Postharvest Fungal Diseases of Orange Fruits and Evaluation of Certain Control Methods

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Dedicated

to

my Family

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Abstract

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This study was conducted to identify the fungal pathogens of different diseased orange fruits of different cultivars that had been collected from different packinghouses and markets in Jordan. Results of isolation on nutritional medium (PDA) and microscopical identification revealed the presence of the following species of fungi: ***Penicillium digitatum*** Sacc. , ***P. italicum*** Wehmer, ***Alternaria citri*** Ell., ***Phytophthora sp.*** (Smith) Leonian, ***Botrytis cinerea*** Pers, ***Trichoderma sp.*** Pers and ***Fusarium sp.*** (Macf) Sacc.

Through studying the symptoms of diseased fruits, the following diseases were found: Green mold, Blue mold, Stem end rot , Brown rot , Gray mold, ***Trichoderma*** rot and ***Fusarium*** white mold . Inoculation of healthy fruits with different fungal isolates confirmed the Koch's postulates. Storage of Shamouti, Navel and Valencia orange cultivars , under cooled (1-3° C) and common storage (at ambient temperature), showed sensitivity of such cultivars to be infected with fungi . ***Penicillium digitatum*** and ***P. italicum*** were the only 2 fungal pathogens found on different cultivars at both conditions of storage with more incidence percent of ***P. digitatum***

compared to *P. italicum*. The three orange cultivars did not show significant differences to infection by green and blue mold fungal pathogens.

Different methods of Green mold control were evaluated on Shamouti orange fruits using artificial inoculation under cold storage (1-3 °C) and storage inside the incubator at 20±1°C. Postharvest fruit dipping , previously inoculated with (20 x 10^6)conidia /ml of ***P. digitatum*** , in different suspensions of fungicides at recommended concentration showed a high efficacy in fungal control with significant differences among these fungicides in comparison with the control. Incidence of green mold was 13, 18.3 and 36.7% when benomyl, carbendazim and thiophanate methyl were used , respectively at 20 ± 1 °C of incubation.

Although ixing of either two fungicides did not increase the percent of decay control, no significant differences were found among fungicides mixtures but highly significant differences were obtained in comparison with the control.

Postharvest fruit waxing and illumination of previously inoculated fruits for 2 , 4 and 8 hours of UV light did not show any decay control of ***P. digitatum*** at 20 ± 1 °C. However , orange fruit decay was delayed for 3 and 6 days when fruits were illuminated for 16 and 24 hours, respectively. Data on the incidence of green mold showed significant differences among different exposure times of UV light when compared with the control 15 days after illumination .

The antagonistic yeast, ***D.hansenii,*** ($15x10^7$cells/ml) showed significance less reduction in the decay in comparison with the control

Preharvest spraying of orange trees in the field with benomyl (12g/20L), carbendazim (20g/20 L) and thiophanate methyl (20g/20 L) gave 56.4 , 56.4 and 60 % of the incidence , respectively .

Results of fungal growth in vitro test showed significant differences between fungicides and mixtures in comparison with the control , except that thiophanate methyl was less effective .

Results of storing fruits at 20 ±1° C and treating inoculated fruits chemically,physically and biologically showed significant differences among those treatments while treating the inoculated fruits with same treatments at 1±3° C did not show any fruit dacay.

Disease severity for all control methods ranged between 0.7 and 2.12 (according to scale 0 - 4) at the end of incubation period .

I : Introduction

Oranges (***Citrus sinensis*** [L.] Osbeck) are popular all over the world and widely planted in Jordan Valley. In 1996, the total area planted with oranges was 14587 dunums with a total production of 36028 tons. It was reported that Jordan has imported 30900 tons of oranges and exported 10300 tons in 1996 (Statistical Year Book, 1996) . Therefore, protecting fruits from diseases during storage is impotrtant.

For many years, ***Penicillium digitatum*** Sacc. the causal agent of the green mold was the dominant postharvest pathogen of citrus fruits; the blue mold, caused by ***P. italicum*** Wehmer, was of minor importance (Gutter, 1975) . Several other species of fungi are responsible for major losses of this crop during harvesting, transportation and storage (Charles *et al*, 1994). The major postharvest diseases of orange fruits include the stem-end rots incited by either ***Diplodia natalensis*** P. Evans , ***Phomopsis citri*** Fawc. or ***Alternaria citri*** Ell. & Pierce. ; Gray mold caused by ***Botrytis cinerea*** Pers. ; sour rot caused by ***Geotrichum candidum*** Link ; and brown rot caused by ***Phytophthora citrophthora*** (Smith & Smith) Leonian (Anna, 1990).

In Jordan, no work was reported on the subject. There have been a number of effective fungicides for the control of postharvest diseases of orange fruits. For example benomyl , as Charles *et al.* (1994) reported , is effective in reducing postharvest decay and extending the shelf life of the produce. He also reported that these fungicides are becoming less effective because postharvest pathogens are developing resistant to them. Also, recent health concerns over pesticide residues in fruits have precipitated the

complete withdrawal of some fungicides from the market. Therefore, antagonistic yeasts, ultraviolet illumination and other natural compounds have been used as alternatives to fungicides (Charles *et al.*, 1994).

In Jordan, plant pathologists have not given postharvest diseases the priority they warrant. Therefore, this research was conducted to study the following objectives:

1. Isolation and Identification of the causal agents for postharvest fungal diseases of oranges in different packinghouses and markets in Jordan.
2. Determination of the incidence of postharvest fungal diseases of orange fruit in refrigerated stores and in common stores at ambient temperature.
3. Evaluation of different treatments for green mold disease control. These treatments include:-

    * Postharvest dipping of fruits in fungicides.
    * Postharvest fruit waxing.
    * Illumination by ultraviolet light (UV).
    * Postharvest dipping by the antagonistic yeast ***Debaryomyces hansenii*** (Zopf) Van Rij.
    * Preharvest spraying of orange trees using different fungicides.

II : Review of Literature

Orange fruits are being frequently subjected to the pathogens attack. Although no serious postharvest diseases of oranges have been attributed to bacteria, several species of fungi are responsible for major losses of this crop during harvesting, transporation, storage and marketing (Charles et al., 1994). Smoot (1969) cited by David *et al.* (1986) found that after two weeks storage at 21°C , 20% decay was a reasonable loss estimate for citrus fruits produced in humid areas and given no postharvest fungicides treatment . Percent of losses in oranges during storage and marketing were 52% and 1 - 25%, respectively (Brown,1979).

2: 1 Postharvest fungal diseases of citrus fruits

Fungi causing postharvest decays in citrus fruits can be divided into two broad groups. Those that infect fruits in the field but remain in quiescent state and those causing decays primarly after harvest.

2 : 1 : 1 Quiescent infections of citrus fruits :

Some decay fungi establish quiescent infections during the growing season and growth resumes on the fruit after harvest (Table 1).

Stem end rot (black rot) caused by ***Alternaria citri*** Ell. and Pierce develops from quiescent infection and occurs predominantly in fruit during long-term storage, but it sometimes develops in the orchards where it may cause premature fruit drop. The infection results from previously latent infection in the button (Calyx & disk) or stylar end of fruit causing brown to blackish discoloration of the fruit peel or grow through the vascular bundles to the center of the fruit causing black rot or center rot (Brown and McCornack, 1972).

Table (1) : Major postharvest diseases of citrus fruits, causal fungi, type and site of fruit infection *

No.	*Disease*	*Causal Fungus*	*Type of infection*	*Site of infection*
1.	Anthracnose	***Colletotrichum gleosporioides*** (Penz.) Sacc.	Quiescent	Intact or injured rind
2.	Blue mold	***Penicillium italicum*** Wehmer	Active	Injured rind
3.	Brown rot	***Phytophthora citrophthora P. parasitica*** Dast	Active or quiescent	Intact rind
4.	Green mold	***Penicillium digitatum*** Sacc.	Active	Injured rind
5.	Gray mold	***Botrytis cinerea*** Pers.	Active or quiescent	Flowering part (petal)
6.	Sour rot	***Geotrichum candidum*** Link	Active	Injured rind
7.	Stem end rot (Black rot)	***Alternaria citri*** Ell.	Quiescent	Stem end at natural openning
8.	Stem end rot	***Phomopsis citri*** Fawc. ***Diplodia natalensis*** P.Evans	Quiescent	Stem end at natural openning
9.	***Trichoderma*** rot	***Trichoderma viridae*** Pers.	Active	Injured rind
10.	***Fusarium*** rot	***Fusarium*** **spp.** (Mart.) Sacc.	Active	Injured rind

* Taken from different sources .

Stem end rot caused by ***Diplodia natalensis*** P. Evans and ***Phomopsis citri*** Fawc. also develops from quiescent infections of the button (David *et al.*, 1986). As a result of ***Phomopsis citri*** infection, the tissue shrinks and a clear line of demarcation is formed at the junction between diseased and healthy rind. No streaks or fingerlike projection of discolored rind, which are characteristic of ***Diplodia*** stem end rot, are normally seen in the rind of fruit affected by ***Phomopsis*** stem end rot (Anna, 1990).

Brown rot caused primarly by ***Phytophthora citrophthora*** (R. E. Smith & E. H. Smith) Leonian and ***P. parasitica*** Dast generally attacks relatively few fruits and only those close to or in contact with the soil. Fruit decay is first observed as a light brown discoloration of the rind. The affected area is firm and leathery, and infected fruits have a characteristic punget,rancid odor, which distinguish this disease from stem end rot (Whiteside *et* al., 1988). Rotting may occur while the fruits on the tree , or fruits with quiescent infection may pass unnoticed at harvest (Schiffmann and Cohen ,1969 .

Gray mold is caused by the widely distributed fungus ***Botrytis cinerea*** Pers. which attacks many different hosts. Rot associated with gray mold on citrus fruit appears as a brown leathery decay. At high humidity, distinctive patches of gray-brown to olive spore masses appear on the fruit surface. (Whiteside *et al.* 1988).

2 : 1 : 2 Wound fungal pathogens of citrus fruits :

Some decay fungi are wound pathogens and only infect through injuries that occur at handling during harvest and packing as in Table 1 (Eckert and Ogawa, 1985).

Green and blue molds caused by ***Penicillium digitatum*** Sacc. and ***P. italicum*** Wehmer, respectively occur in all citrus growing areas and often constitute the predominant type of decay. In both diseases, the earliest symptom is a soft water soaked area on the peel of the fruit, which soon becomes covered with white mold. Colored spores form at the center of lesion; in green mold there is usually a broad band of white beyond the sporing area, whereas in blue mold the white margin is generally not more than about 2 mm wide. The two fungi frequently appear together.During short term transport and storage green mold usually predominates because at moderate temperature it grows more rapidly (Barmore and Brown , 1982 and Whiteside *et al.* , 1988).

Blue mold is capable of spreading directly into uninjured healthy fruits by contact, causing 'nesting' and this can have serious consequences in long term storage. In contrast, green mold is generally unable to infect adjacent fruits unless they are injured (Anna , 1990).

Deep wounds of the fruit peel are necessary for ***Trichoderma viridae*** Pers., a causal fungus of ***Trichoderma*** rot. Diseased fruit become cocoa brown, and the infected peel remains leathery. Under humid conditions, clumps of white hyphae appear on the surface of the fruit ; later the fruit is covered by coarse, white mycelium and masses of yellow-green to dark green spores (Cole and Wood , 1970) .

Fusarium rot is usually considered as a minor disease of citrus and may be found occasionally in any citrus growing area (Waks *et al.* , 1985). Species of ***Fusarium*** recorded in various countries include ***F. solani*** (Mart.) Sacc. ***F. moniliforme*** Sheld and ***F. oxysporum*** Schlect. Lesions are generally dark brown, leathery and sunken . Under humid conditions a white or pinkish mold forms on the surface (Anna 1990) .

2 : 2 Green mold disease of citrus fruits :

Green mold incited by ***Penicillium digitatum*** Sacc. is a limiting factor in long distance marketing of citrus fruits. The disease is the most important postharvest decay of citrus fruits and accounts for the most decay losses of citrus worldwide (Brown , 1973 and Bancroft *et al.*, 1984) . Practical losses due to green mold arise from two sources : individual diseased fruit and sound fruit devalued (soiled) by superficial contamination with spores of ***P. digitatum*** (Eckert *et al.* ,1979).

2 : 2 : 1 Symptoms and habitat:

In the early, pinhole stage, the decay appears as a soft, watery and slightly discolored spot 6-12 mm in diameter. White mycelium appears on the rind surface and after it reaches a diameter of approximately 2.5 cm, olive green spores are produced at the center of the mycelial area. The sporulating area is surrounded by a broad zone of white mycelium and an outer zone of softened rind. The entire fruit is soon encompassed by a mass of olive green spores which are easily dispersed if the fruit is handled, shaken or exposed to air currents. If the relative humidity is low, the whole fruit shrinks to a wrinkled, dry mummy. If the relative humidity is high, other molds and bacteria become involved . Conidia of ***P. digitatum*** is commonly found in the field, packing area, storage room, transit container and in markets (Anna , 1990 and Whiteside *et al.*, 1988) .

2 : 2 : 2 Mechanism of infection:

Conidia of ***P. digitatum*** are invariably present in the atmosphere of citrus growing area and initial infection of fruit is characteristically via

wounds in the peel of the fruit, such wounds usually occur during harvesting and subsequent handling (Kavanagh and Wood,1967).

P. digitatum can also invade fruit through physiologically induced injuries, such as chilling injury and stem end rind breakdown . The infection and sporulation cycle can be repeated many times in a packinghouse (Barmore and Brown , 1982 and Whiteside *et al.* ,1988) .

2 : 2 : 3 Taxonomic characteristics of *Penicillium digitatum:*

P. digitatum Sacc. belongs to the class: Ascomycetes, order: Eurotiales and Family : Eurotiaceae (Alexopoulos and Mims, 1979) . It produces conidiophores which are borne from the aerial hyphae forming smooth walled stipes ranges between 70-150 x 5-7 μ m in length and diameter, respectively. Those stipes form terminal penicilli, when best developed, terverticillate but frequently biverticillate or irregular. Rami 20-30 x 5-6 μ m ; metulae in verticils of 2-3, commonly 15-25 x 5-6 μm, phialides in verticils of 3-5, ampulliform to cylindroidal, 10-15 x 4-5 μ m. Each phialide bear a chain of ellipsoidal to cylindroidal conidia ranges between 6-8 x 2.5-6 μ m. ***P. digitatum*** presents a microscopic morphology distinguished by the size of each element in the penicillus (John, 1979) . Colonies of ***P. digitatum*** on artificial media are similar in appearance to the mold growth that develops on infected fruit (Ramirez , 1980).

2 : 2 : 4 Control of green mold disease:

2 : 2 : 4 : 1 Chemical control measures:

The benzimidazale fungicides benomyl, thiabendazole and carbendazim were manufactured late 1960s to control green mold disease as well as other postharvest diseases of citrus fruits (Eckert and Ogawa, 1985).

Fungicides inhibitory to the pathogen must be applied within 48 hours when ambient temperatures are 20-27 ° C in order to abort the infection

process. However, benomyl (1.9g /1L) residues prevented emergence of hyphae from the fruit surface inoculated by ***P. digitatum*** during 2 weeks of storage at 20 ° C, whereas carbendazim (1.25g/1L) failed to prevent fruit decay at the same condition of incubation (Eckert *et al.*, 1979).

Brown (1984) found that percentage control of green mold by dipping orange fruits in benomyl (600 mg/L) and carbendazim (600mg/L) was 95 and 70% , respectively.

Inspite of inoculation of orange fruit, cv. 'Shamouti', with ***P. digitatum*** conidia, thiabendazole fungicide reduced the decay caused by this fungus (Gutter, 1975). Brown and Albrigo (1970), cited by Brown and Albrigo (1972) , found that grove application of benomyl reduced postharvest decay of citrus fruits caused by ***P. digitatum*** and ***Phomopsis citri***.

Surface residues of flavedo and albedo persisted for at least 70 days after application. However, the quantity of carbendazim remaining in orange peel after 70 days did not control green mold. Some inhibition of ***P. digitatum*** was observed in 'Valencia' oranges inoculated 84 days after grove spraying (Brown and Albrigo, 1970).

Wax is commercially applied to many fruits and vegetables to reduce dehydration and improve consumer appeal (Gerald and Singh, 1985 and Davis and Hoffmann, 1973). However, no significant differences were obtained between wax and water when orange fruits were dipped in both solvents (Brown, 1984). Waks *et al.* (1985) found that waxing of citrus fruits reduced the incidence of green mold in comparison with the control .

2 : 2 : 4 : 2 Physical control measures :

Short-wave ultraviolet (UV) light is known as a specific phytoalexin elicitor. Elicitation of phytoalexin by (UV) illumination increased disease resistance of genetically susceptible genotypes of several species. However, information on phytoalexin induction by abiotic elicitors in citrus and related

fruits has been limited (Bridge and klarman, 1973; Charles *et al.*, 1994 and Rodov *et al.*, 1992).

Riov (1970) identified two physical phytoalexins in the peel of grapefruit (***Citrus paradisi*** Mcfa.) : Scopoletin (6-methoxy, 7-hydroxycoumarin) and Scoparone (6,7-dimethoxycoumarin). Jame and Robert (1977) showed that 6,7-dimethoxycoumarin was found in the peels of several different sweet orange varieties and other citrus species .

Another physical (abiotic) elicitor for postharvest green mold disease control was achieved by heat treatment . Scoparone was detected in large amounts in lemon fruits after heat treatment at 36 °C for 3 days. Heat treatment prevented development of decay in the inoculated fruits with ***P. digitatum*** (Kim *et al.* , 1991).

Ultraviolet illuminated (UV dose: 1.5×10^{3} J.m $^{-2}$) fruits inoculated with dry spores of ***P. digitatum*** 2 days after treatment had a lower incidence of decay than the control. Illumination of previously inoculated fruits failed to prevent decay after 20 days (Rodov *et al*, 1992) . Exposure of harvested grapefruits to (UV) light induced resistance against green mold decay caused by ***P. digitatum*** (Droby *et al.* , 1993 cited in Charles *et al.*, 1994).

Chalutz *et al.* (1992) , cited by Charles *et al.* (1994) , reported that the onset of (UV) induced resistance coincided with the induction of activity of phenyl alanine lyase (PAL) and peroxidase . (PAL) activity in the peel of grapefruit increased within 24 hours after (UV) treatment and remain elevated for 72 hours, while peroxidase activity reached its maximum 72 hours after treatment. Both enzymes are considered to play a role in inducible resistance in plants against pathogens.

2 : 2 : 4 : 3 Biological control with *Debaryomyces hansenii* yeast :

A number of antagonistic microorganisms serves as alternatives to synthetic fungicides. Among these, antagonistic yeasts have proved particularly effective. (Wisniewski and Wilson,1992 and Charles *et al.*, 1994).

De Matos (1983) , cited by Charles and Chalutz (1989) , evaluated a number of antagonists for biological control of ***P. digitatum*** on citrus. De Matos was able to reduce mold incidence from 35% to 8% when a species of ***Trichoderma*** was inoculated with the pathogen into the lemon peel.

The antagonistic yeast ***Pichia guilliermondii*** (US-7) was shown to induce (PAL) in citrus fruit peel indicating the induction of defense response against ***P. digitatum*** decay (Wisniewski and Wilson, 1992) .

Debaryomyces hansenii (Zopf) Van Rij was tested as antagonistic yeast against ***P. digitatum*** mold on citrus fruits. Percentage of infected lemon fruits that dipped in the yeast suspension was 25, whereas 95 percent of fruits were infected in check fruits 13 days after inoculation (Charles and Chalutz , 1989) .

D. hansenii did not inhibit ***P. digitatum*** by producing antibiotics, but it inhibited the incidence of green mold, blue mold and sour rot of several citrus fruits cultivars . Although the antagonist reduced decay on all cultivars tested, its efficacy varied between cultivars. Efficacy of ***D. hansenii*** was mainted when applied simultaneously or prior to inoculation with ***P. digitatum*** . On the other hand, efficacy was reduced when ***D. hansenii*** was applied after inoculation . However , the lack of any known species that cause plant diseases increase the potential applicability of this biological control agent for combating postharvest diseases of fruits (Chalutz and Wilson , 1990) .

III : Materials and Methods

3 : 1 Isolation of postharvest fungal causal agents:

Samples of decayed orange fruits of different cultivars were collected weekly - for one month - from two packinghouses in Jordan. Samples of 'Shamouti' cultivar were collected from carton boxes (30 x 40 x 25 cm) that had been stored for three months at 2-4 °C in Abu-Sham packinghouse in Amman. Samples of 'Valencia' fruits were also collected from South Agricultural Company packinghouses in Al-Qastal area (20 km south of Amman). Fruits were stored at 1-2°C for two months . Other decayed fruits were also collected from the market. Each decayed fruit was placed in a separate plastic bag and given a certain number .

Isolation of fungi was accomplished by removing the fruit skin over the margin of the decayed area by sterilized knife and plated into sterilized 10 cm plastic-dishes containing Potato Dextrose Agar (PDA) medium . The medium was acidified with 10 drops of 25% lactic acid and 0.5 g of PentaChloro-NitroBenzene (PCNB) per liter added after autoclaving to inhibit bacterial growth (Dhingra & Sinclair, 1987). Plates were then incubated for one week at 20 ± 1°C.

3 : 2 Identification of the causal agents

Fungal growth was identified based on mycelial growth, size and shape of conidia and conidiophores. Identification was confirmed with the help of Dr. Abu-Blan , after preparing individual slides and examining them with the microscope following special identification keys (street, 1974; Alexopoulos & Mims, 1979 and John, 1979).

3 : 3 Pathogenicity tests :

All fungal causal agents isolated from decayed orange fruits were inoculated to healthy, water washed and surface sterilized (70% ethyl alcohol) Shamouti orange fruits. Inoculation was accomplished by removing a part of orange fruit peel by using sterilized cork borer and placing fungal mycelium obtained from 7 days old culture . Inoculated fruits were incubated at 20 ± 1°C for one week. Symptoms of inoculated fruits were compared to the diseased fruits collected from the packinghouses & markets . Reisolation of decays was done on 10 cm plastic-dishes containing PDA medium and fungal growth was again examined microscopically.

3:4 Occurrence of postharvest fungal diseases of oranges :

Shamouti, Navel and Valencia orange fruits were picked on Dec.15, 1995, Dec .10, 1995 and Feb. 1, 1996, respectively . Fruits were picked by farm labors from different citrus groves in Jordan Valley. From each orange cultivar, eight boxes (30 x 48 x 12 cm) were packed , each containing 30 fruits . Picked fruits were neither wrapped , washed nor chemically treated. Four boxes of each cultivar were stored in cold room at 1-3 C and 80-90% relative humidity (R.H.). Multi-fan unit was used. The same number of boxes for each cultivar was kept at common store (ambient temperature) at 18-20 C. All boxes were stored for three months in which incidence of diseased fruits was determined at 10 day intervals . To identify the fungi which grows on oranges of different cultivars, decayed fruits were isolated as previously mentioned at the end of the storage period.

3 : 5 Evaluation methods of *Penicillium digitatum* Sacc. control in the laboratory.

3 : 5 : 1 Source of the orange fruits

Fruits of Shamouti orange were picked during mid December, 1995 and 1996 seasons from 15 - year - old trees grafted on sour orange rootstock at the Agricultural Experimental Station in Central Jordan Valley. Fruits were picked carefully by twisting and pulling by hand . Fruits were not allowed to fall onto the ground.

3 : 6 Laboratory procedures :

Fruits were thoroughly washed with water , surface sterilized with 5% sodium hypochlorite (Hypex, 6.5%) and dipped in 70% ethyl alcohol for 30 seconds. Fruits were air dried and wounded at 3 points near stem end by sterilized dissecting needle. The wound size was 5-10 mm wide by 2-4 mm deep. Each fruit was inoculated by dipping in a suspension of ***Penicillium digitatum*** with approximate concentration of 20×10^{6} conidia / ml for 2 minutes.

Fruits were incubated at 20 ± 1°C for 24 hours . Fruits were then subjected to the following treatments:

1. Postharvest dipping in fungicides.
2. Postharvest fruits waxing.
3. Ultraviolet illumination.
4. Dipping in the ***Debaryomyces hansenii*** cells suspension .

3 : 6 : 1 Postharvest dipping of fruits in fungicides :

280 inoculated fruits were dipped separately in the suspension of benomyl, carbendazim, thiophanate methyl and in various mixtures of these fungicides for 2 minutes (Table 2). The suspension of each fungicide was

prepared in accordance with the recommended concentration by the manufacturers. Fruits were then air dried and incubated separately for 15 days at 20 ± 1°C and 85-95% R. H. Similar number of fruits / treatment were placed in a refrigerator at 1-3°C and 80-90% R. H . Fruits dipped in distilled sterilized water served as control. The pH of fresh stock of fungicide suspensions were also measured.

Table (2): Concentration and PH of fungicides suspensions used in the Postharvest fruit dipping.

No.	*Trade name*	*Common name*	*Concentration (g/20L)*	*PH*
1.	Benlate	Benomyl	12	7.2
2.	Carbendazim	Carbendazim	20	7.6
3.	Topsin M	Thiophanate methyl	20	7.1
4.	Benlate + Carbendazim		12 + 20	7.1
5.	Benlate + Topsin M		12 + 20	7.5
6.	Carbendazim + Topsin M		20 + 20	7.4

3 : 6 : 2 Postharvest fruit waxing :

80 inoculated fruits were sprayed with a wax material (Citrashine®, Catania, Italy) at a rate of 3 ml / fruit. Hand sprayer was used to cover all fruit surface. Fruits sprayed with distilled sterilized water served as control . Fruits were then air dried and incubated for 15 days at 20 ± 1°C with 85-95% R.H and at 1-3°C with 80-90% R.H in a refrigerator.

3 : 6 : 3 Illumination by ultraviolet (UV) light:

Inoculated points at the stem end of the fruit were placed approximately 15 cm from two lamps (GRO-LUX / 14 W-output, Sylvania, Great Britain) of UV. (wave length 254 nm) lamps (Plate 1) . Illumination was carried out at controlled temperature inside incubator at 20 ± 1°C . Fruits were exposed for 2, 4, 8, 16 and 24 hours. Light intensity (Table 3) was measured by using Thermopile radiometer (Kipp & Zinnon, Holland). Each exposure time was considered as a separate treatment . Fruits were then transferred to the incubator and incubated for 15 days at 20 ± 1°C with 85-95% R. H. and at 1-3°C with R. H. of 80-90% in a refrigerator. Non-illuminated fruits served as control.

Table (3) : Light intensity measurement for (UV) light at different exposure times

Time (in hours)	*Light intensity (KJ / cm^2)*
2	0.29
4	0.58
8	1.16
16	2.32
24	3.4

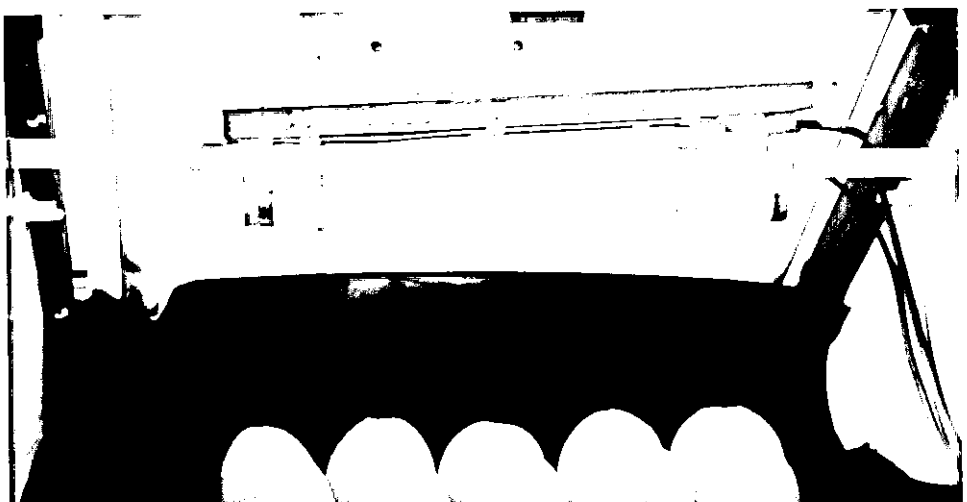


Plate (1) : Inoculated Orange Fruits Exposed to UV Lamps Inside the Incubator .

3 : 6 : 4 Dipping in the *Debaryomyces hansenii* cells suspension:

The antagonistic yeast that used in the control experiment was ***D. hansenii*** (ATCC, 19217). The yeast was supplied by the Catholic University of Leuven (MUCL), Louvain, Belgium . ***D. hansenii*** was cultivated on Dextrose Yeast Peptone Agar (DYPA) slant tube. (Dextrose 20g, Yeast Extract 5g, Peptone 10g, Agar 20g dilluted in 1L. distilled water).

A loopful of ***D. hansenii*** colony from the stock slant was streaked across each (PDA) plate and incubated at 25 ± 1°C for 5 days in the incubator. Yeast growth was transferred into 100 ml dilution bottles with distilled sterilized water. Dilution bottles were shaked for 5 hours at 100 rpm using the Junior Orbit shaker. Fruits inoculated with ***P. digitatum*** spores (20 x 10^6 conidia / ml) were dipped for 3 minutes in cell suspension of ***D. hansenii*** with approximate concentration of 15 x 10^7 cells / ml . Fruits were then air dried and incubated at 20 ± 1°C with 85-95% R. H. and at 1-3°C with 80-90% R.H. in a refrigerator for 15 days . Fruits dipped in distilled sterilized water served as control .

3 : 6 : 5 Preharvest fungicides spraying of orange trees in the field:

Shamouti orange trees of 15 years old, grafted on sour orange root - stock and drip irrigated at the Argicultural Experimental Station in Central Jordan Valley were sprayed, nine days before harvest, with benomyl (12g / 20L.), carbendazim (20g/20L) or thiophanate methyl (20g/20L) fungicides during the first week of December 1995 and 1996 seasons. Separate fungicide spraying was applied twice with one week interval. Two trees for each fungicide separated by a single tree, were sprayed. Fruits were picked randomly from the sprayed trees after 2 days from the second spray and brought to the laboratory. Fruits were then wounded, inoculated with ***P.***

digitatum ($20x10^6$conidia/ml) and incubated as mentioned before . Fruits picked from two water sprayed trees served as control.

3 : 7 Assessment of fruit decay caused by *P. digitatum*:

Fruits decay caused by ***P. digitatum*** as resulting from each of the previous treatments was assessed. Fruits were divided into two 20 fruits lots, 4 replications of 5 fruits each. One lot was incubated at 20 ± 1°C with 85-95% R.H. The other lot was incubated in a refrigerator at 1-3°C with 80-90 % R.H.

Results of decay incidence and severity from the two trials (two seasons) of each treatment were averaged and determined at 1, 3, 6, 9, 12 and 15 days after treatment.

3 : 7 : 1 Incidence of the green mold:

Disease incidence was determined by recording the percent of decayed fruits within the whole inoculated fruits.

3 : 7 : 2 Severity of decay:

Visual estimation was used for disease severity assessment. Scale of disease severity developed by Horsfall and Heuberger (1942) was used in this study for treatments evaluation.

Scale	*% of decay in fruit*
0	Free from infection
1	Trace-25% fruit area killed
2	26-50% fruit area killed
3	51-75% fruit area killed
4	76-100% fruit area killed

The overall assessment of disease severity was calculated by the following equation:

$$Disease\ Severity = \frac{\text{Sum of All Numerical Ratings}}{\text{Total Number of Assessed Fruits}}$$

3 : 8 Statistical analysis:

Data on the incidence and severity values for all treatments were statistically analyzed using completely randomized design (CRD) and means were separated by (LSD) at 0.05 level of probability.

3 : 9 Fungicides in vitro test:

Fungicides used for in vitro tests were benomyl (12g / 20L), carbendazim (20g / 20L) and thiophanate methyl (20g / 20L). Mixtures of each two fungicides were also used.

Fresh stock suspension of each fungicide and their mixtures were prepared in sterilized distilled water and added aseptically to sterile liquified (PDA) after autoclaving and cooling to about 48° C to provide the desired final concentration of each fungicide.

The solution of each fungicide was throughly mixed with (PDA) medium. Plates of solidified medium were inoculated with 6 mm diameter fungal plugs obtained from the edges of 7 days old culture of ***P. digitatum***, plates were then incubated at 20 ± 1° C for 15 days. Plates of solidified medium without fungicides served as control . Six replicates (Plates) were used for each fungicide and each mixture . Mean diameter of the resulting colonies were measured daily. Least significant differences (LSD) had been used to compare between the treatments .

IV : Results

4:1 Symptoms of diseased orange fruits:

Decayed orange fruits collected from the different packinghouses as well as from the markets showed different characteristics symptoms of the causal agents.

P.digitatum showed a mass of olive green spores with a broad zone of white mycelium (Plate 2); while fruits infected with ***P. italicum*** exhibited a mass of blue spores surrounded by a very thin zone of white mycelium color (Plate 3). Fruits infected with ***Fusarium sp.***were covered by a white growth over the surface of the fruit peel (Plate 4) ; while those infected with ***Phytophthora sp.*** were colored with irregular brown spot of leathery appearance (Plate 5). Fruits infected with ***Alternaria citri*** were colored with a light brown - blackish lesion at the stem end of the fruit peel (Plate 6). while fruits infected with ***Botrytis cinerea*** were showed a nests of rot with a mass of gray spores covering the most of the fruit surface (Plate 7) . Fruits infected with ***Trichoderma sp.*** were partially covered by coarse , white mycelium and masses of green to dark green spores on the surface of fruit peel (Plate 8).

4:2 Identification of the fungal causal agents :

The fungal growth 7 days after isolation on PDA medium plates were identified microscopically according to Alexopoulos and Mims (1979) , John (1979) and keys described by street (1974) . Fungi isolated from decayed orange fruits were identified as the following disease causal agents :

1. ***Penicillium digitatum*** was isolated from six fruits from Amman . Conidiophore of ***P.digitatum*** bearing terminal penicilli. Phialides in verticils of 3-4 and conidia ellipsoidal to cylindroidal, 11-13×3-4μ m , borne in disordered chains (Plate 9) .

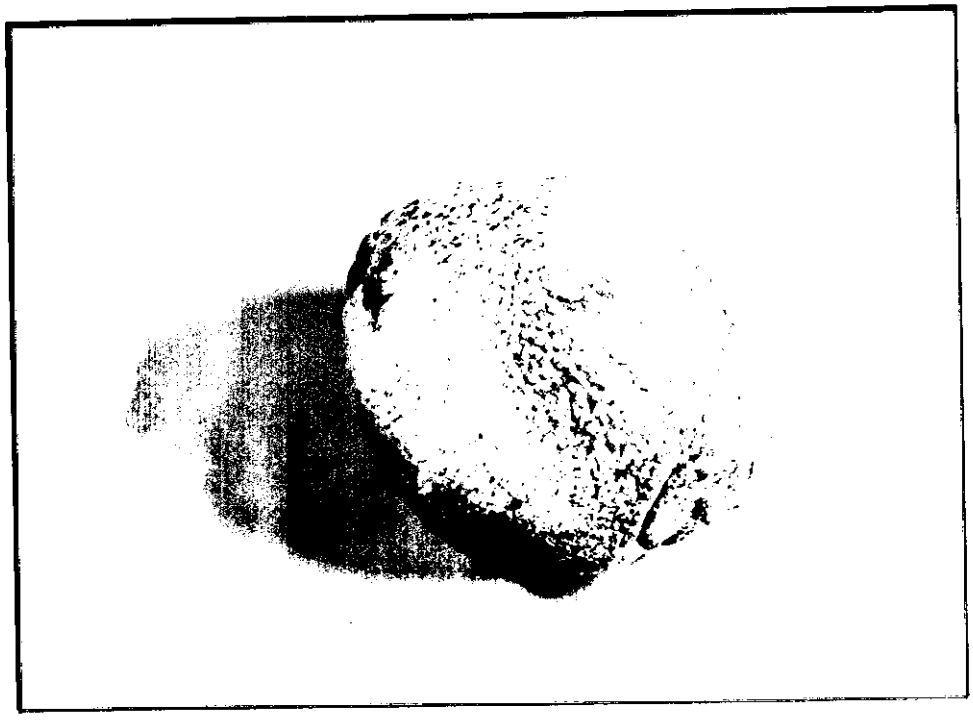


Plate (2) : Symptoms of ***P. digitatum*** on infected Shamouti orange fruits

Plate (3) : Symptoms of ***P. italicum*** on infected Shamouti Orange Fruits

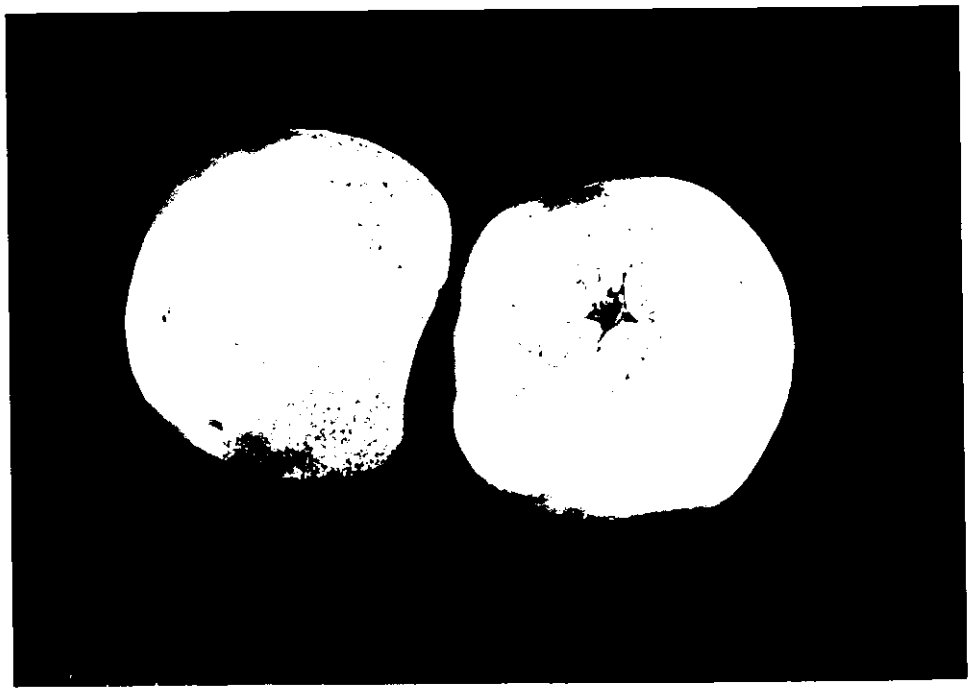


Plate (4) : White mycelium growth caused by ***Fusarium sp.*** on infected Valencia orange fruits

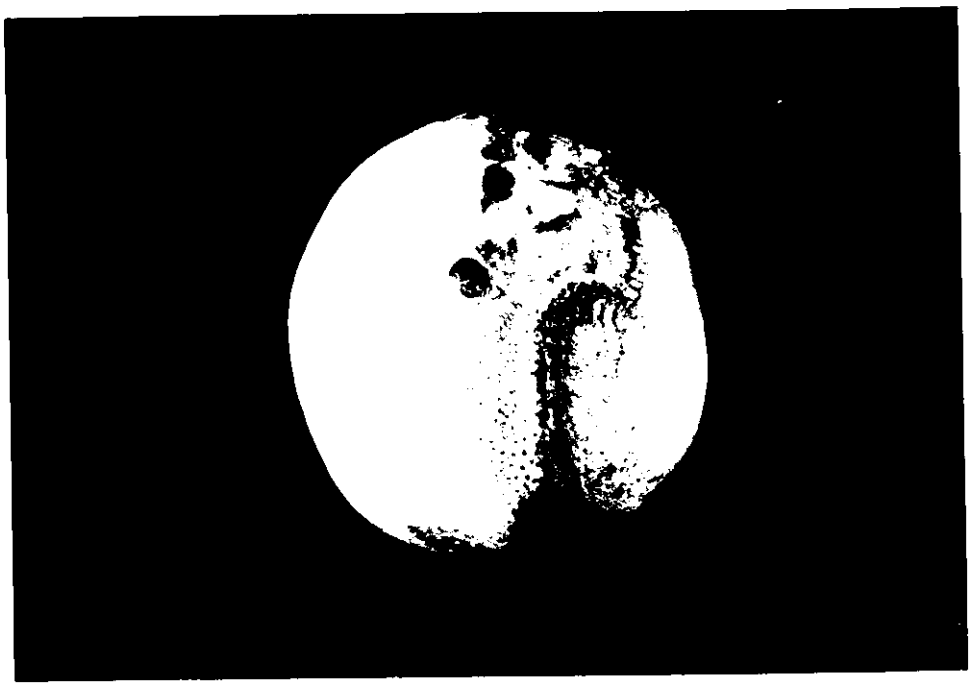


Plate (5) : Symptoms of ***Phytophthota sp***. infection on infected Valencia orange fruits

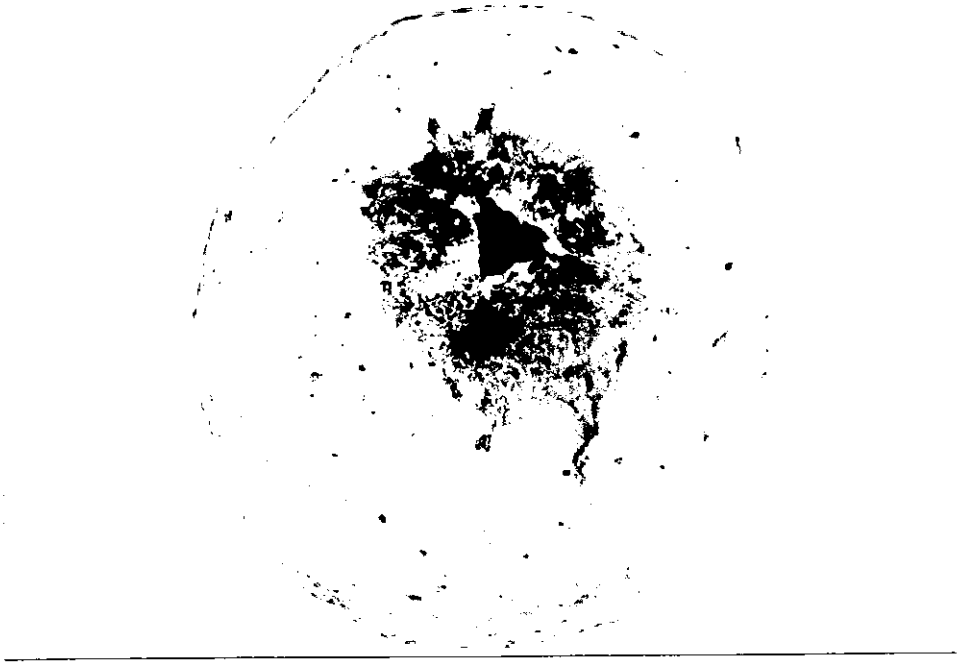


Plate (6) :Symptoms of ***Alternaria citri*** infection at the stem end of Shamouti orange fruits

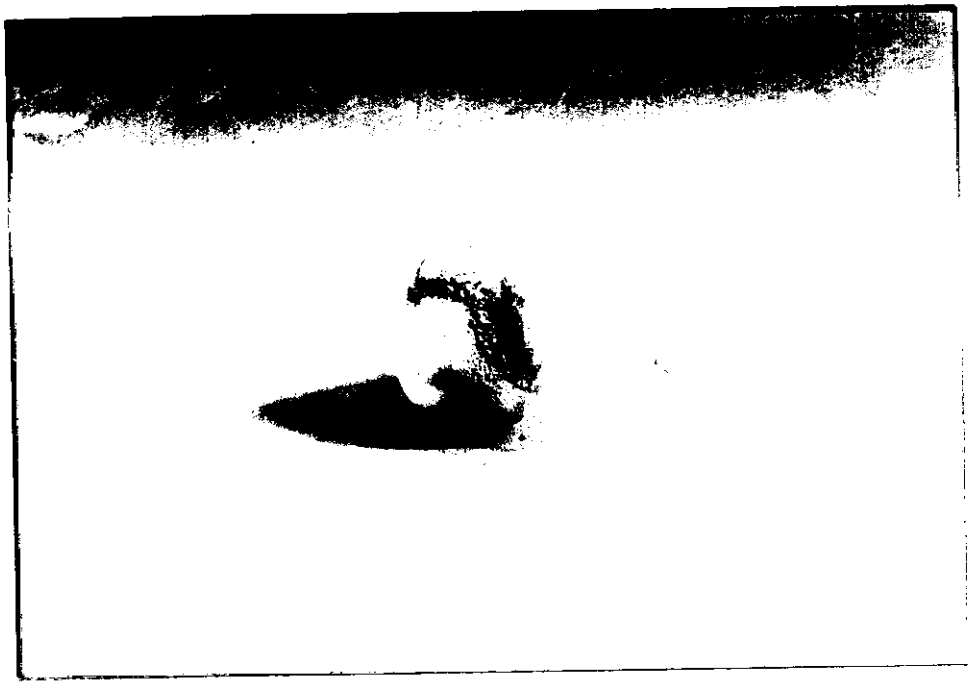


Plate (7) : Nest of rot with a mass of gray spores of ***Botrytis cinerea***

Plate (8) : Symptoms of ***Trichoderma*** infection on Shamouti orange fruits

Plate (9) :Conidiophores and conidia of ***P.digitatum*** . (100 X)

2. ***Penicillium italicum*** was isolated from two fruits from Madaba . Conidiophore bearing terminal penicilli, Phialides in verticils of 4-5 and conidia short cylindroidal, 3-4 × 2-3 µm, borne in a long disordered chains (Plate 10) .
3. ***Fusarium sp.*** was isolated from two fruits from Rosaifa . The macroconidia were several celled, slightly curved or bent at the pointed ends. The microconidia were one - celled and ovoid (Plate 11) .

   Phytophthora sp. was isolated from two fruits from Al- Qastal . ***Phytophthora sp***. produced lemon shaped sporangia and sporangiophore (Plate 12).
4. ***Alternaria citri*** was isolated from four fruits from Amman . Conidiophores were short to elongate, bearing short clavate conidia, 30-35 x 17-20 µ m. They were 4-6 septate and divided by 1-2 longitudinal septa (Plate 13) .
5. ***Botrytis cinerea*** was isolated from three fruits from Amman . Conidiophores were long slender, pigmented and branched . Clusters of ovoid, 1-celled, conidia were borne at apical cell (Plate 14) .

6. ***Trichoderma sp.*** was isolated from two fruits from Amman . Conidiophores were hyaline , much branched and not verticillated . Conidia were hyaline , 1-celled , ovoid were borne in small terminal clusters (Plate 15, A &B) .

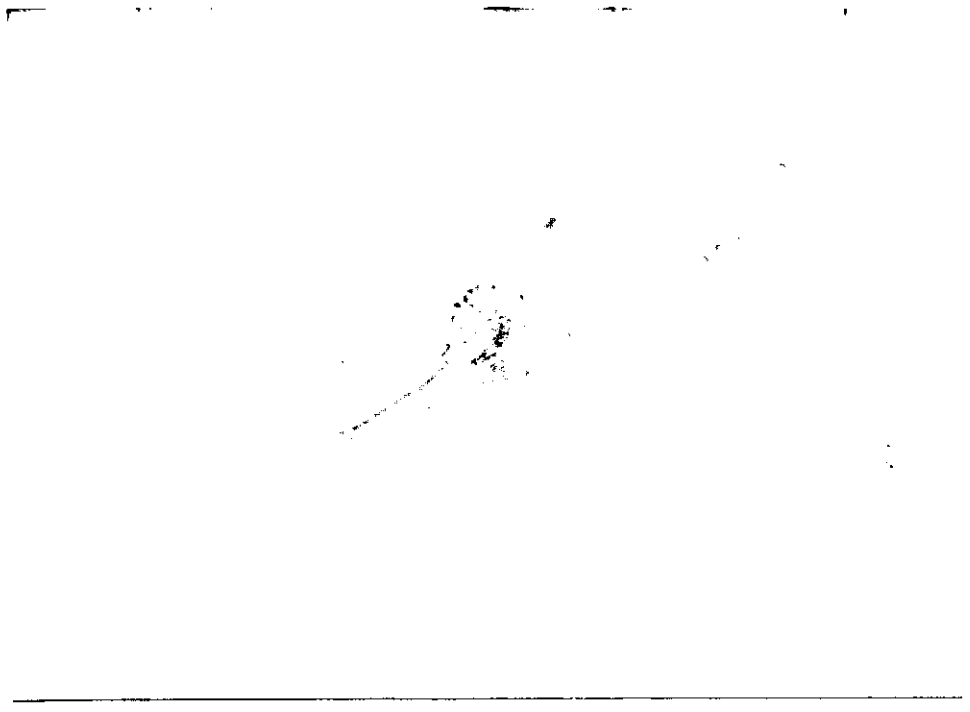


Plate (10):Conidiophores and conidia of ***P. italicum*** . (100X)

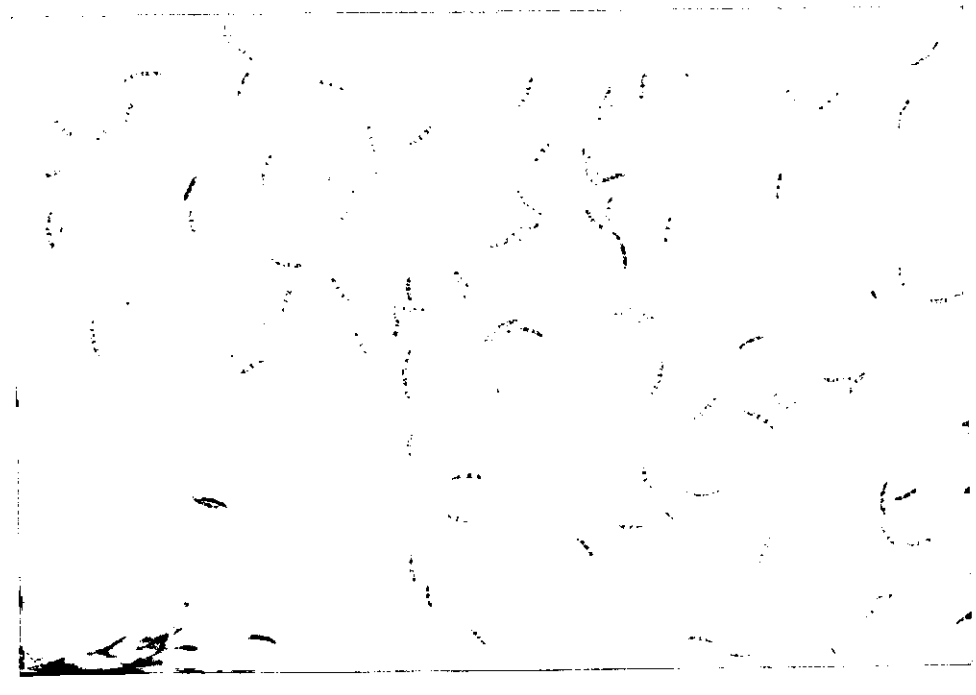


Plate (11): Microconidia and macroconidia of ***Fusarium sp***. (80X)

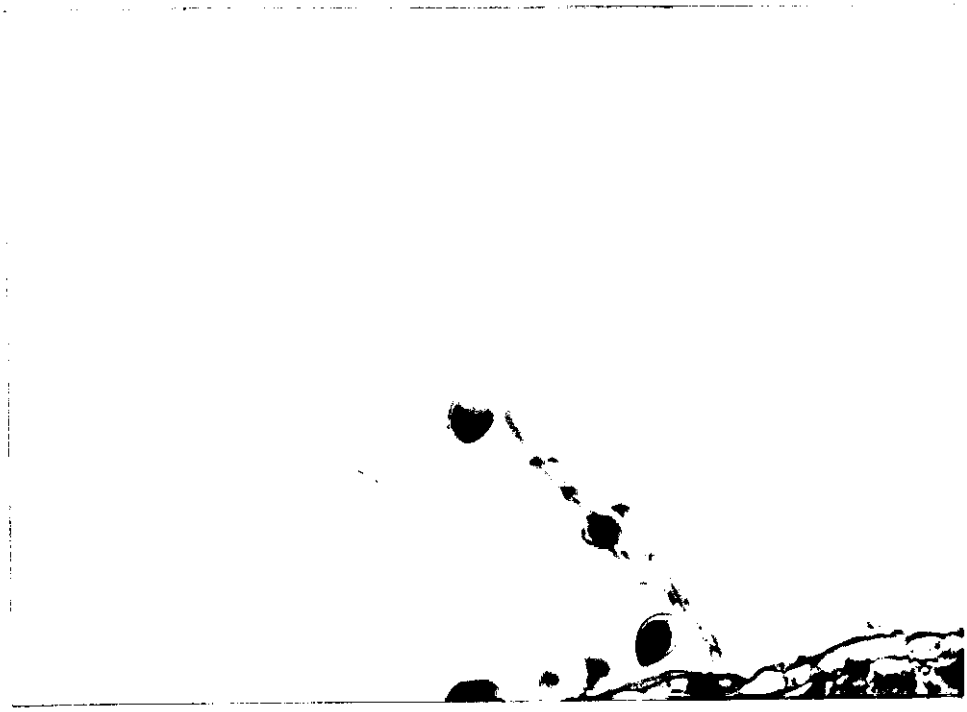


Plate (12): Sporangiophore and sporangia of ***Phytophthora sp.*** (80X)

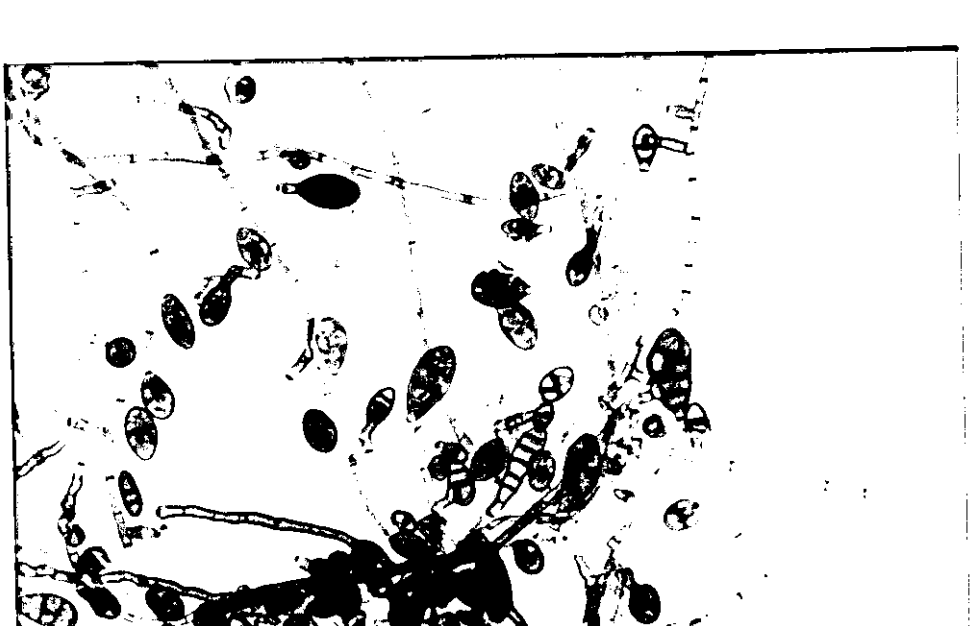


Plate (13) : Transversely and longitudinally septation of ***Alternaria citri*** (80X)

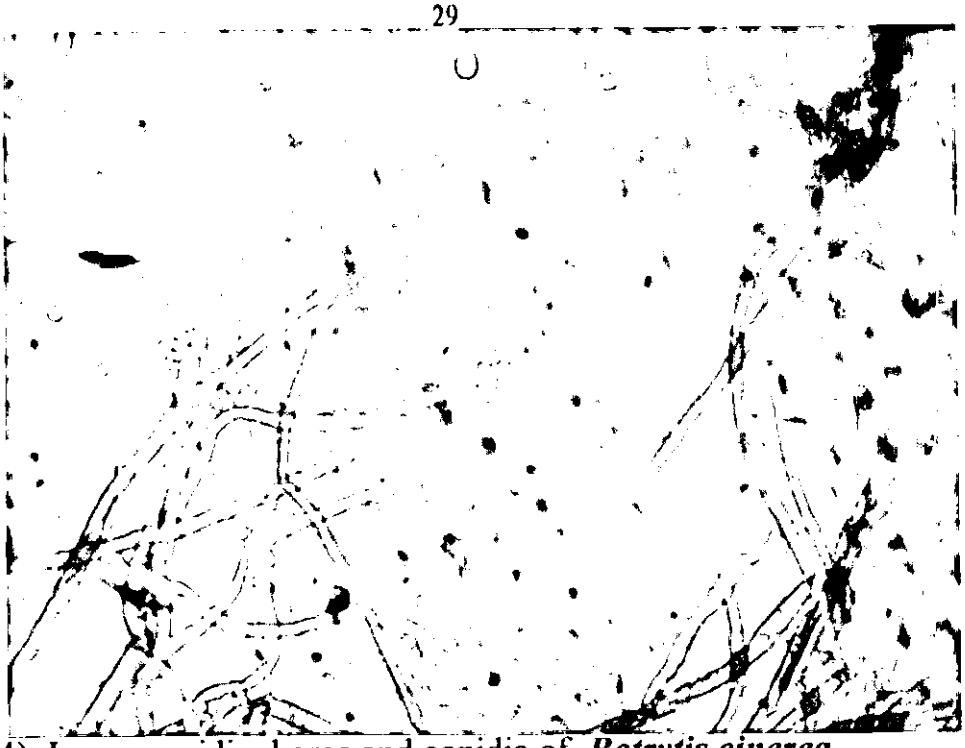


Plate (14): Long conidiophores and conidia of ***Botrytis cinerea*** .
(100X)

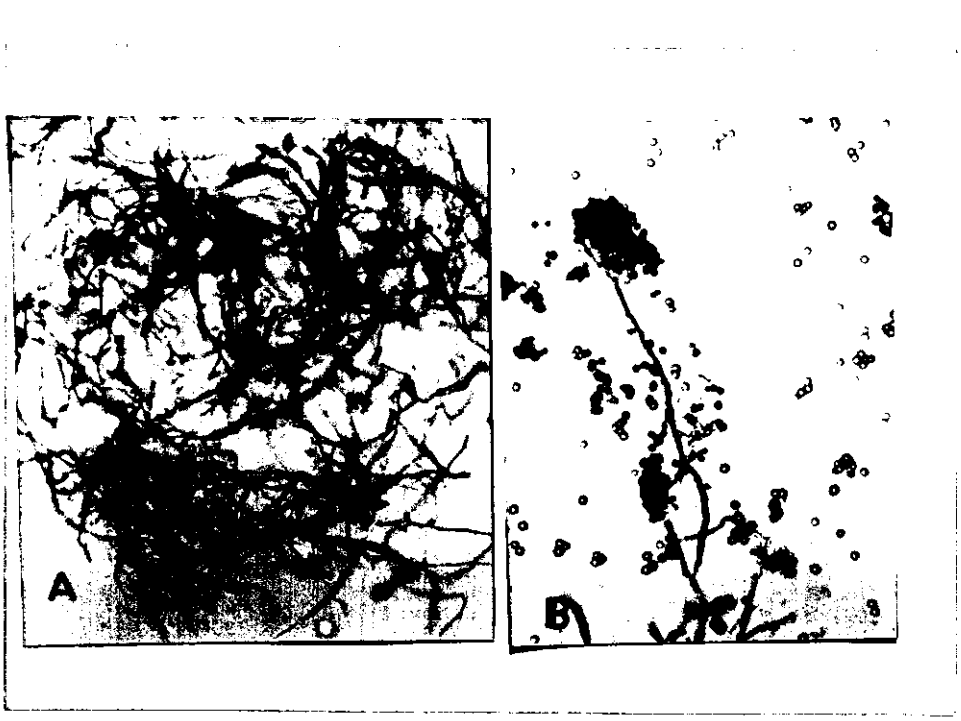


Plate (15): Conidiophores and conidia of ***Trichoderma sp.*** , A. branched condiophores , B . clusters of conidia. (100X)

4:3 Pathogenicity tests:

All fungal isolates fulfilled the Koch's postulates . Symptoms resulted from the inoculation of healthy orange fruits with 7 days old culture of different fungal isolates were similar to symptoms of the diseased fruits that had been collected from packinghouses and markets.

Morphological characteristics including size and shape of conidia and conidiopheres of fungal growth reisolated from the decayed fruits was similar to that of the initially diseased ones .

4:4 Occurrence of postharvest fungal diseases of oranges :

Results on incidence decay percent of different orange fruit cultivars in cold room (1-3°C and 80-90%R.H.) and common store (ambient temperature) are shown in Figures 1,2 and 3 (Appendices1,2and 3) However, results on this experiment can be summerized as follows:-

1. ***Penicillium digitatum*** and ***P. italicum*** were the only two fungal pathogens isolated from the different orange cultivars at the end period of storage at both storage conditions (Plate 16, A&B).
2. No significant differences in the incidence percent of decay caused by both fungi were found between three orange cultivars.
3. For the same orange cultivar, high significant differences in the incidence percent of decay caused by ***P. digitatum*** were found between the two storage conditions.
4. Incidence percent of fruits decay caused by ***P. digitatum*** for both conditions of storage was significantly different in comparison to incidence percent of fruits decay caused by ***P. italicum*** at the same conditions of storage and for all stored cultivars .
5. Slightly significant difference was obtained between both conditions of storage for the incidence percent of fruits decay caused by ***P. italicum*** .

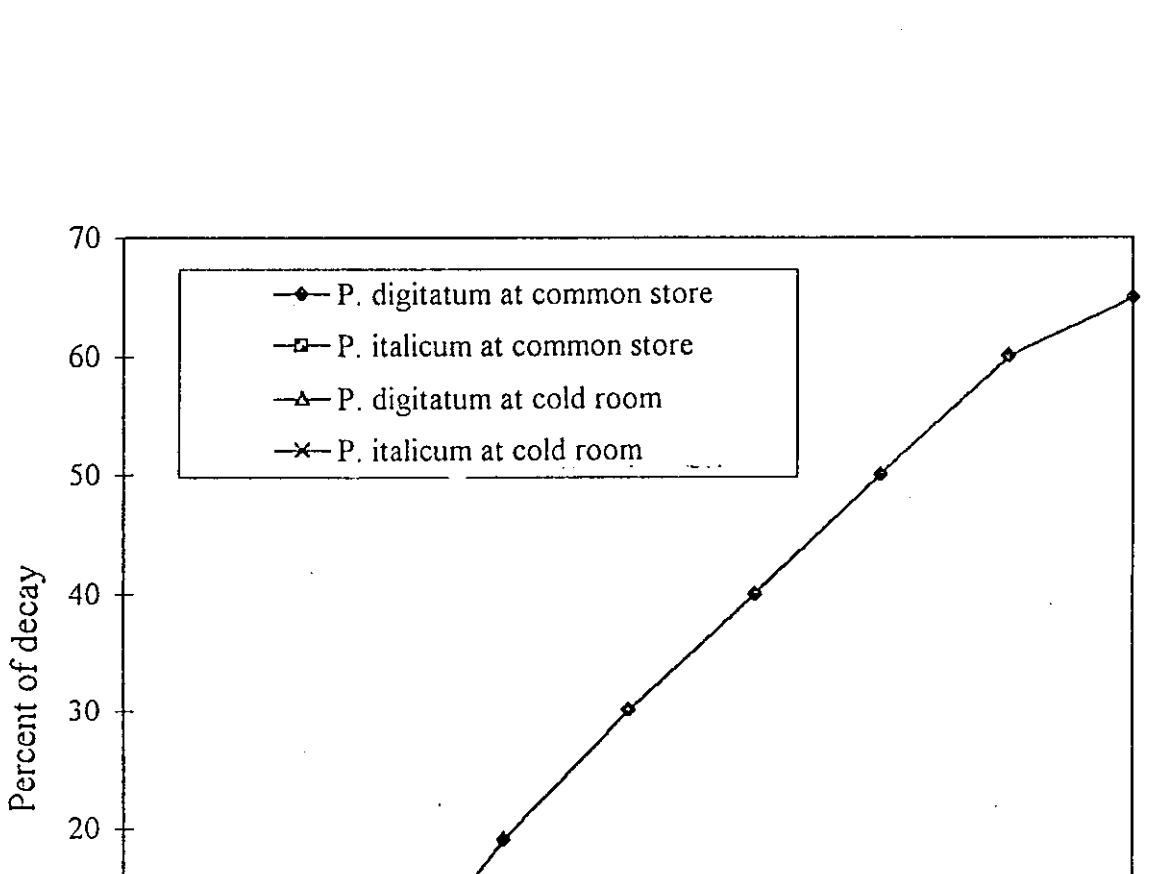


Figure (1) : Percent of Decay Caused by ***Penicillium*** on Valencia orange fruit in cold room (1-3 °C) and common store (ambient temprature)

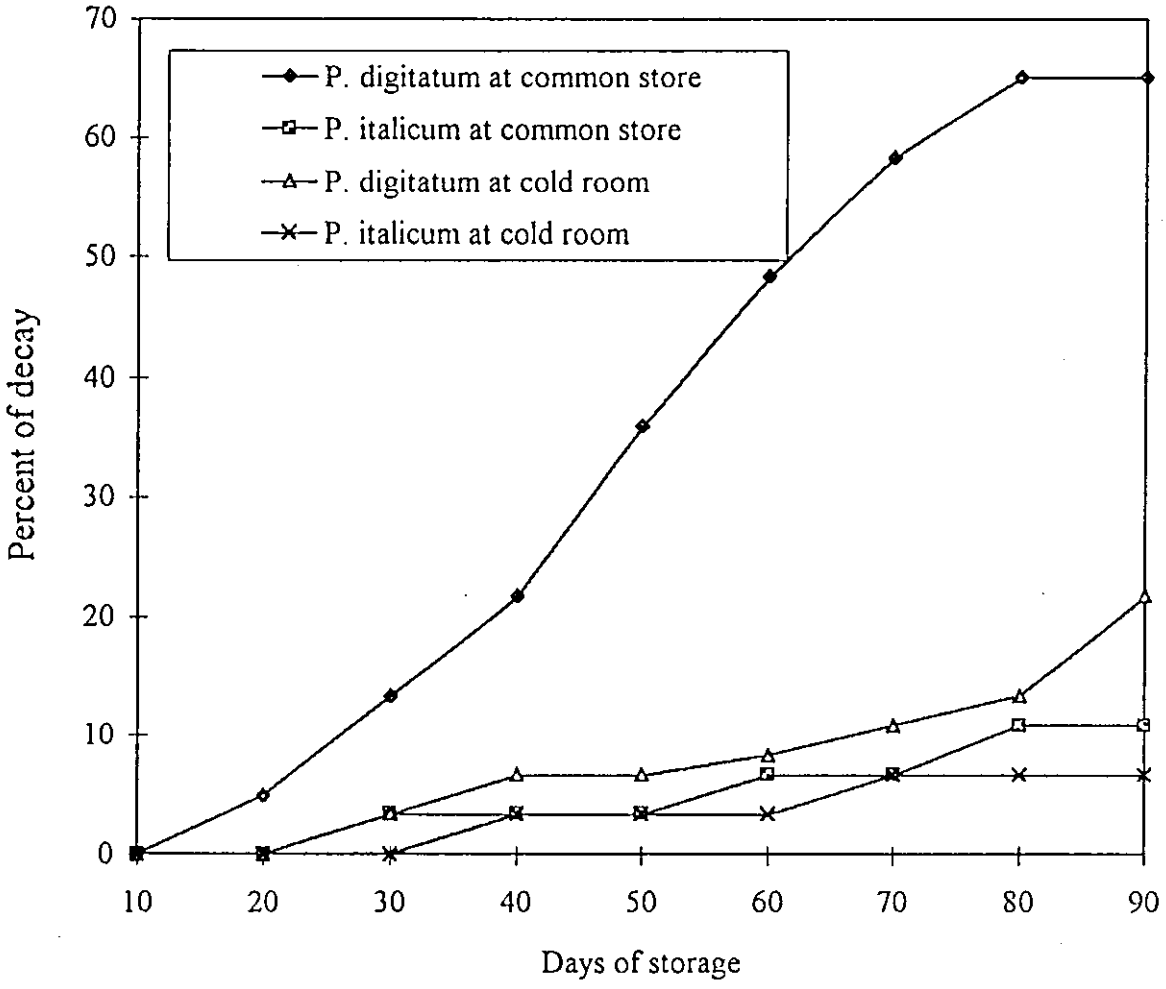


Figure (2) : Percent of Decay Caused by ***Penicillium*** on Navel orange fruit in cold room (1-3 °C) and common store (ambient temprature)

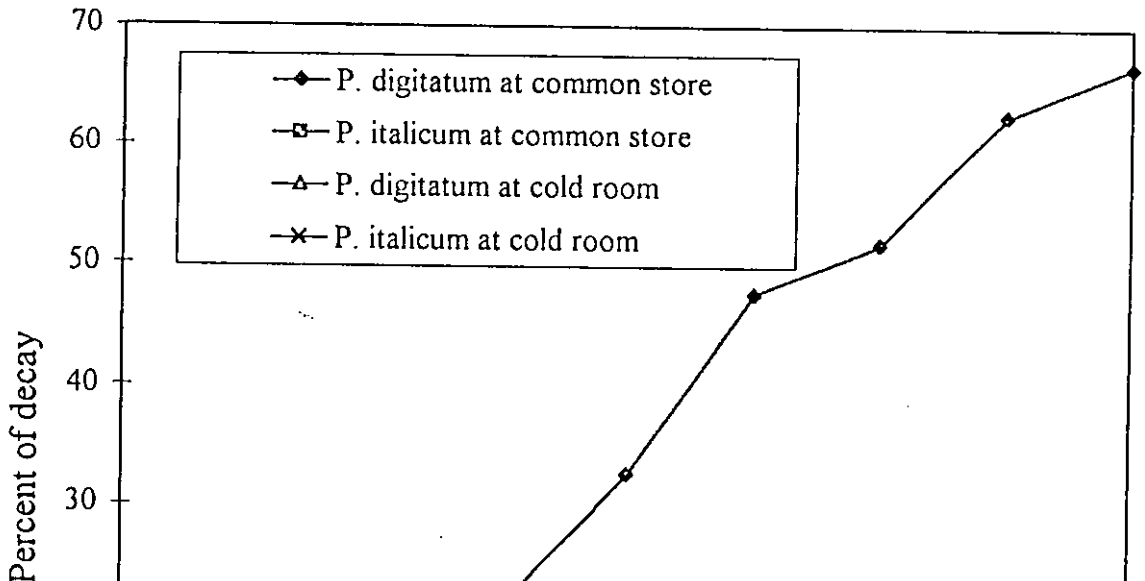


Figure (3) : Percent of Decay Caused by ***Penicillium*** on Shamouti orange fruit in cold room (1-3 $^{\circ}$C) and common store (ambient temprature)

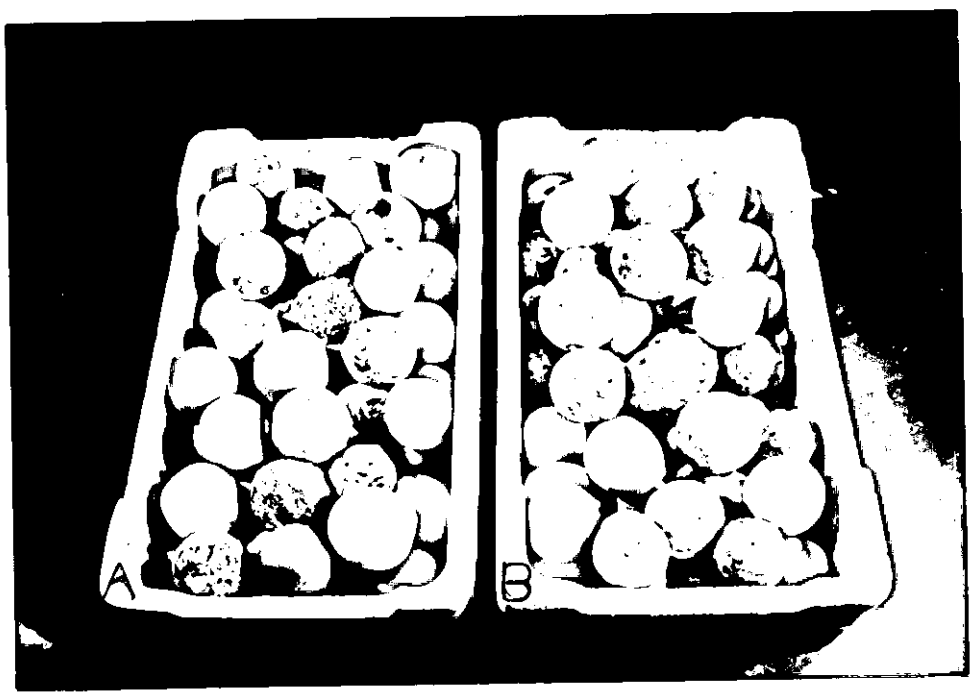


Plate (16) : Occurrence of ***Penicillium digitatum*** and ***P. italicum*** on Shamouti orange fruits during storage .

(A) : Cold room

(B) : Common store (ambient temperature)

4:5 Effect of postharvest dipping of fruits in fungicides :

Benomyl and carbendazim fungicides were similarly effective in controlling green mold after 15 days at 20 ± 1 °C and 85-95 % R.H (Table 4) . However , efficacy of fungicides was in the following order ; benomyl , carbendazim and thiophanate methyl (Plate 17) . Mixture of any two fungicides did not increase the effectiveness against decay . Cold storage at 1-3 C and 80-90% R.H , with or without fungicides , was very effective (incidence = 0 %) for 15 days . Disease severity of tested fungicides and their mixtures ranged between 0.7 - 1.14 during 15 days of incubation at 20 ± 1 °C (Table 5) .

Inoculated fruits that served as control in this experiment and in all experiments for evaluation developed small lesion (less than one cm in diameter) of decay as a soft area at the inoculated point three days after inoculation . Two days later , sporulation of fungus was observed on the decayed area at 20 ± 1 °C and 85-95 % R.H .

4:6 Effect of postharvest fruit waxing :

Fruits waxing reduced the decay caused by ***P. digitatum*** during incubation at 20 ± 1 °C and 85-95 % R.H for 3 days (Table 6) . However, Results of incidence indicated that the whole fruits sprayed with wax were found to decay 6 days after treatment . On the other hand , incidence of green mold was 0 % when fruits stored in a refrigerator at 1-3 C and 80-90 % R.H Severity values of decay of waxed fruits were differ significantly from the control (Table 7) .

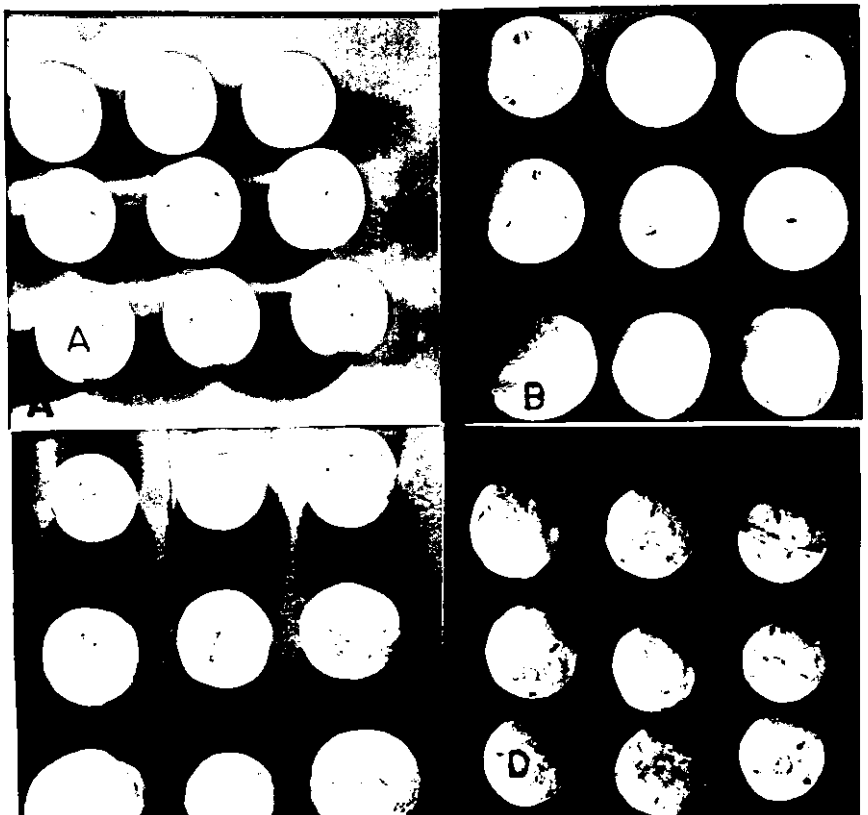


Plate (17): Effect of postharvest fruits dipping in fungicides on green mold 15 days after treatment .

(A) : Benomy .

(B) : Carbendazim .

(C) : Thiophanate Methyl .

(D) : Control .

Table (4) : Effect of postharvest dipping of orange fruits in fungicides and mixtures of these fungicides on incidence of green mold at 20±1 °C and 85-95% R.H

	Days After Treatment					
Fungicides	**1**	**3**	**6**	**9**	**12**	**15**
Benomyl	0* (0) a	0 (0) a	0 (0) a	0 (0) a	5 (13) ab	5 (13) ab
Carbendazim	0 (0) a	0 (0) a	0 (0) a	10 (18) b	10 (18.3) a	10 (18.3) a
Thiophanate Methyl	0 (0) a	0 (0) a	25 (30) b	25 (30) c	33 (35.7) c	35 (36.7) c
B+C	0 (0) a	0 (0) a	0 (0) a	0 (0) a	0 (0) a	0 (0) b
B+T	0 (0) a	0 (0) a	10 (18.3) c	10 (18.3) b	10 (18.3) a	10 (18.3) a
C+T	0 (0) a	0 (0) a	10 (18.3) c	10 (18.3) b	10 (18.3) a	10 (18.3) a
Control	0 (0) a	100 (90) b	100 (90) d	100 (90) d	100 (90) c	100 (90) d
LSD	0	0	5	11	14	14

* Mean of 4 replications .

Values between parenthesis are angular transformed ($\sin^{-1}\sqrt{x}$) .

Values within the column followed with the same letter are not significantly different at P=0.05 according to the LSD .

Table (5) : Effect of postharvest dipping of orange fruits in fungicides and mixtures on severity values of green mold at 20 ± 1 °C and 85-95% R.H .

Treatment	Days After Treatment					
	1	3	6	9	12	15
Benomyl	0.0 (0.7) a	0.0 (0.7) a	0.0 (0.7) a	0.0 (0.7) a	0.05 (0.76) a	0.05 (0.76) a
carbendazim	0.0 (0.7) a	0.0 (0.7) a	0.0 (0.7) a	0.1 (0.76) a	0.1 (0.76) a	0.3 (0.82) a
Thiophanate methyl	0.0 (0.7) a	0.0 (0.7) a	0.23 (0.85) b	0.25 (0.85) b	0.5 (1.0) b	0.85 (1.14) b
B + C	0.0 (0.7) a	0.0 (0.7) a	0.0 (0.7) a	0.0 (0.7) a	0.0 (0.7) a	0.0 (0.7) a
B + T	0.0 (0.7) a	0.0 (0.7) a	0.1 (0.76) a	0.1 (0.76) a	0.2 (0.82) a	0.3 (0.87) a
C + T	0.0 (0.7) a	0.0 (0.7) a	0.1 (0.76) a	0.1 (0.76) a	0.3 (0.82) a	0.45 (0.87) a
Control	0.0 (0.7) a	1.0 (1.22) b	2.0 (1.58) c	3.0 (1.87) c	3.0 (1.87) c	4.0 (2.12) c
LSD	0.0	0.0	0.07	0.08	0.13	0.21

Values between parenthesis are root transformed ($\sqrt{x + 0.5}$) .

Values within the column followed with the same letter are not significantly different at P=0.05 according to the LSD .

Table (6) : Effect of postharvest fruit waxing on the incidence of green mold at 20± 1 °C and 85-95% R.H .

Treatment	Days After Treatment					
	1	3	6	9	12	15
Waxing	0* (0) a	80 (63.4) a	100 (90) a	100 (90) a	100 (90) a	100 (90) a
Control	0 (0) a	100 (90) b	100 (90) a	100 (90) a	100 (90) a	100 (90) a
LSD	0.0	4.0	0.0	0.0	0.0	0.0

* Mean of 4 replications .

Values between parenthesis are angular transformed ($\sin^{-1}\sqrt{x}$) .

Values within the column followed with the same letter are not significantly different at P=0.05 according to the LSD .

Table (7) : Effect of postharvest fruits waxing on severity values of green mold at 20± 1 °C and 85-95% R.H.

Treatment	Days After Treatment					
	1	3	6	9	12	15
Waxing	0.0 (0.7) a	0.8 (1.14) a	1.0 (1.22) a	2.0 (1.58) a	2.0 (1.58) a	3.0 (1.87) a
Control	0.0 (0.7) a	1.0 (1.22) b	2.0 (1.58) b	3.0 (1.87) b	4.0 (2.12) b	4.0 (2.12) b
LSD	0.0	0.0	0.0	0.0	0.0	0.0

Values between parenthesis are root transformed ($\sqrt{x + 0.5}$) .

Values within the column followed with the same letter are not significantly different at P=0.05 according to the LSD .

4:7 Effect of UV illumination on fruit protection :

Results on the incidence and severity values used for evaluation of different exposure times of inoculated fruits to UV-light using two lamps are presented in table (8 and 9). Two hours, 4 hrs and 8 hrs exposure times did not show any significant control of decay in comparison with the control 15 days after UV illumination 20 ± 1 °C . Exposuring inoculated fruits for 16 hrs and 24 hrs delayed the decay appearance for 3 and 6 days , respectively. However, no significant differences in the incidence of green mold were found between 16 hrs. and 24 hrs. exposure times. On the other hand , no decay was observed on fruits stored in a refrige during 15 days at 1-3 C and 80-90% R.H. Severity values of all exposure time , except at 24 hrs, did not differ significantly from the control (Table 9) (Plate 18).

4:8 Effect of postharvest fruit dipping with *Debaryomyces hansenii*:

Results on the incidence of green mold by using the antagonistic yeast were significantly different in comparison with control at 20 ± 1 °C and 85-95 % R.H during 15 days of evaluation (Table 10). On the contrary cold storage at 1-3 C, with or without yeast , was very effective in decay control for 15 days (incidence = 0 %). Significant diferences were found in severity values between treated fruits and control ones (Table 11).

4:9 Effect of preharvest fungicides spraying of orange trees:

Preharvest sprayed fungicides of orange trees , twice with one week interval , showed incidence of fruit decay ranged between 56.4 % and 60% . However , no significant differences were found between fungicides after 15 days of inoculation at 20-1 C and 85-95% R.H but they were differ significantly from the control (Table 12) .

Severity values of the green mold were also differ significantly between sprayed fruits and non-sprayed fruits (control) (Table 13) .

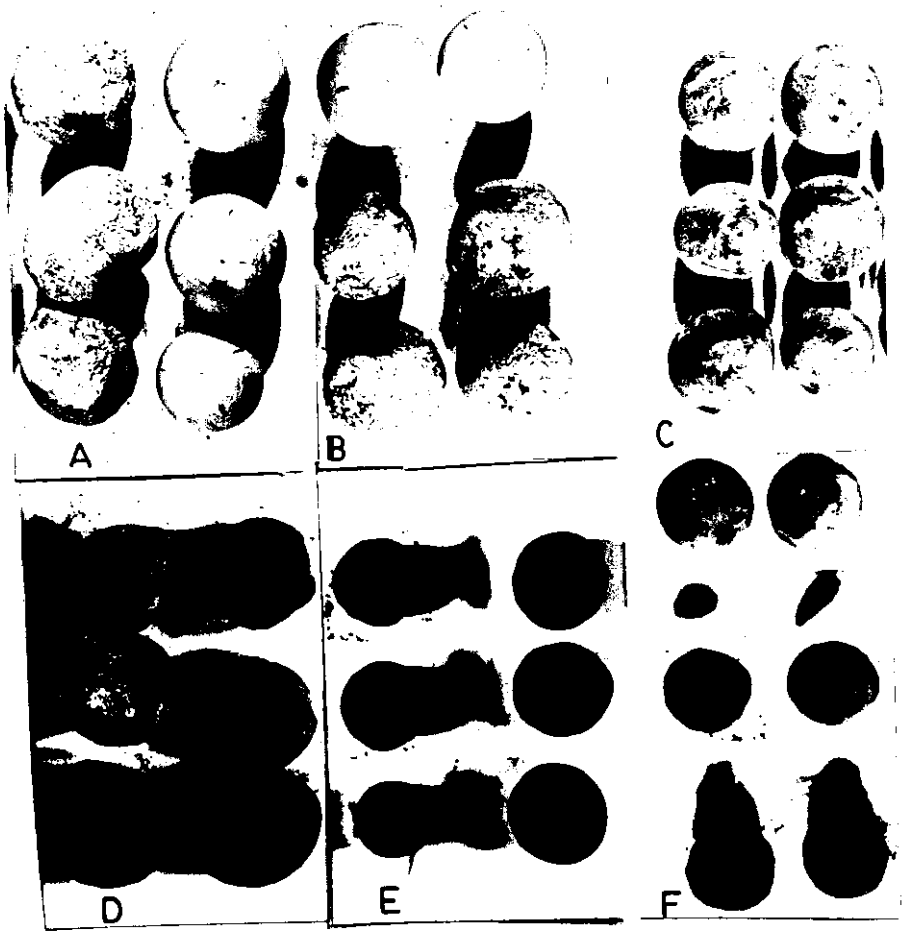


Plate (18) : Effect of UV Illumination of Fruits for Different Exposure Times on Green Mold , A. 24 Hours, B .16 Hours , C . 8 Hours , D. 4 Hours E . 2 Hours , F . Control (Non- Illuminated Fruits)

Table (8) : Effect of (UV) illumination of orange fruits at different exposur times on the incidence of green mold at 20± 1 °C and 85-95 % R.H .

Treatment (Hours)	Days After Treatment					
	1	3	6	9	12	15
2	0* (0) a	35 (36) a	65 (53.8) a	100 (90) a	100 (90) a	100 (90) a
4	0 (0) a	20 (26.5) b	65 (53.8) a	90 (76.7) a	95 (83.3) a	95 (83.3) a
8	0 (0) a	0 (0) c	25 (26.5) b	60 (52.4) b	90 (80) a	90 (80) a
16	0 (0) a	0 (0) c	5 (6.62) c	10 (13.2) c	60 (50.7) b	80 (60) b
24	0 (0) a	0 (0) c	0 (0) c	10 (13.2) c	50 (45) b	60 (50.7) b
Control	0 (0) a	100 (90) d	100 (90) d	100 (90) a	100 (90) a	100 (90) a
L S D	0.0	3.85	9.7	16.2	19.7	14.9

* Mean of 4 replications .

Values between parenthesis are angular transformed ($\sin^{-1}\sqrt{x}$) .

Values within the column followed with the same letter are not significantly different at P=0.05 according to the LSD .

Table (9) : Effect of (UV) illumination of orange fruits at different exposure times on severity values of green mold at 20 ± 1°C and 85-95% R.H .

Treatment (Hours)	Days After Treatment					
	1	3	6	9	12	15
2	0.0 (0.7) a	0.4 (0.92) c	1.5 (1.37) a	3.0 (1.86) a	4.0 (2.12) a	4.0 (2.12) a
4	0.0 (0.7) a	0.2 (0.83) b	0.65 (1.04) b	1.8 (1.45) b	2.85 (1.7) b	4.0 (2.12) a
8	0.0 (0.7) a	0.25 (0.73) a	0.25 (0.83) c	1.2 (1.3) c	2.7 (1.78) b	3.75 (2.06) a
16	0.0 (0.7) a	0.05 (0.73) a	0.05 (0.73) e	0.1 (0.76) e	1.6 (1.45) c	2.9 (2.06) a
24	0.0 (0.7) a	0.0 (0.7) a	0.0 (0.7) e	0.1 (0.76) e	1.0 (1.22) c	2.25 (1.51) b
Control	0.0 (0.7) a	1.0 (1.2) d	1.4 (1.2) d	3.0 (1.58) d	4.0 (2.12) a	4.0 (2.12) a
LSD	0.0	0.05	0.06	0.06	0.12	0.13

Values between parenthesis are root transformed ($\sqrt{x + 0.5}$).

Values within the column followed with the same letter are not significantly different at P=0.05 according to the LSD .

Table (10) : Effect of postharvest dipping of orange fruits in *D. hanseni* on the incidence of green mold at 20± 1 °C and 85-95 % R.H .

Treatment	Days After Treatment					
	1	3	6	9	12	15
Yeast	0* (0) a	57.5 (49.2) a	70 (57) a	72.5 (58.3) a	75 (60) a	75 (60) a
Control	0 (0) a	100 (90) b	100 (90) b	100 (90) b	100 (90) b	100 (90) b
LSD	0.0	3.5	6.3	4	4	4

* Mean of 4 replications .

Values between parenthesis are angular transformed ($\sin^{-1}\sqrt{x}$) .

Values within the column followed with the same letter are not significantly different at P=0.05 according to the LSD .

Table (11) : Effect of postharvest dipping of orange fruits in *D.hansenii* on severity values of green mold at 20 ± 1 °C and 85-95 % R.H .

Treatment	Days After Treatment					
	1	3	6	9	12	15
Yeast	0.0 (0.7) a	0.75 (1.11) a	0.75 (1.11) a	1.52 (1.41) a	2.25 (1.65) a	3.0 (1.86) a
Control	0.0 (0.7) a	1.0 (1.22) b	1.0 (1.22) b	2.0 (1.58) b	4 (2.12) b	4 (2.12) b
LSD	0.0	0.06	0.06	0.09	0.11	0.13

Values between parenthesis are root transformed ($\sqrt{x + 0.5}$).

Values within the column followed with the same letter are not significantly different at P=0.05 according to the LSD .

Table (12) : Effect of preharvest fungicides spraying of orange trees on the incidence of green mold at 20 ± 1 °C and 85-95 % R.H .

	Days After Inoculation					
Treatment	**1**	**3**	**6**	**9**	**12**	**15**
Benomyl	0 (0) a	25 (26.2) a	50 (45) a	65 (56.4) a	65 (56.4) a	65 (56.4) a
Carbendazim	0* (0) a	35 (36) a	55 (47) ab	60 (50.7) a	65 (56.4) a	65 (56.4) a
Thiophanate Methyl	0 (0) a	70 (57.1) b	70 (57.1) b	70 (57.1) a	70 (57.1) a	75 (60) a
Control	0 (0) a	100 (90) c	100 (90) c	100 (90) b	100 (90) b	100 (90) b
LSD	0.0	17.5	11.5	12.8	10.5	11.5

* Mean of 4 replications .

Values between parenthesis are angular transformed ($\mathrm{Sin}^{-1}\sqrt{x}$) .

Values within the column followed with the same letter are not significantly different at P=0.05 according to the LSD .

Table (13) : Effect of preharvest fungicides spraying of orange trees on severity of green mold values at 20 ± 1 °C and 85-95% R.H .

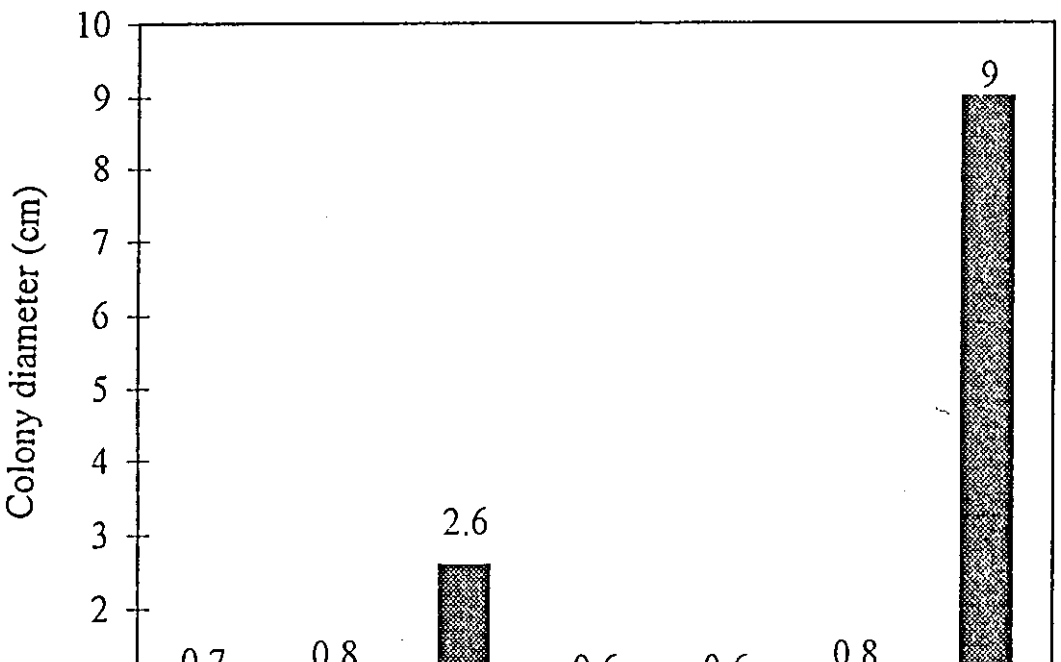
Treatment	Days After Inoculation					
	1*	3	6	9	12	15
Benomyl	0.0 (0.7) a	0.25 (0.68) a	0.65 (1.06) a	1.65 (1.43) a	2.55 (1.7) a	2.9 (1.9) a
Carbendazim	0.0 (0.7) a	0.25 (0.68) a	0.65 (1.06) a	1.65 (1.43) a	2.25 (1.7) a	2.9 (1.9) a
Thiophanate Methyl	0.0 (0.7) a	0.7 (1.1) ab	0.7 (1.09) a	1.65 (1.43) a	2.55 (1.7) a	3.0 (1.83) a
Control	0.0 (0.7) a	1.0 (1.22) b	2.0 (1.6) b	2.75 (1.6) a	3.75 (1.87) b	4.0 (2.12) b
LSD	0.0	0.5	0.18	0.2	0.13	0.12

Values between parenthesis are root transformed ($\sqrt{x + 0.5}$) .

Values within the column followed with the same letter are not significantly different at P=0.05 according to the LSD .

4:10 Effect of fungicides on *Penicillium digitatum* growth in vitro

Fungicides that used to detect their effect on ***P. digitatum*** growth were determined in laboratory experiment at 20 ± 1 °C for 15 days. Results indicated that the daily increase in colonies diameter of ***P. digitatum*** was affected by different fungicides or a mixtures of these fungicides . However, no significant differences were found between benomyl, carbendazim or mixtures of all tested fungicides but high significant differences were noticed between all fungicides or a mixtures and control after 15 days of inoculation (Figure 4) .



B: Benomyl C: Carbendazim T: Thiophanate Methyl

Figure (4) : Effect of different fungicides and their mixtures on colony of diameter of ***P. digitatum*** after 15 days of inoculation

V : Discussion

Several fungi were isolated and identified microscopically depending on morphological characteristics and taxonomic keys made by Alexopoulos and Mims (1979) , Street (1974) and John (1979) . Among these fungi are: ***aPenicillium digitatum*** Sacc., ***P. italicum*** Wehmer, ***Alternaria citri*** Ell., ***Phytophthora sp.*** (Smith and Smith)Leonian, ***Botrytis cinerea*** Pers., ***Trichoderma sp.*** Pers. and ***Fusarium sp.*** (Mart.) Sacc. . These different fungi were responsible for many diseases affecting the quality of the fruits, these diseases were: Green mold, Blue mold, Stem end rot, Brown rot, Gray mold, ***Trichoderma*** rot and ***Fusarium*** mold. These diseases agreed with what Eckert and Ogawa (1985) reported in U.S.A. However, Identification of fungal causal agents was confirmed by pathogenicity tests which resulted in the fulfillment of Koch's postulates for all fungal isolates as well as different symptoms on the diseased fruits .

Penicillium digitatum Sacc. was the most common and dominant fungus in the different packinghouses from which decayed fruits were collected. This finding agreed with what many other authors have reported (Charles and Chalutz, 1989 and Gutter, 1975) and that was confirmed by the high percent of decay incidence , of orange fruits of different cultivars , due to ***P. digitatum*** in common store at ambient temperature .

P. digitatum is a wound pathogen (Kavanagh and Wood, 1967). Method of harvesting fruits facilitate the fungus entrance to the fruits through wounds. Low % of incidence decay of ***P. italicum*** was due to rapid growth of ***P. digitatum*** at modereate temperature and become predominate as explained by Barmore and Wood (1982) report. Very low % of decay incidence, caused by ***P.digitatum*** ,of different orange cultivars at 1-3 C of storage condition revealed that low temperature inhibited the fungus

sporulation. However, since orange fruits were not wrapped or chemically treated and the presence of healthy fruits with infected ones , the high % of decay incidence of ***P. digitatum*** was expected. However, sensitivity to ***P. digitatum*** and ***P. italicum*** did not vary among different orange cultivars at both conditions of storage.

Inoculated fruits with ***P. digitatum*** suspension (20×10^6 conidia / ml) and inocubation for 24 hours before applying different treatment is necessary. Results of evaluation treatments revealed that this fungus need 3-4 days to cause soft water soaked area on the peel of the check fruits after incubation at 20± 1 °C and 85-95 % R.H. Two days later, sporulation on the decayed area took placed. Rapid growth of ***P. digitatum*** occured in the following days of incubation till whole fruit is covered with olive green spores of ***P. digitatum***.

Numerous investigations have shown that postharvest application of benomyl can prevent development of ***P. digitatum*** on citrus fruits (Brown, 1974). The low incidence of the green mold by benomyl as well as carbendazim could be related to the systemic properties of both fungicides . Penetration of benomyl and carbendazim to a depth of 4-6 mm or greater into the flesh of pears was reported by Ben-Arie (1975) . This penetration might explain their effect on orange decay control of ***P. digitatum*** .

However, the best control was achieved with benomyl , less with carbendazim and the least with thiophanate methyl. Benomyl is more effective than carbendazim for the control of ***Penicillium*** sporulation on decaying oranges (Eckert *et al.*,1979) and for protecting the fruit against infection of wounds (Brown ,1984). Benomyl penetrated the cuticle of the orange fruit better than carbendazim and inhibited the hyphae of ***Penicillium*** ramifying in the flavedo layer of the orange peel (Eckert *et al.*,1979). Thiophanate methyl has compared favourably with benomyl and carbendazim for control of ***Penicillium*** decay (Pelser , 1973 cited in Eckert

and Ogawa 1985). Our results on the incidence of green mold were in agreement with the work of Eckert *et al.*, (1979) regarding to benomyl and carbendazim, but in contrast with Brown (1984) work. This might be explained by the size of wounds in the orange fruit peel which influence the control of green mold fungal pathogen.

Postharvest orange fruit waxing was not effective in controlling green mold at 20±1°C and 85-95% R.H in comparison with the control. These results are in agreement with the work of Brown (1985) and Eckert *et al* (1979). However, inoculation method of fruit may contribute in the green mold control. Waks *et al* (1985) found that applying wax to fruits before inoculation of orange fruits with dry spores of ***P. digitatum*** reduced the incidence of mold, this could be related to the action of wax as a barrier which prevents contact between the spores and non-wounded fruits .

Illumination of previously inoculated fruits by (UV) light failed to prevent the decay at 2,4 and 8 hours exposure times . However, delaying of decay development was achieved by illuminating the inoculated fruits for 16 and 24 hours. Rodov *et al* (1992) reported that disease inhibition in kumquat fruit by (UV) treatment was achieved by treating fruit before inoculation with dry spores. The inoculation of fruits before (UV) treatment gave an advantage to the fungal pathogen. The way of (UV) light action on the fungal pathogen was reported by Rodov *et al* (1992) and Jame *et al* (1977) . Decay resistance in UV-illuminated fruit could be related to the phytoalexin elicited in the peel by the treatment.

In spite of high percent of decayed fruits as a result of dipping in ***Debaryomyces hansenii*** suspension ($15x10^7$cells/ml), significant differences were found in comparison with the control. Our results on the incidence of the greem mold agreed with the work of Charles and Chalutz (1989) and Chalutz and Wilson (1990) . However, The effectiveness of ***D. hansenii*** can

probably be enhanced by manipulating their preparation and method of application.

Preharvest spraying of orange trees with different fungicides was not effective in green mold control. However, incidence was not significantly different between tested fungicides but they differ significantly from the control. Brown and Albrigo (1972) and (1970) showed that preharvest application of benomyl to orange trees resulted in fungistatic concentrations of carbendazim in the peel tissues and they suggested that these residues might prevent later development of ***P. digitatum*** in the peel. However , carbendazim efficiency has been shown to decrease with time due to breakdown or complexing (Hock et al., 1970 and Peterson and Edgington , 1971), this may explain the high incidence of green mold after 15 days of incubation at 20 ± 1 °C and 85-95 % R.H .

Results of using fungicides or their mixtures to control ***P. digitatum*** were confirmed by in vitro experiment where such fungicides and mixtures are incorporated into PDA medium which was inoculated with fungal mycelial plug. Daily increase in colony diameter of fungal hyphae revealed significant differences between fungicides and their mixtures from the control with no significant different among fungicides and their mixtures, except that Thiophanate methyl was less effective. However, efficacy of fungicides did not increased when each two fungicides were mixed together. Therefore, postharvest fruits dipping in benomyl and carbendazim was the best control method of green mold disease.

Our results on evaluation methods of the green mold control at two different conditions of incubation (20± 1 °C and 1 -3 °C) revealed that temperature of 20 ± 1 °C of incubation was a suitable temperature to differentiate between all treatments and eventually determining the best method of treatment since incubated fruits at 1-3°C did not show any decay development of inoculated fruit during 15 days of incubation .

VI : Conclusions and Recommendations

1. Several postharvest fungi on orange fruits were isolated and identified, these are: ***Penicillium digitatum, P .italicum , Alternaria citri , Botrytis cinerea , Phytophthora sp. , Fusarium sp*** and ***Trichoderma sp.*** These fungi cause the green mold, blue mold, stem end rot, gray mold, brown rot, ***Fusarium*** rot and ***Trichoderma*** rot diseases in Jordan .
2. ***Penicillium digitatum*** and ***P. italicum*** were most commonly associated with fruits decay of Shamouti , Valencia and Navel .
3. Preharvest fungicide spraying of orange trees was not effective in decay control compared to the postharvest fruit dipping in fungicides.
4. The best control method was achieved by postharvest dipping of fruits in benomyl and carbendazim fungicides .
5. Mixing each two fungicides did not increase the efficacy of decay control .
6. Fruit waxing was not effective in decay control.
7. Illumination by (UV) light and the use of the antagonistic yeast , ***D.hansenii,*** showed an indication of green mold disease control .
8. Cold storage prevent fruit decay development .
9. Fruits must be picked carefully to avoid injuries through which ***P.digitatum*** as well as other fungal pathogens may enter .
10. Further studies must be conducted to investigate the losses caused by postharvest fungal diseases on other citrus spp.
11. Manipulation and integration of different control methods must be studied .

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Appendices

Appendix (1) : Incidence of green and blue mold on Shamouti orange fruits in common store and cold room .

Days	Room Temperature		Cold Room	
	A	B	A	B
10	0 a	0 a	0 a	0 a
20	4.95 a	0 b	0 b	0 b
30	13.3 a	0 b	3.3 b	0 b
40	21.6 a	3.3 b	6.6 b	0 c
50	32.5 a	3.3 b	8.3 c	3.3 b
60	47.5 a	6.6 b	10.6 c	3.3 b
70	51.7 a	6.6 b	13.3 c	6.6 b
80	62.5 a	10.6 b	16.6 c	6.6 d
90	66.65 a	10.6 b	16.6 c	10.6

* Average of 4 boxes , 30 fruits per each

Values within row followed with the same letter are not significantly different at P=0.05 according to the LSD .

A : Green mold

B : Blue mold

Appendix(2) : Incidence of green and blue mold on Valencia orange fruit in common store and cold room .

Days	Room Temperature		Cold Room	
	A	B	A	B
10	0 a	0 a	0 a	0 a
20	4.1* a	0 b	0 b	0 b
30	5.8 a	0 b	0 b	0 b
40	19 a	3.3 b	5.8 b	3.3 b
50	30 a	6.6 b	8.3 b	3.3 b
60	40 a	10 b	10.6 b	6.6 b
70	50 a	10 b	10.6 b	6.6 b
80	60 a	10 b	13.3 b	6.6 c
90	65 a	10 b	13.3 b	6.6 c

* Average of 4 boxes , 30 fruits per each .

Values within row followed with the same letter are not significantly different at P=0.05 according to the LSD .

A : Green mold

B : Blue mold

Appendix (3) : Incidence of green and blue mold on Navel orenge fruits in common store and cold room .

Days	Room Temperature		Cold Room	
	A	B	A	B
10	0 a	0 a	0 a	0 a
20	5 a	0 b	0 b	0 b
30	13.3 a	3.3 b	3.3	0 b
40	21.6 a	3.3 b	6.6 b	3.3 b
50	36 a	3.3 b	6.6 b	3.3 b
60	48 a	6.6 b	8.3 b	3.3 b
70	58 a	6.6 b	10.8 b	6.6 b
80	65 a	10.8 b	13.3 b	6.6 c
90	65 a	10.8 b	21.6 c	6.6 b

* Average of 4 boxes , 30 fruits per each .

Values within row followed with the same letter are not significantly different at P=0.05 according to the LSD .

A : Green mold

B : Blue mold

الملخص

الأمراض الفطرية لما بعد حصاد ثمار البرتقال وتقييم بعض طرق المكافحة

إعداد

أيمن اسماعيل درويش

إشراف

أ. د. حفظي أحمد أبو بلاّن

أجريت هذه الدراسة لمعرفة المسببات الفطرية الممرضة لأصناف مختلفة من ثمار البرتقال المصابة والتي تم جمعها من مخازن التبريد ومن الاسواق . دلت نتائج العزل على البيئات الغذائية والفحص المجهري تواجد الانواع التالية من الفطريات :

Alternaria citri Ell , ***P. italicum*** Wehmer , ***Penicillium digitatum*** Sacc

Phytophthora sp. (Smith and Smith) Leonian, ***Botrytis cinerea*** Pers ***Fusarium sp.*** Sacc و ***Trichoderma sp.*** Pers

تبين من خلال دراسة الاعراض المصاحبة للثمار المصابة ان هذه الفطريات تسبب الامراض الفطرية التالية-:

العفن الاخضر , العفن الازرق , تعفن الطرف الساقي , العفن الفيوزاريومي الابيض , التعفن الرمادي , التعفن البني و تعفن الترايكودرمي. وقد تم تأكيد هذه المسببات المرضية بإتباع فروض كوخ واجراء عمليات العدوى لثمار البرتقال السليمة بجراثيم الفطريات المعزولة.

اظهرت نتائج تخزين ثلاثة اصناف مختلفة من ثمار البرتقال تحت ظروف التخزين المبرد (1-3°م) والتخزين العادي تحت ظروف الجو المحيط بالثمار , حساسية للاصابة بالفطريات. حيث وجد أن فطري ***P.digitatum*** و ***P.italicum*** هما الاكثر شيوعا على جميع الاصناف وتحت كلتا ظروف التخزين مع فروقات معنوية اكبر في نسبة حدوث الاصابة بفطر ***P.digitatum***عند مقارنته بفطر ***P. italicum*** . لم تظهر أصناف البرتقال المختلفة, الشموطي ، البلدي (أبو صرة) والبلنسيا , أية فروقات معنوية عند إصابتها بفطري العفن الاخضر والازرق.

تم تقييم عدة طرق مختلفة من المكافحة بفطر ***P.digitatum*** على صنف البرتقال الشموطي باستخدام العدوى الصناعية وتحت ظروف التخزين المبرد (1-3°م) والتخزين بالحاضنة (20 ± 1°م) حيث بينت طريقة غمر الثمار بعد الحصاد , والتي سبق عدواها بجرايثم الفطر 20 × 10^6 جرثومة/مل , بمحاليل ثلاثة مبيدات مختلفة كفاءة عالية في مكافحة الفطر مع وجود فروقات معنوية بين هذه المبيدات عند مقارنتها بالشاهد. حيث كانت نسبة الأصابة باستخدام كل من مبيد بنوميل , كاربندازيم و ثيوفانيت مثيل 13، 18.3 و 36.7 ٪ على التوالي و على درجة حرارة الحاضنة (20 ± 1°م). لم يظهر خلط المبيدات الفطرية بعضها ببعض أية زيادة في مكافحة مرض العفن الاخضر.

لم تظهر نتائج طريقة رش الثمار بالمادة الشمعية أية مكافحة لمرض العفن الاخضر على حرارة الحاضنة (20 ± 1°م) خلال 15 يوم من التخزين.

عند تعريض الثمار للأشعة فوق البنفسجية (UV) ولمدة 2 ،4 و 8 ساعات كانت نسبة الاصابة 100٪ ، في حين تم تأخير ظهور العفن لثمار البرتقال لمدة 3 و6 أيام عند تعريض الثمار لمدة 16 و 24 ساعة على التوالي.

بينت نتائج غمر الثمار بمحلول الخميرة المثبطة لنمو الفطريات وباستخدام ***Debaryomyces hansenii*** بتركيز 15 × 10^7 جرثومة / مل فروقات معنوية في نسبة الاصابة بمرض العفن الاخضر عند مقارنتها بالشاهد.

وصلت نسبة الاصابة عند رش أشجار البرتقال قبل الحصاد مرتين , يفصلها اسبوع واحد، بمحاليل المبيدات الفطرية : بنوميل ، كاربندازيم و ثيوفانيت مثيل الى 56.4 , 56.4 و 60٪ على التوالي . ولتأكيد نتائج مكافحة الفطر باستخدام المبيدات الفطرية المذكورة اعلاه أو خليط منها ، فإن خلط كل من هذه المبيدات مع البيئة الغذائية (PDA) اللازمة لنمو الفطر أظهر فروقات معنوية بين هذه المبيدات والشاهد ، الا انه لم تظهر فروقات معنوية بين هذه المبيدات باستثناء مبيد الثيوفانيت مثيل الذي كان أقل كفاءة في منع نمو الفطر في البيئة الغذائية بعد 15 يوم من الحضن على درجة حرارة (20 ± 1°م) . دلت نتائج تخزين الثمار المعدية بجراثيم الفطر ***P.digitatum*** و المعاملة بالطرق الكيمائية والفيزيائية والبيولوجية على درجة حرارة الحاضنة وجود فروقات معنوية بين المعاملات في حين لم تظهر حرارة التخزين المبرد من (1-3°م) أي تلف للثمار عند معاملتها بنفس الطرق السابقة .

تراوحت شدة الاصابة بمرض العفن الاخضر لكافة طرق المكافحة بين 0.7 و 2.12عند نهاية فترة الحضن علي درجة حرارة 20 + 1م و رطوبة نسبية 85- 95 %