanal vapor on spore viability of *Penicillium expansum*, lesion development on whole apple fruit, and flavor volatile biosynthesis were investigated. Spore viability was reduced by 94% after

exposure to a hexanal concentration of 40 mmol/L for 24 h, compared with 50% at 18 mmol/L and 20% at 9 mmol/L. Decay on whole apple fruit inoculated with 5×10^4 spores/mL of *P. expansum* was reduced with exposure to hexanal vapor for 48 h. Although almost all of the fruit treated with 8 to 12 mmol/L developed decay lesions, lesion size was reduced compared with the controls. At concentrations of 15 to 19 mmol/L, and 25 to 29 mmol/L, the incidence of fruit with lesions was 44% and 24%, respectively, compared with 100% and 98% in the inoculated control apples and lesion size was further reduced. Apples treated at 4 °C with only 5 to 7 mmol/L hexanal vapor also showed a marked reduction in lesion incidence. Hexanal was rapidly converted to high levels of the aroma volatiles hexanol, hexylacetate, hexylbutanoate, and hexylhexanoate, but these decreased to levels similar to the control after 4 to 7 d of being held in air. There was no detectable hexanal after holding fruit in air for 5 h (Fan et al., 2006)**.**

4. *Trichoderma harzianum* Rifai

4.1. Description:

Trichoderma is among the most common saprophytic fungi. All of them within the subdivision Deuteromycotina. Most *Trichoderma* strains have no sexual stage, but instead produce only asexual spores. For a few strains, the sexual stage is known; however, these do not include strains that have usually been considered for biocontrol purposes. The sexual stage, when found, is within the Ascomycetes in the genus Hypocrea (Monte, 2001). Colonies of *Trichoderma* grow rapidly and mature in 5 days at 25C and on potato dextrose agar, the colonies are wooly and become

compact in time, the color is white, yellow, or green cushions of sporuating filaments (De Hoog et al., 2000; St-Germain and Sumerbell, 1996). Colonies have either floccose or elliptical conidia, or tufted non-floccose globes, conidia are single-celled, usually green (typically 3µm in diameter) while typical fungal Hyphae are 5 to 10 µm diameter. Conidia are smooth- or rough – walled and grouped in sticky heads at the tips of the phialides (hyaline, flask-shaped and inflated at the base). These clusters frequently get disrupted during routine slide prepatation procedure for microsopic examination (Sutton et al., 1998; Kubicek and Harman, 1998). Recent taxonomy has gone from consisting of nine to at least 33 species. As an example, the best biocontrol specis *T. harzianum* which is tolerant to stress imposed by mutrient scarcity, has been separated into an array of species *T. harzianum*, *T. inhamatum*, *T. longibrachiatum*, *T. atroviride* and *T. asperellum*, (Hermosa et al., 2000; Monte, 2001; Hagedorn, 2004; Kuhls et al., 1999). Morphological features of the conidia and phialides help in differentiation of these species from each other, the most secure way for most investigators to identify a species of *Trichoderma* is through DNA sequences, in the mid 1990's DNA sequences provided the much-needed independently derived data that would enable a better understanding of species of *Trichoderma* (Gams and Bissett, 1998; Kinderman et al., 1998; Kulhls et al., 1997).

4.2 Distribution:

Trichoderma is widely distributed in plant material, decaying vegetation, wood, and in almost all soils. *Trichoderma* is able to grow in soils having a PH range from 2.5-9.5, although most prefer a slight to moderately acidic environment (Hagedron, 2004). They have been

considered to be at least partially reponsible for the control of suppressive soils, soils on which crops or trees are unaffected by a given pathogen (Agrios, 1997: Games and Bissett, 1998). *T. harzianum* or *T. hamatum* identified as two of the usual soil species exert their effects by competing for nutrients and producing toxins against phytopathognic species (Bora et al., 2000). Several new species of *Trichoderma* from eastern and Southeast Asian soils have been recently described by John Bissett and his collaborators (Bisset et al., 2003).

4.3.Host plant:

Trichoderma has very wide host range, since *Trichoderma* species are found in almost all soils (Hagerdon, 2004). Once established in a host plant, vegetables, fruits, ornamentals, *Trichoderma* has been shown to co – exist for up to five years, it has been found that plant benefits correlate with increased population of *Trichoderma*. In other words, the more the better, whether its larger doses or more frequent application- or both (Winter, 2000).

4.4. Pathogenicity:

The most commonly reported biocontrol agent of *Trichoderma* is *T. harzianum*. However, this species was implicated as the cause of the green mould epidemic of commercially grown mushrooms in North America and Europe. The consequences of *T. harzianum* being a pathogen of such an economically important crop as mushrooms would have been disastrous to biological control (Seay, 1996; Samuels and Doder, 2002; Savoie and Mata, 2003).

4.5. Role of *Trichoderma* in controlling Fungi

4.5.1. Fungal diseases controlled by *T. harzianum*:

Many *Trichoderma* strains have been identified as having potential applications in biological control, they are effective against a wide range of plant pathogenic fungi inculuding: *Armillaria, Botrytis, Colletotrichum, Dematophora, Endothia, Fulvia, Fusarium*, *Chondrostereum, Fusicladium, Macrophomina, Monilia, Nectria, Phoma, Phytophthora, Plasmopara, Pseudoperospora, Pythium, Rhizoctonia, Sclerotinia, Sclerotium, Venturia, Verticillium*, and wood-rot fungi (Monte, 2001; Harman, 2000, Agrios, 1997; Batta, 2004; Sawant et al., 1995). Many recent studies have been-demonstrated the effect of *T. harzimum* on postharvest diseases which cause fruit rot, for example, significant curative and preventive effect was provided by the antagonistic strain *Trichoderma* – Th 1 of *T. harzianum* against *Alternaria atternata* causing black fruit spot on Persimmon fruits (Batta, 2001). This disease infects fruits in the field near the harvesting time, but develops during the postharvest period causing fruit rot (Batta, 2001). Another significant effect was obtained in controlling *Penicillium expansum*, the causative fungus of blue molde on apples, through studying the effect of treatment with *T. harzianum Rifai*formulated in invert emulsion of postharvest decay of apple blue mold (Batta, 2004). Significant differences were obtained between means of percent reduction in decay-lesion diameter relative to sterile distilled water control in the treatments with formulated and non formulated conidida in invert emulsion (48.8%, 24.8% and 0.6%, respectively). Also, a significant long period of protection from *P. expansum* infection (up to 2 months) was also obtained when unwounded apple fruits were dipped for 30 second period in formulated *T.*

harzianum conidia before being inoculated by P. *expansum* compared to the wounded fruits. This indicates the importance of this type of treatment in protecting apple fruits from blue mold infection for long time at postharvest stage without refrigeration (Batta, 2004). *T. harzianum* are also used in biological control of damping- off diseases caused by *Pythium* species and *Rhizotonia* (Agrios, 1997; Harman, 1998; Biswas, 1999; Dutta and Das, 1999; Omarjee et al., 2001). *Botrytis cinerea* is another postharvest disease that causes grey mold on apple, it was biologically controlled by *T. harzianum Rifai* formulated in invert emulsion (Batta, 1999; Batta, 2003). Formulated *T. harzianum* conidia in invert emulsion had a significant preventive effect against *B.cinerea* on wounded apple fruits compared to non –formulated *T. harziamum* conidia and control treatments. The diameter of typical *Botrytis* lesions on treated apple fruit was significantly reduced. In addition, the application of formulated *T. harzianum* conidia inhibited *Botrytis* sporulation (no production of conidia) on the surface of typical *Botrytis* lesions. Dipping healthy apple fruit in formulated conidia of *T. harzianum,* followed by inoculation with *B. cinerea* by spraying a conidial suspension of the pathogen on the treated fruits, protected treated fruits from infection with *B. cinerea* for 16 days, when using micro- wounded fruits. According to Batta (2003), formulation of invert emulsion was characterized by the low viscosity and contained both coconut and soybean oil with tow emulsifiers (oil-soluble emulsifier tween 20 and water-soluble emulsifier dehymuls k). The invert emulsion produced was stable and compatible with the Th2 strain of *T. harzianum* conidia in this formulation remained viable much longer than non formulated conidia of the same strain held at 20±1 C°and 30 % ambient RH. The ingredients of the invert emulsion especially oils and emulsifiers

are safe and not toxic to apple fruit. These ingredients are also likely to be non- toxic to humans as they are also used as food additives and in the manufacture of cosmetics (Batta, 2003).

4.5.2 The commercial products of T. harzianum

4.5.2.1. Type, formulation and methodof application of commercial strains products.

Biocontrol agents: these fungi are used, with or without legal registration, for control of plant diseases (Harman, 1998), it has been investigated as biological control agent for over 70 years (Samuels, 1996), but only relatively recently have strains become commercially available on the open market (Monte, 2001; Fravel, 2002; Harman, 2000). Other commercial products of *Trichoderma* which are under registration or on the open market are: *Trichodex* (Israel) against Botrytis of vegetables and grape vines, Soil gard (USA), supresivit (Denmark), Tusal (Spain), and *Trichoderma* 200 (Israel) are used against damping – off diseases caused by *Pythium*, *Rhizoctonia spp*.(Monte,2001), and *Macrophomia Phaseolina* (Adekunle et al., 2001) as a seed treatrment.

4.5.2.2 Tolerance assessment of using T. harzianum commercial strains products:

An exemption from the requirement of a tolerance for residues of *T. harzianum Rifai* strain T-39 on all food commodities when used as ground and certain foliar applications. This regulation eliminates the need to establish one maximum permissible level for residues of *T. harzianum Rifai* strain T-39, an exemption had been granted since testing of the

biofungicide showed no toxic effect. Another exemption from the requirement of a tolerance for residues of the microbial pesticide active ingredient T. HKRL – AG2, known as stratin T-22 when used as seed treatment, on cuttings and transplants, or as soil application. In a study of the biological efficiency by *Trichoderma* on the germination of winter wheat grain, the isolates Trichoderma also not toxic for germinating plants and in some cases they stimulated the growth of above ground and under ground wheat organs (Michalikova and kohacik, 1992).

4.5.3. Biological activity and mode of action:

Trichoderma spp. have evolved mumerous mechanisms for attacking othe fungi and for enhancing plant and root growth, several new general methods for biocontrol and for enhancement of plant growth have recently been demonstrated, and it is now clear that there must be hundreds of separate genes and gene products involved in the following processes (Agrios, 1997; vinas, 2004; Monte, 2001), known as modes of action:

1) Mycoparasitism: relies on the recognition, binding and enzymatic disruption of the host-fungus cell wall and death of the pathogen by direct parasitism (Goldman and Goldman, 1998; Monte, 2001).

2) Nutrient or site competition: for example, sugars such as maltose, sucrose and glucose, have been suggested to play a role in the bicontrol of moulds by yeasts against diseases (Filonow, 1998).

3) Antibiosis: direct toxic affection the pathogen by antibiotic substances released by the antagonist, the concentrations of the antibiotic in solution (crude filtrates and crude antibiotic solutions)

will be estimated from the probit regression line of inhibition of germination of spores-log concentration of antibiotic as described by Madrigal et al. (1991). This probit of response-log concentration curve will be calculated from the result of the relative toxicity of different concentration levels of the pure antibiotic on the germination of spores of every pathogen by following the probit analysis method (Finney, 1971). From these curves the effective doses (ED) of 50%inhibition for both the germination and the germ tube growth will be calculated.

4) Production of volatile compounds: volatile compound from the biological control agents can be an important factor of the inhibitory mechanism, especially under closed storage condition, such as ethylene, released by the metabolic activities of the antagonist. Effects will be recorded as changes in radial growth, spore formation and CFU·S of the target fungi such as, *Penicillium expansum*, *Botrytis cinerea*, *Rhizopus stolonifer* (Vinas, 2004). If inhibition by volatile compounds is indicated, this will be confirmed by investigating whether the effects can be removed by continuous ventilation. For biological control agents showing a high degree of inhibition through the gas phase a tentative identification of volatile agents will be done through gas- chromatography, using known controls.

5) Induced host resistance: a state of enhanced defensive capacity developed by a plant or plant part when appropriately stimulated and can occur naturally as a result of limited infection by a pathogen (Harman, 2000).

6) Solubiliaztion and sequestration inorganic nutrients: production of hydrolytic enzmes through direct interactions between the Biocontrol agent and the pathogen (vinas, 2004; Altomare et al., 1999).A major part of *Trichoderma* antifungal system consists of a number of genes encoding for an astonishing variety of secreted lytic enzymes, including endochitinases, N-acetyl-ß – glucoseminidases, chitin 1,4-ß-chitobiosidases, proteases, endo-and exoglucan ß -1,3-glucosidases (Haran et al., 1996a), endoglucan ß -1,6-glucosidases, lipases, xylanases, mannanases, pectinases, pectin lyases, amylases, phospholipases, RNases, and DNases (Haran et al., 1996b; De la Cruz et al., 1992; Lorito et al., 1994). Particularly useful for biocontrol applications are chitinolytic and glucanolytic enzymes because of their efficient ability to degrade the cell wall of palnt pathogenic fungi by hydrolyzing biopolymers not present in plant tissues. Studies performed previously have indicated that cell-wall-degrading enzymes (CWDEs) from *Trichoderma* strains have great potential in agriculture as active components in new fungicidal formulations (Benitez et al., 1998). This is because purified CWDEs from different strains of *T. harzinum* are highly effective in inhibiting spore germination and mycelial growth in a broad range of pathogens. In contrast to plant enzymes, chitinases and glucanases from *Trichoderma* can degrade not only the immature wall at hyphal apices but also the strong chitin-glucan complexes of mature cell walls, as well as survival structures such as sclerotia and chlamydospores, which reduce not only disease symptoms but also pathogen spead. In particular, enzymes absent from plants such as ß -1,6-glucanases can degrade important fungal cell –wall structures

such as ß -1,6glucans by linking chitin or ß -1,3-glucans to cell-wall proteins. The antifungal activity of *Trichoderma* CWDEs can be enhanced synergistically by combining enzymes with different lytic activites (such as exo – and endochitinases and / or glucanases). for instance, a combination of an endochitinase, an exochitinase and ß -1,3- glueanase purified from *T. harzianum* has an effective dose (ED50) on *Botrytis* of about 1ppm, which is comparable to the effective dose of most chemical fungicides, fungicides synergistic with the *Trichoderma* CWDEs include several compounds used for chemical control of plant diseases, such as azoles, benzimidazoles and pyrimidines. Tests show that *Trichoderma* chitinases and glucanases have no effect on the plant even when relatively large quantities are injected into plant tissues. CWDEs aer not harmful to humans or animals, as indicated by EPA tests for registration of strains of *Trichoderma* for use as biocontrol agents in the United States, and they degrade into environmentally friendly resideues. CWDEs are particularly suited to postharvest control. Low-temperature controlled storage conditions will favor these applications as the level of enzyme activities will be more easily predicted than in the greenhouse or the field. Purified CWDEs or mixtures of CWDEs with high antifungal activity obtained from *Trichoderma* culture filtrates can be included in commercial formulations since they are easily characterized, stable, resistant to drying, freezing, temperatures up to 60°C, and have broad PH (Monte, 2001).

Chapter Three

Materials and Methods

3. Materials and Methods

3.1 fungal strains used

Pure fungal cultures were used in the experiments. They were: *Trichoderma harzianum* (strain: Th2) obtained from laboratory of plant protection(An- Najah National University), *Botrytis cinarea* (strain: BC1), *Rhizopus stolonifer* (strain: RS1), *Penicillium expansum* (strain: PE 8) isolated by the laboratory of plant protection (An- Najah National University) from infected peach and strawberry fruits.

3.2 Types of fruits used

Four types of mature fruit were picked at harvesting stage to be used in the experiments. They were: grape berries (*Vitis vinifera*; cultivar: Halawani), pear fruits (*Pyrus communis*; cultivar: Costhia), kiwi fruits (*Actinidia chinenesis*; cultivar: Triumph) and strawberry fruits (Fragaria × ananassa; cultivar: 40). All fruits were firstly washed with tap water then disinfected with sodium hypochlorite (0.025 % V/V) before being rinsed 3 times in sterile distilled water then put in closed plastic cans to be protected from contamination during the experiment and to obtain humid chamber conditions.

3.3 Chemical material

Chemicals used in preparation of invert emulsion were: water – soluble wax (Dehymuls K®) (Düsseldors) glycerine (sun pharm drug), vegetable oils (coconut and soybean oils)(Vegetable Oil Industry Co.), tween 20, sterile deionized water. Other chemicals used were: oat meal

used agar (OMA) (Fluka) potato dextrose agar (PDA) (Fluka) sodium hypochlorite for disinfection of fruits.

3.4 Preparation for fungal cultures for experiments

The strains of *Trichderma harzianum* and *Botrytis cinerea*, were subcultured on oat meal agar, whereas *Penicillium expansum* and *Rhizopus stolinifer* were subcultured on potato dextroze agar under aseptic conditions, then plates were incubated at 20±2ºC and 16 hours of illumination per day (growth chamber conditions) for 10-14 days in order to obtain enough quantities of fungal conidia or sporangiospores for inoculation. The fungal growth on plate surface for each strain were scraped with sterile scalpel to make the conidia or spores become suspended in sterile deionized water covering the plate surface. Before being used, the suspension was sieved through 75 – µm mesh then counted using haemocymeter. The concentration of the suspension prepared for the four fungi were: 2.15×10^6 conidia/ ml, 2.1×10^6 conidia/ ml, 2.6×10^6 sporangiospores/ ml, and 1.88×10^8 conidia/ ml for *Botrytis cinerea, Penicillium expansum*, *Rhizopus stolinifer*, and *Trichderma harzianum*, respectively.

3.5 Preparation of invert emulsion and introduction of *Trichoderma harzianum*

The invert emulsion used in our experiments to formulate *Trichoderma harzianum* conidia (strain: Th2) was prepared as described previously (Batta, 2004). Accordingly, it contains the following ingredients (w/w): sterile distilled water (45.25 %), glycerine (4.00 %), water – soluble wax (Dehymuls K®) (0.75 %), tween 20 (2.50 %), and a mixture

of 19.00 % coconut oil + 28.50 % soybean oil. The fungus (*Trichoderma harzianum*) was introduced as conidia into the invert emulsion as described previously (Batta, 2004). The concentration of introduced *Trichoderma harzianum* conidia in the invert emulsion was titrated at 7.2 $\times 10^7$conidia /ml.

3.6 Evaluation of treatment efficacy with *Trichoderma harzianum*

To test the treatment efficacy with *Trichoderma harzianum* against each of the pathogens used in experiments (*Botrytis cinerea*; *Rhizopus stolonifer* and *Penicillium expansum*) on the different types of fruits, 4 types of treatments were used:i) pathogen + *Trichoderma harzianum* in invert emulsion formulated ii) pathogen+ *Trichoderma harzianum* suspended in sterile deionized water (unformulated) iii) pathogen + sterile deionized water as control, and iv) pathogen + blank formulation invert emulsion. The effect of the four treatments on the lesion development resulted from these pathogens on the different fruits was tested at the same time of pathogen inoculation on wounded and unwounded fruits. This is done by depositing 25 –µl droplet taken from formulated *Trichoderma harzianum* conidia in invert emulsion (concentration = 7.2 $\times 10^7$ conidia / ml) or unformulated *Trichoderma harzianum* conidia (suspended in sterile deionized water at a concentration = 3.75 $\times 10^8$ conidia / ml) was applied per fruit. The same droplet size (25µl) was also applied for sterile deionized water treatment (control) and for blank formulation of invert emulsion for the purpose of comparison of inoculation of each pathogen on the different types of fruits was done by depositing 25 – µl droplet of the pathogen suspension (concentrations were indicated in section 3.4) per wound. Iincubation of fruits after inoculation and treatment was carried out

at 18 ± 2ºC and 16 hours of illumination per day under humid conditions in closed plastic cans. The assessment of treatment effect was done by measuring the disease lesion diameter formed around the wounds after 4 days of inoculation and treatment. The means of pathogen – lesion diameter was then calculated.

3.7 Determination of protection period from infection with pathogens after treatment with *Trichoderma harzianum.*

This determination was done on wounded fruits in comparison with the unwounded fruits. Two types of treatment were used during the determination: i) formulated *Trichoderma harzianum* on wounded fruits inoculated with the pathogen (mentioned above), ii) control treatment with blank formulation of invert emulsion on wounded fruits inoculated with the pathogens. The same types of treatment were applied on unwounded fruits for comparison. To carry out each one of the treatment mentioned above, fixed quantity of 2 ml of formulated *Trichoderma harzianum* conidia (concentration1.88×10^8 conidia/ ml) was sprayed per fruit using small hand sprayer. The same quantity (2 ml) was also sprayed per fruit in the control treatment with blank formulation of invert emulsion. Inoculation of each pathogen was carried out by spraying 1 mL of each pathogen conidial suspension (concentrations: 2.15×10^6 conidia/ ml for *Botrytis cinerea,* 2.1×10^6 conidia / ml *Penicillium expansum*, 2.6×10^6 sporangiospores/ ml for *Rhizopus stolinifer*, and 1.88×10^8 conidia/ ml for *Trichderma harzianum*) per fruit. Microwounds were made by needle pricks. Incubation of fruits after inoculation and treatment was conducted at 18±2ºC in closed plastic cans until being evaluated. The minimum protection period from infection with each pathogen on each fruit type after

treatment with *Trichoderma harzianum* formulated in invert emulsion was determined.

3.8 Experimental design and analysis of data

The completely randomized design (CRD) was used in the experiments with 4 experimental treatments. Each treatment was replicated 4 times representing 4 fruits. Mean lesion diameter in each treatment was calculated for comparison and analysis. Data were analyzed using statistical program for calculating ANOVA and mean separation by using Scheffee test.

Chapter Four

Results

4. Results

4.1. Effect of treatment with *Trichoderma harzianum* on gray mold of grape

There were significant differences ($p<.05$) between mean lesion diameters of *Botrytis cinerea* in the different treatments (Table 1). Treatment with *Botrytis Cinerea* + formulated *Trichoderma harzianum* in invert emulsion was significantly different from treatment with *Botrytis cinerea* + sterile distilled water as control treatment and *Botrytis cinerea* + Blank formulation of invert emulsion (Table 1).

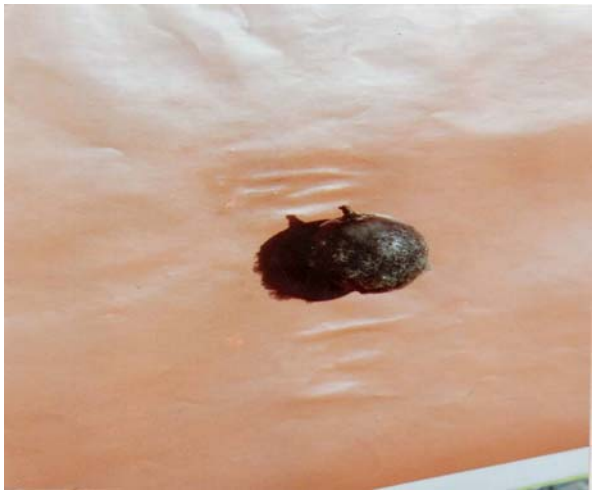
The mean lesion diameter (Figure 2) decreased significantly from 20.4 mm in case strile distilled water and 19.6mm in case blank formulation of invert emulsion to 12.2 mm and 8.8mm in case of unformulated and formulated *T. harzianum* respectively.

This demonstrated the efficacy of treatment with formulated *Trichoderma* in invert emulsion. However, no significant differences were observed betetween *Botrytis cinerea* + formulated *Trichoderma harzianum* in invert emulsion and *Botrytis cinerea* + unformulated *Trichoderma harzianum*. The same result of non – significant differences was obtained between *Botrytis cinerea* + sterile distilled water and *Botrytis cinerea* + Balnk formulation of invert emulsion (Table 1).

Table No. (1) Gray mold lesion diameter developed on grape berries 4 days after inoculation and treatment at 18±2°C and 16 hours of illumination per day under humid conditions.

Treatments	Mean Lesion diameter of gray mold in mm
Bortrtis cinerea + formulated *Trichoderma harzianum* in invert emulsion	8.8 a*
Botrytis cinerea + unformulated *Trichoderma harzianum*	12.2 a
Botrytis cinerea + sterile distilled wate	20.4 b
Botrytis cinerea + blank formulation of invert emulsion	19.6 b

* Means followed by different letters are significatly different at ($p < .05$) using ANOVA and scheffee test.



Figure(2) Typical symptoms of *Botrytis cinerea* on grape

4.2. Effect of treatment with *Trichoderma harzianum* on blue mold of grape

There were significant differences ($p<.05$) between mean lesion diameters of *Penicillium expansum* in the different treatments (Table 2). Treatment with *Penicillium expansum*+ formulated *Trichoderma harzianum* in invert emulsion was significantly different from treatment with *Penicillium expansum* + unformulated *Trichoderma harzianum* and

Penicillium expansum + sterile distilled water as control treatment and *Penicillium expansum* + blank formulation of invert emulsion (Table 2). The mean lesion diameter (Figure 3) decreased significantly from 17.0 mm in case strile distilled water and 17.2mm in case blank formulation of invert emulsion to 12.0 mm and 8.6mm in case of unformulated and formulated *T. harzianum* respectively. This demonstrated the efficacy of treatment with formulated *Trichoderma* in invert emulsion. However, no significant differences were observed between *Penicillium expansum* + unformulated *Trichoderma harzianum* and *Penicillium expansum* + sterile distilled water and *Penicillium expansum*+ blank formulation of invert emulsion (Table 2).

Table No. (2) Blue mold lesion diameter developed on grape berries 4 days after inoculation and treatment at 18±2°Cand 16 hours of illumination per day under humid conditions.

Treatment	Mean lesion diameter of gray mold in mm
Penicillium expansum+ formulated *Trichoderma harzianum* in invert emulsion	8.6 a*
Penicillium expansum+ unformulated *Trichoderma harzianum*	12.0 b
Penicillium expansum+ sterile distilled water	17.0 b
Penicillium expansum+blank formulation of invert emulsion	17.2 b

* Means followed by different letters are significatly different at ($p < .05$) using ANOVA and scheffee test.

Figure No. (3) Typical symptoms of *Penicillium expansum* on grape

4.3. Effect of treatment with *Trichoderma harzianum* on Gray mold of pear

There were no significant differences ($p < .05$) between lesion diameters of *Botrytis cinerea* in the different treatments (Table 3). Treatments with *Botrytis cinerea*+ formulated *Trichoderma harzianum* in invert emulsion was not significantly different from treatment with *Botrytis cinerea*+ unformulated *Trichoderma harzianum* and *Botrytis cinerea*+ sterile distilled water as control treatment and *Botrytis cinerea*+ blank formulation of invert emulsion (Table 3 ; Figure4). This indicates that formulation of *Trichoderma harzianum* in invert emulsion didn't decrease the lesion diameter significantly.

Table No. (3) Gray mold lesion diameter developed on pear fruits4 days after inoculation and treatment at 18±2°C and 16 hours of illumination per day under humid conditions.

Treatment	Mean Lesion diameter of gray mold in mm
Botrytis cinerea+ formulated *Trichoderma harzianum* in invert emulsion	12.67a*
Botrytis cinerea+ unformulated *Trichoderma harzianum*	17.67 a
Botrytis cinerea+ sterile distilled water	23.00 a
Botrytis cinerea+ blank formulation of invert emulsion	23.67 a

* Means followed by different letters are significatly different at (p <.05) using ANOVA and scheffee test.

Figure No. (4) Typical symptoms of *Botrytis cinerea* on pear

4.4. Effect of treatment with *Trichoderma harzianum* on blue mold of pear

There were significant differences (p <.05) between mean lesion diameters of *Penicillium expansum* in the different treatments (Table 4). Treatment with *Penicillium expansum*+ formulated *Trichoderma harzianum* in invert emulsion was significantly different from treatment with *Penicillium expansum* + sterile distilled water as control treatment and *Penicillium expansum* + blank formulation of invert emulsion (Table 4). The mean lesion diameter (Figure 5) decreased significantly from 19.67mm in case strile distilled water and 21.00 mm in case blank formulation of invert emulsion to 14.0 mm and 8.67 mm in case of unformulated and formulated *T. harzianum* respectively. However, no significant differences were observed between *P.expansum*+ formulated *T.harzianum* in invert emulsion and unformulated.

Table No. (4) Blue mold lesion diameter developed on pear fruit 4 days after inoculation and treatment at 18±2°C and 16 hours of illumination per day under humid conditions.

Treatment	Lesion diameter of gray mold in mm
Penicillium expansum+ formulated *Trichoderma harzianum* in invert emulsion	8.67a*
Penicillium expansum+ unformulated *Trichoderma harzianum*	14.0 a
Penicillium expansum+ sterile distilled water	19.67 b
Penicillium expansum+blank formulation of invert emulsion	21.00b

* Means followed by different letters are significatly different at (p <.05) using ANOVA and scheffee test.

Figure No. (5) Typical symptoms of *Penicillium expansum* on pear

4.5. Effect of treatment with *Trichoderma harzianum* on gray mold of kiwi

There were significant differences (p<.05) between mean lesion diameters of *Botrytis cnerea* in the different treatments (Table5). Treatment with *Botrytis cinerea* + formulated *Trichoderma harzianum* in invert emulsion was significantly different from treatment with *Botrytis cinerea* + sterile distilled water as control treatment and *Botrytis cinerea* + blank formulation of invert emulsion (Table 5). The mean lesion diameter (Figure

6) decreased significantly from 17.6 mm in case strile distilled water and 18.6mm in case blank formulation of invert emulsion to 11.8 mm and 8.6 mm in case of unformulated and formulated *T. harzianum* respectively. This demonstrated the efficacy of treatment with formulated *Trichoderma* in invert emulsion. However, no significant differences were observed betetween *Botrytis cinerea* + formulated *Trichoderma harzianum* in invert emulsion and *Botrytis cinerea* + unformulated *Trichoderma harzianum* (Table5).

Table No. (5) Gray mold lesion diameter developed on kiwi fruits 4 days after inoculation and treatment at 18±2°C and 16 hours of illumination per day under humid conditions.

Treatment	Mean Lesion diameter of gray mold in mm
Bortrtis cinerea + formulated *Trichoderma harzianum* in invert emulsion	8.6 a*
Botrytis cinerea + unformulated *Trichoderma harzianum*	11.8 a
Botrytis cinerea + sterile distilled water	17.6 b
Botrytis cinerea +blank formulation of invert emulsion	18.6 b

* Mans followed by different letters are significatly different at ($p < .05$) using ANOVA and scheffee test.

Figure No. (6) Typical symptoms of *Botrytis cinerea* on kiwi

4.6. Effect of treatment with *Trichoderma harzianum* on gray mold of strawberry

These results showed significant differences ($p < 0.05$) between mean lesion diameters of *Botrytis cinerea* in the different treatments (Table 6). Treatment with *Botrytis cinerea* + formulated *Trichoderma harzianum* in invert emulsion was significantly different from treatment with *Botrytis cinerea* + unformulated *Trichoderma harzianum* and *Botrytis cinerea*+ sterile distilled water as control treatment and *Botrytis cinerea*+ blank formulation of invert emulsion (Table 6). The mean lesion diameter (Figure 7) decreased significantly from 25.67 mm in case strile distilled water and 25.00 mm in case blank formulation of invert emulsion to 15.67 mm and 8.67 mm in case of unformulated and formulated *T. harzianum* respectively. This demonstrated the efficacy of treatment with formulated *Trichoderma* in invert emulsion. However, no significant differences were observed betetween *Botrytis cinerea*+ unformulated *Trichoderma harzianum* and *Botrytis cinerea*+ sterrile distilled water and *Botrytis cinerea*+ blank formulation of invert emulsion. The same result of non – significant differences was obtained between *Botrytis cinerea*+ sterile distilled water and *Botrytis cinerea*+ balnk formulation of invert emulsion (Table 6).

Table No. (6) Gray mold lesion diameter developed on strawberry fruit 4 days after inoculation and treatment at 18±2°Cand 16 hours of illumination per day under humid conditions.

Treatment	Lesion diameter of gray mold in mm
Bortytis cinerea + formulated *Trichoderma harzianum* in invert emulsion	8.67 a*
Botrytis cinerea + unformulated *Trichoderma harzianum*	15.67 b

Treatment	Lesion diameter of gray mold in mm
Botrytis cinerea + sterile distilled water	25.67 c
Botrytis cinerea +blank formulation of invert emulsion	25.00 c

* Means followed by different letters are significatly different at (p <.05) using ANOVA and scheffee test.

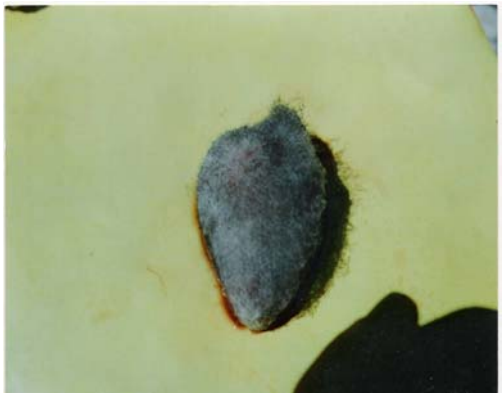


Figure No. (7) Typical symptoms of *Botrytis cinerea* on strawberry

4.7. Effect of treatment with *Trichoderma harzianum* on Rhizopus soft rot of strawberry

There were significant differences (p <.05) between mean lesion diameters of *Rhizopus stolonifer* in the different treatments (Table7). Treatment with *Rhizopus stolonifer*+ formulated *Trichoderma harzianum* in invert emulsion was significantly different from treatment with *Rhizopus stolonifer*+ unformulated *Trichoderma harzianum* and *Rhizopus stolonifer*+ sterile distilled water as control treatment and *Rhizopus stolonifer*+ blank formulation of invert emulsion (Table7). The mean lesion diameter (Figure 8) decreased significantly from 36.67 mm in case strile distilled water and 35.33 mm in case blank formulation of invert emulsion to 30.00 mm and 23.67mm in case of unformulated and formulated *T. harzianum*

respectively. This demonstrated the efficacy of treatment with formulated *Trichoderma* in invert emulsion. However, no significant differences were observed betetween *Rhizopus stolonifer*+ unformulated *Trichoderma harzianum* and *Rhizopus stolonifer*+ sterrile distilled water and *Rhizopus stolonifer*+ Blank formulation of invert emulsion (Table 7).

Table No. (7) Rhizopus soft rot lesion diameter developed on strawberry fruits 4 days after inoculation and treatment at 18±2°Cand 16 hours of illumination per day under humid conditions.

Treatment	Mean Lesion diameter of gray mold in mm
Rhizopus stolonifer + formulated *Trichoderma harzianum* in invert emulsion	23.67 a*
Rhizopus stolonifer + unformulated *Trichoderma harzianum*	30.00 b
Rhizopus stolonifer + sterile distilled water	36.67 b
Rhizopus stolonifer +blank formulation of invert emulsion	35.33 b

* Means followed by different letters are significatly different at ($p < .05$) using ANOVA and scheffee test.

Figure No. (8) Typical symptoms of *Rhizopus stolonifer* on strawberry

4.8. Protection period from infection with gray mold, blue mold and Rhizopus soft rot on different types of fruits

The longest minimum protection period from infection with *Botrytis cinerea* due to the treatment with formulated *Trichoderma harzianum* in invert emulsion was obtained on unwounded grape berries, kiwi fruits, pear fruits and strawberry fruits were 25, 22, 18 and 14 days, respectively (Table 8). Much shorter periods than the above periods were obtained on wounded fruits (5 to 6 days) on the same fruits for the same pathogen (Table 8). For *Penicillium expansum*, the longest minimum protection period from infection with the disease due to the treatment with formulated *Trichoderma harzianum* in invert emulsion was obtained on unwounded grape berries (19 days) then, on pear fruits (16 days) (Table 8). For *Rhizopus stolonifer*, the minimum protection period on unwounded strawberry fruits was 10 days only (Table 8). Shorter minimum protection periods from infection with *Penicillium expansum* and *Rhizopus stolonifer* on the different types of fruits due to treatment with formulated *Trichoderma harzianum* in invert emulsion were obtained (Table 8).

Table No. (8) Minimum protection period in days for the treatment with *Botrytis cinerea, Penicillium expansum*, *Rhizopus stolonifer*, on different types of fruit after incubation and treatment with formulated *Trichoderma* at 18± 2°C and 16 hours of illumination per day under humid conditions.

Pathogen type	Fruit type	Wounded fruit (1)		Unwounded fruit (2)	
		Formulated *Trichod-erma*	Blank formulat-ion of invert emulsion	Formul-ated *Tricho-derma*	Blank formulation of invert emulsion
Botrytis cinerea	Grape pear Kiwi Straw-berry	14 days 7 days 7 days 9 days	6 days 5 days 5 days 6 days	25 days 18 days 22 days 14 days	11 days 12 days 10 days 9 days
Penicillium expansum	Grape Pear	11 days 8 days	5 days 6 days	19 days 16 days	10 days 10 days
Rhizopus stolonifer	Straw-berry	5 days	3 days	10 days	5 days

1) No. of replicates = 6 represent 2 treatment of wounded and unwounded fruit.

2) Lesions appeared at end of protection period ranged from 4 – 7mm according to fruit type.

Chapter Five

Discussion and Conclusion

Postharvest diseases are considered the most serious diseases on various fruits. Infection with these diseases may occur in the field during harvesting or earlier, but development usually occurs during the postharvest stage. Therefore, it may causes serious economic losses on a wide range of ripened fruit (Batta, 2004)).The maine control measure practiced against the postharvest diseases is done by applying fungicides such as Iprodine (Roveral®), fenhexamide (Teldor®), dichlofluanid +tebuconazole (Silvacur®) vinclozolin (Ronilan®) or folpet+prochloraz (Mirage-F®) (Batta, 2004). However, chemical treatment of ripened fruit has many serious side- effects especially leaving residus and sometimes causing fruit injury, in addition to presence offensive odors under modified condition. The advantage of biological control are decrease disease intensity leading to higer production, reduce the use of chemical fungicide and nematicides, reduce likelihood of undesirable effects (environment pollution, effects on non –target organisms, resistance development against fungicide) from chemical fungicide, provide greater flexibility in disease management, safe for the user and the farming community (International rice research institute, 2003). Examples on postharvest disease control is the use of antagonistics microorcanisms (bacteria or fungal) as *Trichoderma* sp.(Batta, 1999), *Erwinia* sp. against *Botrytis cinerea* growth on apples stored under modified atmospheres (Dock et al, 1998), *Bacillus subtilus* (Esterio, 2000), *Candida sake* against major postharvest pathogen (Nunes et al, 2002). In this stdy we used T. harzianum to assess its biological effictiveness against gray mold caused by the fungus *Botrytis cinerea* on four types of fruits (grape, pear, kiwi, strawberry), blue mold

caused by *Penicillium expansum* on two types of fruits (grape, pear) and rhizopus soft rot caused by *Rhizopus stolonifer* on two types of fruits (strawberry, grape) at temperature 18 ± 2°C, and 16 hours of illumination per day under humid conditions. The laboratory experiment indicated that when using formulated form of *T. harzianum* in invert emulsion, the mean lesion diameter of the diseases of infected fruits decreased significantly after 4 days after inoculation and treatment. This demonstrated the efficacy of treatment with formulated *Trichoderma* in invert emulsion. A similer significant effect was obtained in previous study in controlling *Botrytis cinerea* on apples through stydying the effect of treatment with *T. harzianum* Rifai formulated in invert emulsion on postharvest decay of apple gray mold (Batta, 2004).Significant differences were obtaiened between means of percent reduction in diameter treated with formulated and non-formulated conidia of *T.harzianum* relative to sterile distlled water (control treatment). This could be explained by the disruption of the host fungus cell wall by direct parasitism of *Trichoderma* (Goldman and Goldman, 1998; Monte, 2001), or by competing on the site or nutrientof the host fungus cell (Filonow, 1998), or by producing toxic substance or volatile compounds as ethylene, released by the metabolic activites of the antagonist, that may change the redial growth, spores formation and CFUs of the target fungi (Vinas, 2004). The smallest minimum protection period that was obtained in the present study on strawberry was attributed mainly to its soft flesh nature. This is in agreement with the results of a previous study carried out on *P. expansum* infection on unwounded apple fruits (Batta, 2004). When these fruits were dipped for 30- second period in formulated *T. harzianum* conidia befor in invert emulsion being inoculated by *P.expansum* compered to the wounded fruits. This indicates the

importance of this type of treatment in protection apple fruits from blue mold infection for long time at postharvest stagewith out refrigeration (Batta, 2004).

In conclusion, since the present study represents a trial to use the antagonistic fungus *T. harzianum* (especially in formulated form using invert emulsion) against *B.cinerea*, *P.expansum* and *R.stolonifer*, it may be considered as the first step towards using the *T.harzianum* in biocontrol of these postharvest disease management programs. However, further experiments are recommended to be conducted before this commercial use such as confirmation of the fungus efficacy against the three postharvest diseases are to be applied under natural conditions of fruit storage and marketing; the side effects (if any) of the formulation when applied under natural conditions should be also investigated in the future.

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Appendix A

Gray mold –lesion diameter developed on grape fruit 4days after noculation and treatment at 18±2°C.

Treatments	Replicates (lesion diameter in mm)					
	R1	R2	R3	R4	R5	Mean
1.Botrytis cinerea + formulated *Trichoderma harzianum* in IE.	8	7	11	6	12	8.8 [a]*
2.Botrytis cinerea + Unformulated *Trichoderma harzianum*	13	16	10	8	14	12.2 [a]
3.Botrytis cinerea + S.D.W as control	23	18	19	20	22	20.4 [b]
4.Botrytis cinerea + blank formulation of invert emulsion	20	21	19	18	20	19.6 [b]

*Means folllowed by different letters are significantly different at P ≤.05 Using ANOVA and Schefee test.

$C = Y^2 \ldots / rt = \underline{(8 + 7 + \ldots 20)^2} = \underline{93025} = 4651.2$

$5 \times 4 \qquad 20$

SS total $= \sum Yij^2 - C = (8)^2 + \ldots (20)^2 - C = 5223 - 4651.2 = 541.7$

SS treatment $(\sum Yij)^2 / r - C = \underline{25665} - 4651.2 = 481.8$

5

SS error = SS total – SS treatment = 541.7 - 481.8 = 59.9

$H_\circ$: $M_1 = M_2 = M_3 = M_4$

H_1 : at least two means are different

ANOVA Table

Source of variation	SS	dF	Ms	Fc
Treatment	481.8	3	160.6	42
Error	59.9	16	3.7	
Total	541	19		

F, 05 (3, 16) = 3.24 Science > F tabulated, we reject H◦ so at least two means are different and it is significant.

According to Schiffee test:

1- $H_◦: M_1 = M_2$, $H_{1:} M_1 \neq M_2$, we reject H◦ if:

$$| X_1 - X_2 | \geq \sqrt{MSE.\ (K-1).\ F\ \alpha\ (k-1.\ n-k).\ \frac{1}{n_1} + \frac{1}{n_2}}$$

$$| 8: 8 - 12.2 | \geq \sqrt{3.7x\ 4\ x\ 3.24\ x.45}$$

$3.4 \geq 4.6$, we don't reject H◦, so $M_1 \neq M_2$

2- $H_{◦:} M_1 = M_3$

$H_{1:} M_1 \neq M_3$, we reject H◦ if:

$$| X_1 - X_3 | \geq 4.6$$

$$| 8.8 - 20.4| \geq 4.6$$

$11.6 \geq 4.6$, we reject H◦, so $M_1 \neq M_3$

3- $H_{◦:} M_1 = M_4$, $H_{1:} M_1 \neq M_4$, we reject H◦ if:

$$| X_1 - X_3 | \geq 4.6$$

$$| 8.8 - 19.6 | \geq 4.6$$

$10.8 \geq 15.6$,we reject $H_\circ$, so $M_1 \neq M_4$

4- $H_\circ : M_1 = M_3$, $H_1 : M_2 \neq M_3$, we reject $H_\circ$ if:

$| X_2 - X_3 | \geq 4.6$

$| 12.2 - 20.4 | \geq 4.6$

$8.2 \geq 4.6$,we reject $H_\circ$, so $M_2 \neq M_3$

5. $H_\circ : M_1 = M_4$, $H_1 : M_2 \neq M_4$, we reject $H_\circ$ if

$| X_2 - X_4 | \geq 4.6$

$| 12.2 - 19.6 | \geq 4.6$

$7.4 \geq 4.6$, we reject $H_\circ$, so $M_2 \neq M_3$

6. $H_\circ : M_3 = M_4$, $H_1 : M_3 \neq M_4$, we reject $H_\circ$ if

$| X_3 - X_4 | \geq 4.6$

$| 20.4 - 19.6 | \geq 4.6$

$0.8 \geq 4.6$, we don't reject $H_\circ$, so $M_3 \neq M_4$

Appendix B

Blue mold-lesion diameter developed on grape fruit 4days after inoculation and treatment at 18±2°C.

Treatments	Replicates (lesion diameter in mm)					
	R1	R2	R3	R4	R4	Mean
1.Penicillium expansum + formulated *Trichoderma harzianum* in IE.	10	8	10	7	8	8.6[a]*
2.Penicillium expansum + Unformulated *T. harzianum*	13	12	10	12	13	12.0 [a]
3.Penicillium expansum + S.D.W as control	18	17	16	16	18	17.0 [b]
4.Penicillium expansum + blank formulation of invert emulsion	16	19	18	17	16	17.2[b]

*Means followed by different letters are significantly different at P ≤ .05 Using ANOVA and Schefee test.

$$C = Y^2 \ldots / rt = \frac{(10+ \ \ldots \ 16)^2}{5 \text{ X } 4} = 3753.8$$

$$\text{SS total} = \sum Y\,ij^2 - C = (10)^2 + \ldots (16)^2 - C = 4038 - 3753.8 = 284.2$$

$$\text{SS treatment} \ (\sum Y\,ij)^2 / r - C = \frac{20070}{5} - 3753.8 = 260.2$$

$$\text{SS error} = \text{SS total} - \text{SS treatment} = 284.2 - 260.2 = 24$$

$H_\circ$: $M_1 = M_2 = M_3 = M_4$

H_1 : at least two means are different

ANOVA Table

Source of variation	**SS**	**dF**	**Ms**	**Fc**
Treatment	260.2	3	86.7	57.8
Error	24	16	1.5	
Total	284.2	19		

F, 05 (3,16) = 3.24 Science Fc > F tabulated, we reject H◦ so at least two means are different and it is significant.

According to Schiffee test:

1- H◦: $M_1 = M_2$, $H_{1:}\ M_1 \neq M_2$, we reject H◦ if:

$$| X_1 - X_2 | \geq \sqrt{MSE.\ (k-1)\ .\ F\alpha\ (k-1.\ n-k).\ \frac{1}{n_1} + \frac{1}{n_2}}$$

$$| 8: 6 - 12.0 | \geq \sqrt{1.5 \times 4 \times 3.24 \times .45}$$

$3.4 \geq 2.96$, we reject H◦, so $M_1 \neq M_2$

2 -H◦ : $M_1 = M_3$

$H_{1:}\ M_1 \neq M_3$, we reject H◦ if:

$| X_1 - X_3 | \geq 2.96$

$| 8.6 - 17.0| \geq 2.96$

$8.4 \geq 2.96$, we reject H◦ , so $M_1 \neq M_3$

3- H ◦ : $M_1 = M_4$, $H_{1:}\ M_1 \neq M_4$, we reject H ◦ if:

$| X_1 - X_3 | \geq 2.96$

$| 8.6 - 17.2 | \geq 2.96$

$8.6 \geq 2.96$,we reject $H_{\circ}$, so $M_1 \neq M_4$

4- $H_{\circ}$: $M_1 = M_3$, $H_1 : M_2 \neq M_3$, we reject $H_{\circ}$ if:

$$| X_2 - X_3 | \geq 2.96$$

$$| 12.0 - 17.0 | \geq 2.96$$

$5 \geq 2.96$,we reject $H_{\circ}$, so $M_2 \neq M_3$

5. $H_{\circ}$: $M_1 = M_4$, H_1 : $M_2 \neq M_4$, we reject $H_{\circ}$ if

$$| X_2 - X_4 | \geq 2.96$$

$$| 12.0 - 17.2 | \geq 2.96$$

$5.2 \geq .985$, we reject $H_{\circ}$, so $M_2 \neq M_3$

6. $H_{\circ}$: $M_3 = M_4$, H_1 : $M_3 \neq M_4$, we reject $H_{\circ}$ if

$$| X_3 - X_4 | \geq 2.96$$

$$| 17.0 - 17.2 | \geq 2.96$$

$.2 \geq 2.96$, we don't reject $H_{\circ}$, so $M_3 \neq M_4$

Appendix C

Gray mold –lesion diameter developed on pear fruit 4days after inoculation and treatment at 18±2°C.

Treatments	Replicates (lesion diameter in mm)					
	R1	R2	R3			Mean
Botrytis cinerea + formulated *Trichoderma harzianum* in IE.	8	12	18			12.67 [a*]
Botrytis cinerea + Unformulated *T. harzianum*	18	17	18			17.67 [a]
Botrytis cinerea + S.D.W as control	21	29	19			23.0 [a]
Botrytis cinerea + blank formulation of invert emulsion	30	23	18			23.67 [a]

*Means followed by different letters are significantly different at P ≤.05 Using ANOVA and Schefee test.

$$C = Y^2 \ldots / rt = \frac{(8 + \ldots 18)^2}{3 \text{ X } 4} = \frac{53361}{12} = 4446.7$$

SS total $= \sum Y\,ij^2 - C = (8)^2 + \ldots (18)^2 - C = 4865 - 4446.7.\ 2 = 418.4$

SS treatment $(\sum Y\,ij)^2 / r - C = \frac{14055}{3} - 4446.7 = 238.3$

SS error = SS total – SS treatment - = 418.4 - 238.3 = 180.1

$H_\circ$: $M_1 = M_2 = M_3 = M_4$

H_1 : at least two means are different

ANOVA Table

Source of variation	**SS**	**dF**	**Ms**	**Fc**
Treatment	238.3	3	79.4	3.52
Error	180.1	8	22.5	
Total	418.4	11		

F, 05 (3,8) = 4.07 Science Fc < F tabulated, we don't reject $H_{\circ}$, so M1=M2=M3=M4 and there is no significant difference .

Appendix D

Blue mold-lesion diameter developed on grape fruit 4days after inoculation and treatment at 18±2°C.

Treatments	Replicates (lesion diameter in mm)					
	R1	R2	R3			Mean
Botrytis cinerea + formulated *Trichoderma harzianum* in IE.	11	7	8			8.67 [a*]
Botrytis cinerea + Unformulated *T harzianum*	13	15	14			14.0[a]
Botrytis cinerea + S.D.W as control	20	22	21			21.0 [b]
Botrytis cinerea + blank formulation of invert emulsion	17	19	23			19.67 [b]

*Means folllowed by different letters are significantly different at P ≤.05 Using ANOVA and Schefee test.

$$C = Y^2 \ldots / rt = \frac{(11 + \ldots 23)^2}{3X\,4} = \frac{36100}{12} = 3008.3$$

$$SS\ total = \sum Y\,iJ^2 - C = (11)^2 + \ldots (23)^2 - C = 3328 - 3008.3 = 319.7$$

$$SS\ treatment\ \frac{(\sum Y\,iJ)^2}{r} - C = \frac{9890}{3} - 3008.3 = 288.3$$

SS error = SS total – SS treatment - = 319.7- 288.3 = 31.3

$H_\circ$: $M_1 = M_2 = M_3 = M_4$

$H_{1:}$ at least two means are different

ANOVA Table

Source of variation	**SS**	**dF**	**Ms**	**Fc**
Treatment	288.3	3	96.1	24.6
Error	31.3	8	3.9	
Total	319.7	11		

F, 05 (3,8) = 4.07 Science Fc > F tabulated, we reject H◦ so at least two means are different and it is significant.

According to Schiffee test:

1- $H_◦$: $M_1 = M_2$, $H_{1:}\ M_1 \neq M_2$, we reject $H_◦$ if:

$$| X_1 - X_2 | \geq \sqrt{MSE.\ (k-1)\ .\ F\ \alpha\ (k-1.\ n-k)\ \frac{1}{n_1} + \frac{1}{n_2}}$$

$$|8.67 - 14.0 | \geq \sqrt{3.9 \times 2 \times 4.07 \times .58}$$

$5.3 \geq 4.2$, we reject $H_◦$, so $M_1 \neq M_2$

2- $H_{◦:}\ M_1 = M_3$

$H_{1:}\ M_1 \neq M_3$, we reject $H_◦$ if:

$| X_1 - X_3 | \geq 4.2$

$|8.67 - 21.0| \geq 4.2$

$2.3 \geq 4.2$, we don't reject $H_◦$, so $M_1 = M_3$

3- $H_{◦:}\ M_1 = M_4$, $H_{1:}\ M_1 \neq M_4$, we reject $H_◦$ if:

$| X_1 - X_3 | \geq 4.2$

$| 8.67 - 19.67 | \geq 4.2$

$11 \geq 4.2$,we reject $H_{\circ}$, so $M_1 \neq M_4$

4- $H_{\circ}$: $M_1 = M_3$, $H_1 : M_2 \neq M_3$, we reject $H_{\circ}$ if:

$| X_2 - X_3 | \geq 4.2$

$| 14.0 - 21.0 | \geq 4.2$

$7.0 \geq 4.2$,we reject $H_{\circ}$, so $M_2 \neq M_3$

5. $H_{\circ}$: $M_1 = M_4$, H_1 : $M_2 \neq M_4$, we reject $H_{\circ}$ if

$|X_2 - X_4 | \geq 4.2$

$|14.0 - 19.67 | \geq 4.2$

$5.67 \geq 4.2$, we reject $H_{\circ}$, so$M_2 \neq M_3$

6. $H_{\circ}$: $M_3 = M_4$, H_1 : $M_3 \neq M_4$, we reject $H_{\circ}$ if

$| X_3 - X_4 | \geq 4.2$

$| 21.0 - 19.67 | \geq 4.2$

$1.33 \geq 4.2$,we don't reject $H_{\circ}$, so$M_3 = M_4$

Appendix E

Gray mold –lesion diameter developed on kiwi fruit 4days after inoculation and treatment at 18±2°C.

Treatments	Replicates (lesion diameter in mm)					
	R1	R2	R3	R4	R5	Mean
Botrytis cinerea + formulated *Trichoderma harzianum* in IE.	11	7	10	8	7	8.8 [a*]
Botrytis cinerea + Unformulated *T.harzianum*	13	14	12	10	10	12.2 [a]
Botrytis cinerea + S.D.W as control	19	18	15	20	20	20.4 [b]
Botrytis cinerea + blank formulation of invert emulsion	17	18	17	16	16	19.6 [b]

* Means followed by different letters are significantly different at P ≤.05 Using ANOVA and Schefee test.

$$C = Y^2 \ldots / rt = \frac{(11 + \ldots 20)^2}{5 \mathbf{X} 4} = \frac{80089}{20} = 4004.4$$

$$SS\ treatment\ (\sum Y\ ij)^2 / r - C = \frac{21723}{5} - 4004.4 = 340.2$$

SS error = SS total – SS treatment - = 396.6 - 340.2 = 56.4

$H_\circ$: $M_1 = M_2 = M_3 = M_4$

H_1 : at least two means are different

ANOVA Table

Source of variation	**SS**	**dF**	**Ms**	**Fc**
Treatment	340.2	3	113.4	32.2
Error	56.4	16	3.52	
Total	396.6	19		

F, 05 (3,16) = 3.24 Science Fc > F tabulated, we reject H◦ so at least two means are different and it is significant.

According to Schiffee test:

1. H◦: $M_1 = M_2$, $H_{1:} M_1 \neq M_2$, we reject H◦ if:

$$| X_1 - X_2 | \geq \sqrt{MSE.\ (k-1)\ .\ F\ \alpha\ (k-1.\ n-k)\ \frac{1}{n_1} + \frac{1}{n_2}}$$

$$| 8.6 - 11.8 | \geq \sqrt{3.52 \times 4 \times 3.24 \times .45}$$

$3.2 \geq 4.5$, we reject H◦, so $M_1 \neq M_2$

2. $H_{\circ:} M_1 = M_3$

$H_{1:} M_1 \neq M_3$, we reject H◦ if:

$| X_1 - X_3 | \geq 4.5$

$| 8.6 - 18.6| \geq 4.5$

$10 \geq 4.5$, we don't reject H◦, so $M_1 \neq M_3$

3- $H_{\circ:} M_1 = M_4$, $H_{1:} M_1 \neq M_4$, we reject H◦ if:

$| X_1 - X_3 | \geq 4.5$

$| 8.6 - 17.6 | \geq 4.5$

$9 \geq 4.5$,we reject $H_{\circ}$, so $M_1 \neq M_4$

4- $H_{\circ}$: $M_1 = M_3$, $H_1 : M_2 \neq M_3$, we reject $H_{\circ}$ if:

$| X_2 - X_3 | \geq 4.5$

$| 11.8 - 18.6 | \geq 4.5$

$6.8 \geq 4.5$,we reject $H_{\circ}$, so $M_2 \neq M_3$

5. $H_{\circ}$: $M_1 = M_4$, H_1 : $M_2 \neq M_4$, we reject $H_{\circ}$ if

$| X_2 - X_4 | \geq 4.5$

$| 11.8 - 17.6 | \geq 1.56$

$5.8 \geq 4.5$, we reject $H_{\circ}$, so $M_2 \neq M_3$

6. $H_{\circ}$: $M_3 = M_4$, H_1 : $M_3 \neq M_4$, we reject $H_{\circ}$ if

$| X_3 - X_4 | \geq 4.5$

$| 18.6 - 17.6 | \geq 4.5$

$1 \geq 4.5$, we don't reject $H_{\circ}$, so$M_3 = M_4$

Appendix F

Gray mold –lesion diameter developed on strawberry fruit 4days after inoculation and treatment at 18±2°C.

Treatments	Replicates (lesion diameter in mm)					
	R1	R2	R3	R4	R5	Mean
Botrytis cinerea + formulated *Trichoderma harzianum* in IE.	9	8	9	6	12	8.67 [a*]
Botrytis cinerea + Unformulated T harzianum	15	14	18	8	14	15.67[a]
Botrytis cinerea + S.D.W as control	23	26	28	20	22	25.67 [b]
Botrytis cinerea + blank formulation of invert emulsion	24	23	28	18	20	25.0 [b]

*Means followed by different letters are significantly different at $P \leq .05$ Using ANOVA and Schefee test.

$$C = Y^2 \ldots / rt = \frac{(9 + \ldots 28)^2}{3 \times 4} = \frac{50625}{12} = 4218.7$$

$$SS\ total = \sum Y\ ij^2 - C = (9)^2 + \ldots (28)^2 - C = 4849 - 4218.7 = 630.3$$

$$SS\ treatment\ (\sum Y\ ij)^2 / r - C = \frac{14439}{3} - 4218.7 = 594.3$$

SS error = SS total – SS treatment - = 630.3 - 594.3 = 36

H_o: $M_1 = M_2 = M_3 = M_4$

H_1 : at least two means are different

ANOVA Table

Source of variation	**SS**	**dF**	**Ms**	**Fc**
Treatment	594.3	3	198.1	44
Error	36	8	4.5	
Total	630.3	11		

F, 05 (3,8) = 4.07 Science Fc > F tabulated, we reject H◦ so at least two means are different and it is significant.

According to Schiffee test:

1- H◦: $M_1 = M_2$, $H_{1:}\ M_1 \neq M_2$, we reject H◦ if:

$$| X_1 - X_2 | \geq \sqrt{MSE.\ (k-1)\ .\ F\ (k-1.\ n-k)\ \frac{1}{n_1} + \frac{1}{n_2}}$$

$$| 8.67 - 15.67 | \geq \sqrt{4.5 \times 2 \times 4.07 \times .58}$$

$7 \geq 5.6$, we reject H◦, S◦ $M_1 \neq M_2$

2-H◦: $M_1 = M_3$

$H_{1:}\ M_1 \neq M_3$, we reject H◦ if:

$| X_1 - X_3 | \geq 4.6$

$| 8.67 - 25.67| \geq 4.6$

$17 \geq 5.6$, we reject H◦, so $M_1 \neq M_3$

3- H◦: $M_1 = M_4$, $H_{1:}\ M_1 \neq M_4$, we reject H◦ if:

$| X_1 - X_3 | \geq 5.6$

$| 8.6 - 25 | \geq 4.6$

$16.33 \geq 5.6$,we reject $H_{\circ}$, so $M_1 \neq M_4$

4- $H_{\circ}$: $M_1 = M_3$, $H_1 : M_2 \neq M_3$, we reject $H_{\circ}$ if:

$| X_2 - X_3 | \geq 4.6$

$| 15.67 - 25.67 | \geq 4.6$

$10 \geq 5.6$,we reject $H_{\circ}$, so $M_2 \neq M_3$

5. $H_{\circ}$: $M_1 = M_4$, H_1 : $M_2 \neq M_4$, we reject $H_{\circ}$ if

$| X_2 - X_4 | \geq 4.6$

$| 15.67 - 25.0 | \geq 4.6$

$9.33 \geq 5.6$, we reject $H_{\circ}$, so $M_2 \neq M_3$

6. $H_{\circ}$: $M_3 = M_4$, H_1 : $M_3 \neq M_4$, we reject $H_{\circ}$ if

$| X_3 - X_4 | \geq 4.6$

$| 25.67 - 25.0 | \geq 4.6$

$.67 \geq 5.6$, we don't reject $H_{\circ}$, so $M_3 = M_4$

Appendix G

Rhizopus soft rot –lesion diameter developed on strawberry fruit 4days after inoculation and treatment at 18±2°C.

Treatments	Replicates (lesion diameter in mm)					
	R1	R2	R3	R4	R5	Mean
Botrytis cinerea + formulated *Trichoderma harzianum* in IE.	24	25	22			23.67 [a*]
Botrytis cinerea + Unformulated *T. harzianum*	30	32	28			30.0[a]
Botrytis cinerea + S.D.W as control	37	35	38			36.67 [b]
Botrytis cinerea + blank formulation of invert emulsion	35	37	34			35.33 [b]

*Means followed by different letters are significantly different at P ≤.05 Using ANOVA and Schefee test.

$$C = Y^2 \ldots / rt = \frac{(24 + \ldots 34)^2}{3 \times 4} = \frac{142129}{12} = 11844$$

$$\text{SS total} = \sum Y\,ij^2 - C = (24)^2 + \ldots (34)^2 - C = 12181 - 11844 = 336.9$$

$$\text{SS treatment} = \frac{(\sum Y\,ij)^2}{r} - C = \frac{36477}{3} - 11844 = 325$$

$$\text{SS error} = \text{SS total} - \text{SS treatment} = 336.9 - 315 = 21.9$$

$H_\circ$: $M_1 = M_2 = M_3 = M_4$

H_1 : at least two means are different

ANOVA Table

Source of variation	**SS**	**dF**	**Ms**	**Fc**
Treatment	315	3	105	38.8
Error	21.9	8	2.7	
Total	336.6	11		

F, 05 (3,8) = 4.07 Science Fc > F tabulated, we reject $H_\circ$ so at least two means are different and it is significant.

According to Schiffee test:

1- $H_\circ$: $M_1 = M_2$, $H_{1:}\ M_1 \neq M_2$, we reject $H_\circ$ if:

$$| X_1 - X_2 | \geq \sqrt{MSE.\ (k-1)\ .\ F\ (k-1.\ n-k)\ \frac{1}{n_1} + \frac{1}{n_2}}$$

$$| 8.67 - 15.67 | \geq \sqrt{2.7 \times 2 \times 4.07 \times .58}$$

$7 \geq 3.5$, we reject $H_\circ$, so $M_1 \neq M_2$

2- $H_{\circ:}\ M_1 = M_3$

$H_{1:}\ M_1 \neq M_3$, we reject $H_\circ$ if:

$| X_1 - X_3 | \geq 3.5$

$| 8.67 - 25.67| \geq 3.5$

$17 \geq 3.5$, we reject $H_\circ$, so $M_1 \neq M_3$

3- $H_{\circ:}\ M_1 = M_4$, $H_{1:}\ M_1 \neq M_4$, we reject $H_\circ$ if:

$| X_1 - X_3 | \geq 3.5$

$| 8.6 - 25 | \geq 3.5$

$16.33 \geq 5.6$,we reject $H_{\circ}$, so $M_1 \neq M_4$

4- $H_{\circ}$: $M_1 = M_3$, $H_1 : M_2 \neq M_3$, we reject $H_{\circ}$ if:

$| X_2 - X_3 | \geq 3.5$

$| 15.67 - 25.67 | \geq 3.5$

$10 \geq 3.5$,we reject $H_{\circ}$, so $M_2 \neq M_3$

5. $H_{\circ}$: $M_1 = M_4$, H_1 : $M_2 \neq M_4$, we reject $H_{\circ}$ if

$| X_2 - X_4 | \geq 3.5$

$| 15.67 - 25.0 | \geq 3.5$

$9.33 \geq 3.5$, we reject $H_{\circ}$, so $M_2 \neq M_3$

6. $H_{\circ}$: $M_3 = M_4$, H_1 : $M_3 \neq M_4$, we reject $H_{\circ}$ if

$| X_3 - X_4 | \geq 3.5$

$| 25.67 - 25.0 | \geq 3.5$

$.67 \geq 5.6$, we don't reject $H_{\circ}$, so$M_3 = M_4$

جامعة النجاح الوطنية
كلية الدراسات العليا

المكافحة البيولوجية لمرض (العفن الرمادي والعفن الأزرق والعفن الطري) على ثمار العنب والكمثرى والكيوي والفراوله باستعمال الفطر المضاد (ترايكوديرما هارزيانم)

إعداد
محمد إبراهيم أحمد عوده

بإشراف
د. يعقوب بطه

قدمت هذه الأطروحة إستكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم البئية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس- فلسطين
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الملخص

يهدف هذا البحث إلى تقييم فعالية الفطر المضاد (ترايكوديرما هارزيانم) ضد مرض التعفن الرّمادي (جري مولد) والتعفّن الأزرق (بلو مولد) والتعفّن الطّري (رايزوبس سوفت روت) في ثمار العنب والكمّثرى والكيوي والفراولة الذي يسببه كلّ من فطر (بوتريتس سينيريا) و (بينيسيليوم إكسبانسيم) و(رايزوبس ستولونيفير). وأيضاً تحديد فترة الوقاية من الإصابة بهذه الأمراض على الأنواع الأربعة من الفواكه. لقد تمّ استعمال الفطربشكل رئيسي كمستحلب منعكس بعد إدخاله إلى المستحلب بشكل كونيدنا. بالإضافة إلى استعمال الفطر بشكل محلول مائي يحتوي على الكونيديا. تم إجراء (تقييم الفعالية) في المختبر عند درجة حرارة (18 ± 2°م). و16 ساعة إضاءة في اليوم وتحت ظروف رطبة. أثبتت النتائج التي حصلنا عليها أن الفطر ترايكوديرما هارزيانم) بصيغة المستحلب المنعكس كان فعّالاً في تقليل قطر الإصابة لمرض التعفّن الرّمادي والأزرق والطّري مقارنةً بغيره من المعاملات .لقد وجد أن هناك فروق معنويّة (الاحتمالية 0.05) عند مقارنة متوسط قطر الإصابة للأمراض في المعاملات في المستحلب المنعكس المحتوي على الفطر والشاهد. كذلك أشارت النتائج إلى أن فطر (ترايكوديرما) بصيغة المستحلب المنعكس يعطي في ثمار العنب غير المجروحة أطول فترة حماية ممكنة ضد مرض التعفّن الرّمادي وهذا يثبت الفعالية البيولوجيّة لفطر (ترايكوديرما هارزيانم). ومع ذلك فإنّه ينصح بإجراء مزيد من التجارب لزيادة التأكد من فعالية الفطر ضد مرض التعفّن الرّمادي (بوترايتس سينيريا) والتعفّن الأزرق (بينيسيليوم إكسبانسيوم) وكذلك التعفّن الطري (رايزوبس سوفت روت) لغرض الاستعمال في ظروف طبيعيّة تتعلّق بخزن وتسويق الفواكه وقبل استعمالها التجاري للفطر.