THE INVESTIGATION OF POST HARVEST INFECTION STRATIGY OF PENICILLIUM MOULD ON CITRUS FRUITS

By

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الإهـــــــداء

إلـــــي مـــــن جرع الـكأس فـارغـاً لـيسقيـنـي جرعـة الأمـــل

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CONTENTS

CHAPTER 4: RESULTS

LIST OF TABLES

LIST OF FIGURES

Abstract

Fungal disease of post harvest which infect fruits from acceutuated and dangerous pathogenic micro- organism which cause large economical losses reach to 50% , developed and spreads during transport and storage. The citrus fruits are one of the major important agricultural product especially in western region of Libya .

In this study we founed on post harvest citrus green mould disease which caused by *Penicillium digitatium* after running Kock pastulate . In this research we traying to investigate and trace the mode and mechanism of infection of *Penicillium digitatium* on local and imported four citrus varities (Orange , Mandarine , Lemon and Grapefruits) . The optained results of pathogenicity test indicate that *Penicillium digitatum* and is the main causal agent .

The ability of this fungus to infect four citrus varities with same virulence after application three methods of artificial inoculation . Through cooling experiment we founed the optimum growth temperature ranged betwwen 25-27°C . Study the effect of depth and wound location on the previous citrus varities does not show any effect role on infection in varities sampled . After study natural micro-flora associated with studied fruits surface three species of bacteria are identified : *Staphylococcus hominis* , *Staphylococcus haemolyticus* and *Staphylococcus warneri* . After artificial inoculation with *Penicillium digitatum* with inoculum density (10) the washed fruits speed infected compared with non – washed fruits with dispersal time about 12 hours . In test of three types of juice (Peel Juice – Sac Juice – Whole fruit) , all tested juice from citrus varities studied showed *Penicillium digitatum* growth with more rapidly on peel juice compared with other two types of juice extracts , which make ensure that the tested fungus was this obligate aerobic .

Through intervene modulation to change acidic medium of studied fruits by inject two concentrations of apple vineiger and solution of sodium bicarbonate , solution of sodium chloride , sterile distill water as control showed that there is no role in prevent infection by *Penicillium digitatum* but just delaying infection time about five days by used solution of sodium bicarbonate NaHCO3. In study aerodynamic spore mechanism and epidemology of *Penicillium digitatum* the results showed the spores

of *Penicillium digitatum* have low weight to became longtime before fall down , indicating that *Penicillium digitatum* have dry spores . The sedimentation of these spores on fruit surfaces was more vertically faster than horizontally , conferming the way of storage fruits above long distance from ground surface does not prevent the infection incidence .

From studying of lignification effect on peels of studied citrus varities after injuires and kept for 12 hours then injection showed the lignin formation do not prevent the infection but just lated about 6 days . From study specific different isolates showed all species have specific to infect the host fruit without the other . Through study the maturity of lemon fruits on enhance or lated infection with *Penicillium digitatum* show the mature lemon fruits infected earlier through 4 days compared with immature lemon fruits infected after 6 days .

The result obtained from Interaction experiment of different isolates in vitro on Sabroid Agar showed the all isolates followed to same genus of *Penicillium* . Finally the results optained from programs to this disease on injected and non-injected fruits by selective two physical methods (Hot water – Sea water) , two natural oils (Eucalyptus oil – Olive oil) and one mineral oil (Johnson's baby oil) and wax , showed all of these method can not prevent the invection but just delated the infection period , wax test delay five days and the other two treatments were delay about three days only .

CHAPTER ONE

1. INTRODUCTION

It has been recognized that fruits are commercially and nutritionally important food product. Fruits play an important role in human nutrition by contributing the necessary growth factors such as vitamins and essential minerals in human daily diet maintaining a good and normal health. Rot diseases caused by fungal pathogens provoke severe losses of agricultural and horticultural crops every year **(Salman, 2005 and Parveen, *et al.*, 2016)** . Fruits have wide distribution in nature. The relatively short shelf-life period provoked by pathogens is one of the most important limiting factors that impact the economic value of fruits. Approximately 20-25% of the harvested fruits are deteriorated by pathogens during post-harvest handling even in advanced countries **(Droby , 2006 and Zhu, 2006) .**

Commonly cultivated citrus belongs to three genera; Citrus spp., Fortunella spp. and Poncirus spp. of the family Rutaceae **(Cohn, 1972)**. These species can grow in tropical and subtropical regions around the world at both sides of the equator to a latitude of 35°N and 36°S. Many serious pests and diseases are reported on citrus , that reduce the quality of the fruits and the longivity of the trees . The citrus fruit famous by every green . Other factors that limit the distribution of citrus are : soil types , water (quality and quantity) and temperature . Fruits of citrus are the marketable commodity , fall into four groups such as oranges , mandarins , pummelous (Grape fruit) and the common acid group such as lemon and lime **(Swingle, 1967)** .

Citrus Sinensis is a member of the family of "Rutaceae" which contains about150 genera and nearly 2000 species, probably originated in North Eastern India, in Burma and in the adjoin areas **(FAO, 2004).** The genus citrus contain all the species widely cultivated in West Africa including Nigeria **(Nasiru, *et al.*, 2015) .** The approximate composition of edible portion is water (86%), protein (0.6%), fat (0.1%). Micro-nutrients per 100%; calcium (24mg), vitamin A (12mg), thiamine (0.06mg), riboflavin (0.02mg) niacin (0.1mg), also, one medium orange supplies about 66mg of vitamin C, a 100 % of the daily dietary requirement for adults **(Alfred and Patrick, 1985).**

Diseases that harm or destroy the citrus fruits are through the impairment of beneficial physiological or biochemical processes caused by continuous irritation initiated by primary causal agent/pathogens resulting in the reduction of nutritional and market values of the fruits in a given environment **(Nnadi and Madabuike, 2000).**

Citrus is the second largest fruit crop worldwide **(Spiegel-Roy and Goldschmidt, 1996)** and it is one of the foremost export fruit Crops in Libya and in the rest of the world. It is primarily valued for its fruit, which is either consumed (sour orange, sweet orange, tangerine, grapefruit, etc.) or used in processing industry. All species have traditional medicinal value. Citrus has many other uses including animal fodder and craft and fuel wood **(Manner *et al.*, 2006).** In addition, their essential oils are used in the cosmetic and pharmaceutical industries **(Frazier and Westhofe, 1978)** and a highly fermentable energy source with sweet taste and aroma **(Lawal *et al.*, 2013).** The most important diseases to reduce the quantity and quality of citrus. Postharvest fungal decay may cause significant losses to the citrus industry worldwide **(Holmes, *et al.*, 1999 , Barkai - Golan, 2001 and Plaza *et al.*, 2003).** Injuries on citrus fruit caused during harvest, provide entries to wound pathogens, including *Penicillium digitatum* Sacc. and *P. italicum* Wehmer, causal agents of green and blue mold respectively. The mold invades the fruit much more rapidly and predominates in mixed infections, causing approximately 60-80% of decay **(Palou *et al.*, 2001, Skaria *et al.*, 2003 and Plaza *et al.*, 2004) .**

Citrus cultivation is an important commercial and industrial agronomic activity worldwide . Citrus fruit is widely consumed , both as fresh fruit and as juice , not only for its flavor but also because of its vitamin C and antioxidant content . Citrus is the main fresh fruit exported by the Australian horticultural industry , with fruit mainly grown in New South Wales (NSW) , Queensland , South Australia and Victoria . The main markets for Australian citrus are fresh fruit (domestic and export) , fresh juice (domestic and export) and drink manufactured from frozen concentrated juice . In 2002/2003 record export volumes of 167.000 tonnes were achieved , with an estimated gross value production of AUD$201 million (Australian Citrus Limited , 2009) . In particular , South Australia exported fresh oranges to the value of $58.1 million in 2007 (Primary Industries and Resources SA, 2010) . Citrus is an non-

climacteric fruit , therefore it has a relatively long shelf-life . Its cultivation is an important commercial agronomic activity worldwide . Citrus fruits are the most valuable fruit crop in international trade , and include ; mandarins , sweet oranges , limes and grapefruit **(Erminawati Wuryatmo , 2011) .**

Citrus fruits are an essential component of some of the human nutritional requirements like vitamins, minerals and organic acids. Preservation of these products, however, is one of the central problems encountered by producers worldwide. The postharvest losses of fruit and vegetable stands at 20-40% in the average **(Irtwange, 2006) .**

Citrus fruits are a major export commodity of Egypt, with production estimated to be 2.5 million tonnes ⁄ year. The most common and serious postharvest diseases of citrus fruits are green and blue moulds, caused by Penicillium digitatum and P. italicum, respectively **(Plaza *et al.*, 2003).** During April 2009, oranges (Citrus sinensis) from three Egyptian cultivars Baladi, Sukhary, and Abu-surra were collected from commercial markets and packinghouses in the Giza Governorate. After 3 weeks storage at room temperature and high relative humidity, a morphologically distinct Penicillium spp. was observed as a mixed infection with P. digitatum and P. italicum **(Holmes *et al.*, 1993).** The pathogen was isolated on potato dextrose agar (PDA), and identified as P. ulaiense, according to its morphological and cultural characteristics. Penicillium ulaiense was distinguished from P. digitatum by its blue-grey spore mass and from P. italicum by its ability to form coremia (1–7 mm tall) with white stalks **(Youssef, *et al.*, 2010) .** Citrus fruits are infected with many fungal disease especially after harvest in the orchard which transported to the supermarkets and developed in stored from between these disease : Penicillium rots , this pathogen caused 90% of citrus losses during the storage **(Henik, *et al.*, 2012)** .

According to **(Pitt, 1979)** . The genus *Penicillium* includes 150 species. Relatively few species are economically important plant pathogens . Among the most notable species are *P. italicum* (Wehmer) and *P. digitatum* (Pers. Fr.) Sacc. , which cause blue mold and green mold of citrus fruits, respectively. Postharvest losses of citrus fruit caused by *P. digitatum* and *P. italicum* can account for more than 90% of all postharvest losses in semiarid production areas of the world . For this reason ,

virtually all decay control strategies in California citrus packinghouses are aimed at controlling blue and green molds **(Holmes, *et al.*, 1994) .**

Green and blue mold caused by *Penicillium digitatum* (Pers.:Fr) Sacc. and *P. italicum* (Wehmer) are the most important postharvest diseases of citrus fruits, and caused losses of 20% to 30% during storage and marketing. Postharvest decay results in major losses of fruit and vegetables. The USDA estimates that Green Mold destroys approximately 5% of California fresh citrus fruit, amounting to an annual loss of $30- 50 million half of all fruits harvested is lost due to fungal and pests decay worldwide . Losses from postharvest fruit diseases range from 1- 20 % in the United States, depending on the commodity . Postharvest fungal decay may cause significant losses to the citrus industry. Injuries on citrus fruit caused during harvest, provide entries to wound pathogens, including *P. digitatum* Sacc. and *P. italicum* Wehmer, causal agents of green and blue mould **(Oadi, *et al.*, 2012)**

Green mould is the most serious postharvest disease of citrus and generally more common than blue mould . The moulds develop in damaged areas in the fruit rind. Initial symptoms are a softening of the tissue which turns into a water-soaked area . The infection progresses into a white fungal growth which turns blue or green , but retains a white margin. This margin is larger with green mould . Fungal pathogens of *Penicillium digitatum* (green mould) and *P. italicum* (blue mould) . Green mould is more common than blue mould , but blue mould grows faster **(El-Gali , 2014) .**

Both infections develop in damaged areas in the rind. Initial symptoms include a softening of the tissue followed by development of a water soaked area . The infection site then develops into a white fungal growth which turns blue or green as spores are produced . The white margin is larger (10-20mm) with green mould . The optimum temperature for mould growth is 27°C . No growth occurs above 30°C and growth is slow below 10°C **(Taverner, *et al.*, 1999)** .

Post-harvest diseases account to about 50% losses in fruits stored in poor storage conditions especially under high humidity. They are posing a major problem to the agriculture industry **(Agrios, 2005)** . Citrusm fruits are among the crops susceptible to post-harvest diseases caused by fungi under poor storage conditions **(Ogawa *et al.*,**

1995) .The most important fungi causing post-harvest diseases include: *Penicillium spp*, *Aspergillus spp*, *Alternaria spp*, *Botrytis cinerea*, *Monilinia lax* and *Rhizopus stolonifer* **(Harbant Singh *et al.*, 2011)** .

Postharvest fungal diseases result in significant economic losses in the citrus industry . The most common postharvest fungal diseases affecting citrus fruits worldwide are green mould , blue mould and sour rot , which are caused by the filamentous fungi *Penicillium digitatum* (pers.:Fr.) Sacc., *Penicillium italicum* Wehmer and *Geotrichum citri-aurantii* Link ex Pers. Respectively **(Plaza *et al.*, 2003 , Cunningham and Taverner, 2007 and Droby, 2006) .** These pathogens may infect fruit in the packing-house, in transit, in storage and in the market **(Erminawati Wuryatmo , 2011) .**

To control of postharvest disease evaluated the effect of temperature and time on oranges decay development of *Penicillium digitatum* and *Penicillium italicum*, the most postharvest diseases of citrus fruit. The assays carried out in inoculated 'Valencia late' oranges showed in both seasons an excellent control of both pathogens, when fruits were exposed to treatments at 40ºC for 18 h, and stores for 5 days at 5ºC plus 7 days at 20ºC. Concerning quality changes slightly effects were observed on fruits submitted to curing treatment. These results suggest that this treatment could be an environmental friendly alternative to chemical fungicides in oranges packinghouses **(Carla Nunes, *et al.*, 2007) .**

Aim of the study :

1. Isolation and identification of *Penicillium spp*. from infected citrus fruits include local and imported , Orange (Sweet, Navel, Sour, Blood Orange) , Mandarin , Lemon and Grapefruit .

2. Pathogenicity test by application Kock Pastulate .

3. Study the *Penicillium* behavior (mode of action) .

4. Testing control measures of *Penicillium* rot on artificially inoculated citrus fruits by using some physical method (hot water , sea water) , natural oil of plant extracts and mineral oil (Johnson's baby oil) .

CHAPTER TWO

2. LITERATURES AND REVIEWS

2.1 Citrus crop

Citrus species are major exports products of Egypt . The total cultivated area for citrus fruits are 159,446,143 ha with total production estimated to 3,311,300 ton (FAO , 2010) . The orange cultivation accounts 63% of Egypt's total citrus production . Three main varieties of oranges are produced in Egypt : Navel , Valencia and Baladi . Washington Navel orange (*Citrus sinensis, L. Osbeck*) is the most popular orange cultivar among other citrus species in Egypt **(Tarabih, *et al.*, 2013) .**

The genus Citrus, belonging to the Rutaceae or Rue family, comprises of about 140 genera and 1,300 species. *Citrus sinensis* (Orange), *Citrus paradise* (Grapefruit), *Citrus limon* (Lemon), *Citrus reticulate* (tangerine), *Citrus grandis* (shaddock), *Citrus aurantium* (sour orange), *Citrus medica* (Citron), and *Citrus aurantifolia* (lime) are some important fruits of genus *Citrus* **(Kamal, *et al.*, 2011) .**

Citrus are well known as one of the world's major fruit crops that are produced in many countries with tropical or subtropical climate. Brazil, USA, Japan, China, Mexico, Pakistan, and countries of the Mediterranean region, are the major *Citrus* producers. Worldwide, *Citrus* production is estimated to be at levels as high as 105 million metric tons (MMT) per annum, Brazil being the largest producer with contribution of 19.2 MMT followed by the United States. Pakistan with an annual production ca. 1.76 MMT of *Citrus* fruits stands among the ten top *Citrus* producing countries of the world . *Citrus* fruits and their by-products are of high economic and medicinal value because of their multiple uses, such as in the food industry, cosmetics and folk medicine **(Kamal, *et al.*, 2011)** .

The world production of citrus is greater than that of any other fruit crop . citrus is the world number one fruit crop in terms of production being 78.2 million metric tones in 1991 ; followed by grape , banana and apple which were 55.9 , 47.8 and 39.6 million metric tones respectively . The Florida state University (2004) reported that citrus contributed 10 billion dollars to the US economy in the year 2004 . Citrus is sold both as fresh and processed product in both developed and developing countries **(Arekemase and Oyeyiola 2007) .**

In addition to large scale consumption as fresh fruits, the *Citrus* fruits are mainly processed to produce juice. The waste of *Citrus* processing industry, left after juice extraction, such as peels, seeds and pulps, corresponding to about 50% of the raw processed fruit, can be used as a potential source of valuable by-products . Specifically, the *Citrus* peels, commonly treated as agro-industrial waste, are a potential source of valuable secondary plant metabolites and essential oils *Citrus* peel essential oils are reported to be one of the rich sources of bioactive compounds namely coumarins, flavonoids, carotenes, terpenes and linalool etc. Recently, *Citrus* peel essential oils have also been searched for their natural antioxidant and antimicrobial properties . It is widely accepted that biological activities of plant materials are strongly linked with their specific chemical composition, mainly the secondary metabolites such as plant phenolics and flavonoids . Furthermore, studies revealed that drying the plant materials under different conditions can exert significant effect on the chemical profile and biological attributes of the essential oils derived **(Kamal, *et al.*, 2011)**.

The citrus fruits are a rich source of vitamin 'C' and a good source of vitamin 'P'. The citrus production is facing the main threat of postharvest losses. These losses are due to many factors, among which postharvest fungal diseases are considered as a principal cause. Sweet orange at retail are more vulnerable to post harvest diseases. It was observed that in India, the extent of damage varied from 29.9 to 43.8% in sweet orange and 25.5 to 36.8% in acid lime . The improper handling, packaging, storage and transportation may result in decay and growth of microorganisms, which become activated because of the changing physiological state of the fruits and vegetables . Fruit, due to their low pH, higher moisture content and nutrient composition are very susceptible to attack by pathogenic fungi, which in addition to causing rots, may also make them unfit for consumption by producing mycotoxins . The chemical composition of citrus fruits species vary according to citrus varity . Orange fruits have Vitamin C 60mg/100ml juice , Lemon 56.6mg/100ml juice , Mandarin 42mg/100ml juice , Grapefruit 38.3/100ml juice , and Orange fruits have TA % Citric Acid 1.82% , Lemon 6.7% , Mandarin 0.93 % , Grapefruit 2.56 % **(Pankaj Sharma and verma , 2013) .**

Citrus Sinensis is a member of the family of "Rutaceae" which contains about150 genera and nearly 2000 species, probably originated in North Eastern India, in Burma and in the adjoin areas . The genus citrus contain all the species widely cultivated in West Africa including Nigeria. The sweet orange are evergreen trees of small to medium stature. They often have thorny (prickly) stem 1.6 – 1.9m tall with a rounded symmetrical spreading crown. The leaves are shiny and leathery they are elliptical and up to 10.2cm long, they have wings on their petioles (leaf steams). The approximate composition of edible portion is water (86%), protein (0.6%), fat (0.1%). Micro-nutrients per 100%; calcium (24mg), vitamin A (12mg), thiamine (0.06mg), riboflavin (0.02mg) niacin (0.1mg), also, one medium orange supplies about 66mg of vitamin C, a 100 percent of the daily dietary requirement for adults . Diseases that harm or destroy the Citrus fruits are through the impairment of beneficial physiological or biochemical processes caused by continuous irritation initiated by primary causal agent/pathogens resulting in the reduction of nutritional and market values of the fruits in a given environment **.** Citrus sinensis is a medium size tropical plant generally cultivated in the Tropical, Sub-Tropical and Mediterranean regions. It is the most popularly cultivated fruits accounting to about 78.2% million metric tons in 1991; followed by grape, banana, and apple which were 55.9, 47.8 and 39.6 million metric tons respectively . In Nigeria, commercial cultivation in large plantations is mostly within the middle belt. However, some Southern states and those in the far North also engage in Citrus large scale cultivation . In the South-South region including Akwa Ibom cultivation is mainly by individual farmers **(Edward Ntui Okey, 2015) .** Mature Citrus sinensis fruits are reported to contain 87.67% moisture, 11% carbohydrates with a calorific value of 48cal/100g . These fruits are also rich in organic acids, essential oils beta carotenes, vitamin C, effective antioxidants and their pulps contain significant amounts of protein and soluble fibres . The presence of flavonoids, carotenoids and limonoids in these fruits make them useful as anti-cancer, anti-viral as well as anti-inflammatory products . On the other hand, Citrus fruits have high susceptibility to attacks by pathogenic fungi, due to their low pH high moisture content and nutritional composition. These attacks result in fruit rots and other diseases which make them unsuitable for human consumption due to mycotoxin production **(Edward Ntui Okey, 2015) .**

Reports on Citrus post-harvest fungi rots are extensive and world-wide. A number of fungal species including Phythium, Phytophthora, Botryodiplodia, Aspergillus, Penicillium, Rhizopus, Glasdosporium, Mucor, have been associated with Citrus fruit rot . Harvesting injuries, improper handling during transportation and poor storage facilities are general predisposing conditions for fruit rot development especially in economically stressed communities. In Nigerian markets, 20-90% fruits displayed for sale are reported to show symptoms of microbial infection . Due to environmental concerns, the control of plant diseases is now focusing on the use of natural products in place of traditional chemicals with hazardous effects. This study was therefore, conducted to identify the pathogenic fungi and also to determine the possibility of using water leaf extracts from A. indica and C odorata as biocontrol agents of these pathogens **(Edward Ntui Okey, 2015) .**

2.1.1 Description of citrus

2.1.1.1 Orange (*Citrus sinensis*)

Orange (Citrus sinensis) is one of the economic fruits in Thailand. Most production areas are in the northern part of the country especially Chiang Mai. In 2004, Thailand exported 2,189 tons of oranges to Asian countries with a value of 50.8 million Baht . Fungal diseases are one of the major problems facing the citrus production in all areas of the world and resulted in enormous economic losses . Green mold is the most important postharvest disease of citrus in many Asian countries including Thailand. The responsible agent is the fungus, Penicillium digitatum (Pers. Fr.) Sacc. This fungus survives in the orchard from season to season mainly in the form of conidia and causes infection by airborne spores where there are injuries or blemishes **(Kreuawan, *et al.*, 2007) .**

2.1.1.2 Mandarin(*Citrus sinensis*)

Mandarin is very important fruit crop, second only to banana. It is usually consumed in raw form or in fruit salads as well as juice. The fruit consists of three layers . The outer yellow/orange peel is with oil glands which exude the essential oils, producing the typical orange odor . The whitish thread like mesocarp . The endocarp consisting of 8 - 10 segments filled with juice sacs (vesicles) . Mandarins are rich in Ascorbic acid (13 – 54 mg per 100 g of edible portion) and Calcium (25 – 46 mg per 100 g of edible portion). They are a great source of Vitamin C. One orange actually

has all the Vitamin C that one needs for the day. The water content in the fruit is nearly 80 per cent to 90 per cent of edible portion. The chemical composition of the Mandarin is as under **(Somayeh, *et al.*, 2012) .**

2.1.1.3 Lemon (*Citrus limon*)

Lemons are nonclimacteric fruit and have low respiration rates. They are therefore able to be stored for long periods of time. In contrast to other citrus varieties there are significant changes in the internal quality of lemon fruit during storage. During storage the percentage of juice increases (by up to16%) primarily due to the water stored in the peel. The acid content of fruit also increases (by up to 24%) during storage and the peel colour changes from green to yellow **(Somayeh, *et al.*, 2012) .**

Lemons are sensitive to cold temperatures and should not be stored at temperatures below 10°C as they develop chilling injury. The length of time lemons can be stored depends on the stage that they are picked. Fruit harvested with a yellow tinge can be stored for a few weeks, silver-green fruit 6 weeks, light green fruit 2 months and dark green fruit 5-6 months. In Australia long term storage is not common practice. However, in California they store fruit for long periods of time and harvest fruit using four colour grades . Lemon (*Citrus limon*) is the third most important species of citrus after orange and mandarin, with a production totaling more than 4.4 million tones during the 2001/2002 season. Argentina with 1.2 million tones is currently the world's largest producer of lemons **(Somayeh, *et al.*, 2012) .**

The peel is a by-product of lemon juice processing, with a high potential use. Two different tissues are found in what is colloquially called lemon peel, flavedo and albedo . Flavedo is the peel's outer layer, whose colour varies from green to yellow. It is a rich source of essential oils , which have been used since ancient times by the flavour and fragrance industry . Albedo is the major component of lemon peel, and is a spongy and cellulosic layer laid under flavedo. The thickness of the albedo fluctuates according to several variables, among them variety and degree of ripeness. Albedo has high dietary fiber content, and if added to new meat products permits to formulate healthier products like beef burgers , bologna and dry cured sausages . Furthermore, the presence of associated bioactive compounds (flavonoids and vitamin C) with antioxidant properties in fresh lemon albedo involves healthier benefits than other sources of dietary fiber . In the present work, a low cost agricultural waste

material, lemon peel has been used for a source of protein, fats, and essential macro minerals necessary for the growth of animals is being investigated . Citrus is most commonly thought of as a good source of vitamin C. However, fruits also contain an impressive list of other essential nutrients, including both glycaemic and non-glycaemic carbohydrate (sugars and fibre), potassium, folate, calcium, thiamin, niacin, vitamin B6, phosphorus, magnesium, copper, riboflavin, pantothenic acid and a variety of phytochemicals. Citrus contains no fat or sodium. The average energy value of fresh citrus is also low, which can be very important for consumers concerned about putting on excess body weight. A medium orange contains 60 to 80 kcal, a grapefruit 90 kcal and a tablespoon (15 ml) of lemon juice only 4 kcal **(Somayeh, *et al.*, 2012) .**

2.2 Post-harvest diseases

2.2.1 Infection and Losses

Post-harvest diseases account to about 50% losses in fruits stored in poor storage conditions especially under high humidity. They are posing a major problem to the agriculture industry . Citrus fruits are among the crops susceptible to post-harvest diseases caused by fungi under poor storage conditions . The most important fungi causing post-harvest diseases include: *Penicillium* spp, *Aspergillus* spp, *Alternaria* spp, *Botrytis cinerea*, *Monilinia lax* and *Rhizopus stolonifer* . Many fruits are prone to damage caused by insects, animals, early splits, and during mechanical harvesting. This damage predispose the fruits to the wound invading pathogen *Aspergillus flavus* , and other fungi, that causes decay on stored citrus fruits. *Aspergillus flavus* can pose a health problem, especially it produces aflatoxin, a group of toxic, carcinogenic compounds **(Harbant, *et al.*, 2011) .**

The postharvest losses are often more harsh in developing countries due to lack of storage and transportation facilities. Fruit infections by fungi may appear during the growth period, harvesting, handling, transportation and post-harvest stockpile and marketing conditions, or after procuring by the consumer. Fruits incorporate high levels of nutrients element and sugars and their low pH values make them exceptionally desirable to fungal decay . Fungi are considered as an essential post-harvest losses agent of different fruits, based on cultivar, season and production area amid other factors . Fungi are the most crucial and common pathogens and the mean

cause of crop diseases. It Infect a wide range of fruits and vegetables during storage and transportation . Rotted fungi are ubiquitous biological agents that are able to infect fruits because of their ability to produce a wide range of hydrolytic enzymes. Mould growth depends on many factors such as pH, water activity (aw), temperature, atmosphere, and time . As reported by Yemeni Ministry of Agriculture and Irrigation, (2011) the total cultivated area of fruits was reported to be 93,989 acre yielding 991,091 tons per year **(Qais Abdullah, *et al*., 2016)**

Kreuawan, *et al*., 2007 reported that aimed in isolation and identification of the mycobiota associated with post-harvest decay of apples, oranges, bananas, mangoes and grapes from different localities in Sana'a markets. Yemen . The disease is generally controlled by fungicides such as imazalil, sodium ortho-phenyl phenate, or thiabendazole . The use of these chemical agents has been applied for many years with few or limited success due to the development of resistance by the fungus . In addition, the accumulation of hazardous chemicals in the environment raises public concern about their effect on human health. There is an urgent demand for new methods to supplement the existing regimes to achieve better disease control. Biological control offers an environment friendly alternative to the use of chemicals for controlling plant diseases . The increasing interest in the development of such control has led to our investigation of microorganisms associated with orange trees as potential microbial agents for the control of *P. digitatum*. In this study, we reported the isolation of antagonistic microorganisms from various parts of healthy orange trees and their inhibitory effect on growth and spore germination of *P. digitatum* in vitro**.**

Postharvest diseases caused by *Geotricum candidum* (sour rot) *Penicillium digitatum* (green mould) and *Penicillium italicum* (blue mould) are the very significant negative agents affecting in handling and marketing of citrus fruits in Egypt . They cause dangerous problems to the harvested citrus fruits during handling , transporting , exportation and the storage process . Although the use of chemical fungicides gave satisfactory control against mould infection , the fungicide residual can have a harmful effect on people and the environment . Moreover , successive use of fungicides could lead to some fungal isolates to develop a significant resistance to

the applied fungicides therefore , alternative fungicide treatments are needed for the management of postharvest diseases of citrus fruits **(Abd El-Motty *et al*., 2007)** .

Postharvest decays are caused by latent or wound induced fungal infections which causes as great as 25-50% loss and remain an important challenge in sustainable food production **(Wilson, *et al.*, 1991)** . Oranges (*Citrus sinensis*) are usually stored after harvest and postharvest decay is the major factor limiting their shelf life. Increasing the storage time of the fruit can reduce the economic losses due to decay. Increasing pathogen resistance to key fungicides, lack of replacement fungicides, consequent restrictions on fungicide use and high cost of chemicals requires alternative methods which are safer and ecofriendly to reduce postharvest decays. Further, use of fungicides generates health concerns due to their carcinogenic and teratogenic properties **(Sharma, *et al.*, 2009 and Janisiewicz, *et al.*, 2002)**

Qin *et al*., 2010 showed that boron strongly inhibited spore germination , germ tube elongation and mycelial spread of Botrytis cinerea in the culture medium . Moreover , application of boron at 1% caused the appearance of abnormal spores (disrupted) in some cases . Furthermore , boron led to the leakage of cellular constituents (soluble proteins and carbohydrates) from hyphae of *Bacteria cinerea* . The quantities of boric acid and its sodium salts applied as pesticides are modest compared to amounts used for the other non-pesticidal purposes . Further, boric acid , borax and boron – containing salts are ubiquitous in the environment . Boron occurs naturally in water , fruits , forage crops and is an essential nutrient for plants as well as an essential element for many organisms .

The mechanisms by which boron decreased gray mold decay , may be directly related to the disruption effect of boron on cell membrane of the fungal pathogen that resulted in the breakdown of the cell membrane and loss of cytoplasmic materials from the hyphae . Exogenous application of boron was shown to alleviate the occurrence of browning injuries in pears during controlled atmosphere storage **(Xuan *et al.*, 2005)** . Nevertheless , little information is available concerning the effect of boron for control of plant disease caused by microbial pathogens and the physiological basis involved . The inhibition effect of *P. expansum* may be related to the increased oxidative stress caused by boron through suppressing the expression of antioxidant enzymes in the pathogen **(Qin *et al*., 2010)** .

Jojoba oil is commonly known as liquid wax , colorless and odorless with unique physical and chemical properties . Also , jojoba oil can easily be hydrogenated into a soft wax that can be used in candle wax , various kinds of polishes , coating material for fruits and pills **(Nagvi and Ting, 1990) .**

Abd El-Moneim and Abd El-Mageed 2006 , studied that coating Washington Navel orange fruits with jojoba oil led to reduce fruit decay , weight loss and increasing fruits storage life . As storage days progressed total soluble sugar (TSS) increased , but the titratable acidity and ascorbic acid contents decreased . **Abd-Allah *et al.*, 2012** found that coating persimmon fruits with jojoba oil helped to delay ripening and reduced weight loss and decay percentage . Postharvest fungal decay may cause significant losses to the citrus industry. Injuries on citrus fruit caused during harvest, provide entries to wound pathogens, including *Penicillium digitatum* Sacc. and *P. italicum* Wehmer, causal agents of green and blue mould, respectively. These pathogens occur in almost all citrus growing regions of the world . The synthetic fungicide Imazalil, has been routinely used to control postharvest diseases including green and blue moulds . Attempts to find alternatives to chemical control have been ongoing for some time, and indeed many fungi have become resistant to commonly used fungicides. In the mean time, there is increasing consumer concern in respect to chemical residues on fruit products regarding health and environmental issues. However, alternative measures are generally less effective than fungicides. **(Tarabih, *et al.*, 2013**).

Toker and Bicici 1996 studied the stressed on mandarin, orange, grapefruit and lemon were stored at ambient temperature for two months and kept at storage conditions for two and four months give 16.8, 25.1 and 65.4% crop losses were observed, respectively, due to development of total postharvest diseases. The most important ones of them are caused by fungi such as *Penicillium spp*., *Aspergillus spp*., *Alternaria spp*., *Botrytis cinerea*, *Monilinia laxa and Rhizopus stolonifer* (Ladaniya 2008). *P. digitutum* and *P. italicum* are wound invading pathogens that causes decay on stored citrus fruits damaged by insects, animals, early splits, mechanical harvesting, chilling and environmental stresses. Green and blue mould caused by *P. digitutum* and *P. italicum*, respectively, are the most important postharvest disease of fruits worldwide **(Oadi, *et al.*, 2012) .**

2.2.2 Control

The control of plant diseases is still mainly dependent on the use of chemical fungicides. Synthetic fungicides such as thiabendazole, imazalil and sodium ortho-phenylphenate have been used traditionally to control the postharvest diseases, but their excessive use, complemented with high costs, residues in plants and development of resistance, has left a negative effect on human health and the environment. Further, withdrawal of some chemical pesticides, such as benomyl and captan, for control of postharvest diseases in the USA and ethylene dibromide for sterilization of Queensland fruit fly in Australia, is a clear signal that new technologies and new fungicides for control of plant diseases are needed **(Jameel Jhalegar, *et al.*, 2013) .**

Synthetic fungicides, such as, thiabendazole, imazalil and sodium ortho-phenyl phonate has been used traditionally to control the postharvest diseases, but their excessive use complemented with high costs, residues in plants, and development of resistance, has left a negative effect on human health and the environment . Environmentally friendly plant extracts agents have shown to be great potential as an alternative to synthetic fungicides . Recently, the antimicrobial activity of some higher plant products that are biodegradable and safe to human health has attracted the attention of microbiologists in the control of plant disease, but the actual use of these products for the control of postharvest pathogens of fruits generally, and in particular for citrus pathogens is, however, still limited. The purpose of our research is to test the possibility of using extracts from chilly and ginger to control or inhibits the pathogens causing post-harvest diseases in citrus fruit and is presented in this paper **(Harbant, *et al.*, 2011)** .

The United States of America Environmental Protection Agency has classified benomyl as a possible human carcinogen, which can also act as a chronic and reproductive toxicant. However, worldwide ‘organically grown’ fruit, which has not been treated with fungicide, is becoming popular among consumers. Under these circumstances, an alternative method of disease control for Kinnow mandarin without the use of synthetic chemicals is urgently needed. Some environmentally friendly plant extracts have been shown to have great potential as an alternative to synthetic fungicides . Recently, the antimicrobial activity of some higher plant products that are

biodegradable and safe to human health has attracted the attention of microbiologists in the control of plant disease, but the actual use of these products for the control of postharvest pathogens of fruits generally, and in particular for citrus pathogens is, however, still limited. Among the safer alternatives to synthetics, use of plant products has attracted researchers for the management of diseases of several fruits**(Jameel Jhalegar, *et al.*, 2013) .**

The major purpose of our research was to extend the marketable period of 'Kinnow' mandarin through approaches such as using botanicals to control or inhibit the pathogens causing postharvest diseases in Kinnow mandarin, as the fruits are susceptible to postharvest diseases such as green mold and blue mold caused by *P. digitatum* and *P. italicum*, respectively, which reduce its availability for a longer time in the market. **(Jameel Jhalegar, *et al.*, 2013) .**

The genus *Penicillium* was first described in the scientific literature by Link ex Gray in his 1809 work *Observationes in ordines plantarum naturales* . *Penicillium* is classified as a genus of anamorphic fungi in the division Ascomycota (Order Eurotiales, Class Eurotiomycetes, Family Trichocomaceae) . The genus name is derived from the Latin root *Penicillium*, meaning " painter's brush " , and refers to the chains- of conidia that resemble a broom . In a 1979 monograph, John I. Pitt divided *Penicillium* into four subgenera based on conidiophore morphology and branching pattern : *Aspergilloides, Biverticillium, Furcatum* and *Penicillium* **(Pitt, 1979)** .

The main pathogens found in the oranges were *Lasiodiplodia theobromae* and *Penicillium digitatum* . Brazil is the first world producer of citrus fruit, with 20 million tons for each year . São Paulo State is responsible for 81% of the Brazilian production, from which 82% goes to juice production, 17% goes to the domestic market and only 1% is destined to fresh fruit exports . This low exporting/production ratio is due to the cultivars, taxes, phytosanitary barriers, and quality demands by the international market . European Union, for example, classifies citrus black spot as a quarantine disease . Therefore, a careful selection process is necessary at the packinghouse in order to avoid exports of injured fruits. Other typical postharvest diseases such as green mold and stem-end rot also reduce fruit quality. Therefore, fruit has to be treated in order to minimize problems with the importer (**Ivan, *et al.*, 2009) .**

In Spain, the percentage of the fruit rotting after harvest in a typical season is 3 to 6 . However, under favorable disease conditions, losses up to 50% can occur during marketing . In Northeastern Brazil, a survey of postharvest diseases in citrus indicated a 21.9% incidence of fungal rots . Several kinds of injuries may affect fruit's quality after harvest. The damaged tissue becomes susceptible to infections caused by pathogenic microorganisms, besides showing scars visually detrimental to the fruits, reducing their market value . In (1991) found that 80% of impacts on fruits along a citrus sorting line varied from 25 and 150 G. These impacts can injure the fruits. The purposes of this work were to characterize injuries in 'Valencia' orange and 'Murcott' tangor, destined for foreign markets, at different stages in the packinghouse and to identify critical points, as well as the extent of impact on fruits along the citrus processing lines in packinghouse (**Ivan, *et al*., 2009) .**

Green mold caused *by Penicillium digitatum* Sacc . is one of the major decays of fresh citrus . Infection by the causal organism occurs by germination of spores within injuries formed in the peel during handling at harvest and packing . Success of infection is influenced by location and depth of the injury . Shallow injuries between oil glands are generally more resistant to infection than deeper injuries into the albedo and injuries involving oil glands . The purpose of this paper is to report the association of lignin with injuries that are resistant to penetration by *P. digitatum* and observations of factors that curtail or promote the lignification process **(Eldon and Ismail , 1980) .**

Green and blue moulds, caused by *Penicillium digitatum* (Pers. Fr.) Sacc. and *Penicillium italicum* (Wehmer) , respectively , are the most significant postharvest diseases of citrus in all production areas that like Spain, California **(Eckert and Eaks 1989)** . Both *P. digitatum* and *P. italicum* are severe wound pathogens that can infect the fruit in the orchard, the packinghouses and during allocation and marketing . They reproduce very quickly and their spores are ubiquitous in the atmosphere and on fruit exterior and are simple distributed by air currents . Therefore, the source of fungal inoculum in citrus orchards and packing houses is virtually continuous during the season **(Kanetis, *et al.*, 2007)** . Citrus fruit can become " soiled" with conidia of the two fungi that are loosened in handling of diseased fruit . The conidia located in damage that laceration oil glands or penetrate into the albedo of the peel usually bring

irreversible infection within 48 h at 20 – 25°C **(Plaza, *et al.*, 2003 , Lahlali, *et al.*, 2006 and Stange, *et al.*, 2002) .** The germination of conidia of both *Penicillium* species inside rind wounds requires free water and nutrients **(Tarabih and El-Metwally , 2013) .**

Green mold caused by *Penicillium digitatum* is one of the major fungal decays of Florida fresh citrus. Injuries to the fruit peel are required for penetration of the fugus . Penetration by *P. digitatum* was inhibited in injuries to oranges and grapefruit where cells at the injured surface produced lignin before fungal entry. Accumulation of lignin occurred most rapidly at 30°C and at relative humidities above 90%. Under these conditions, lignin developed within 12 hours following injury. Lignification was delayed or inhibited by peel oil or desiccation which caused damage to cells near the injury. These injuries were easily penetrated by *P. digitatum* as were injuries into the albedo where cells were incapable of producing lignin **(Eldon and Ismail , 1980) .**

Green mold, caused by the pathogen *Penicillium digitatum* (Pers.:Fr.) Sacc., is the most economically important postharvest disease of citrus fruits in all production areas that, like Spain and other Mediterranean countries, are characterized by low summer rainfall . Actual losses due to green mold are quite variable and, beyond postharvest factors, depend upon the area of production, citrus cultivar, weather and orchard conditions, and especially the extent of physical or mechanical injury to the fruit during harvest and subsequent handling . *P. digitatum* is a strict wound pathogen that can infect the fruit in the grove, the packinghouse, and during distribution and marketing **(Palou, 2013) .** It reproduces very rapidly and the spores are ubiquitous in the atmosphere and on fruit surfaces and are readily disseminated by air currents . Furthermore, citrus fruit can become “soiled” with conidia that are loosened in handling of decayed fruit. For these reasons, any successful cost-effective postharvest disease management program for citrus fruit is primarily based on the control of green mold . The disease has been primarily controlled worldwide for many years by the application of conventional fungicides such as imazalil, sodium ortho-phenyl phenate, thiabendazole or, more recently, new active ingredients like pyrimethanil, azoxystrobin or fludioxonil . Different mixtures of these compounds are also commercially available. Postharvest treatments with these synthetic chemicals typically are relatively inexpensive, easy to apply, have curative action against pre-

existing or established infections and persistent preventive action against potential new infections, and many also inhibit the sporulation from lesions on decaying fruit . However, concerns about environmental contamination and human health risks associated with fungicide residues periodically led to regulatory reviews and restrictions or cancellations, and export markets are increasingly more sensitive to the use of chemicals for disease control. Further, the widespread and continuous use of these synthetic fungicides has led to the proliferation of resistant biotypes of *P. digitatum* in commercial packinghouses that seriously compromises the effectiveness of these treatments **(Palou, 2013) .**

There is, therefore, a clear need to find and implement methods alternative to conventional fungicides as part of integrated disease management (IDM) programs for the control of postharvest green mold of citrus fruits . According to their nature, these alternative decay control methods can be physical, chemical or biological. Among them, physical methods are advisable because they are absolutely residue-free. Among physical methods, heat treatments are the most important and popular because they are relatively effective, simple, cheap, easy to apply and easy to combine with other disease control methods . The purpose for this mini-review is to describe significant research work focused on the evaluation of heat treatments for the control of citrus postharvest green mold, either alone or in combination with other antifungal treatments **(Palou, 2013)** .

Biological control is becoming increasingly effective in replacing chemicals used to control plant diseases . This method has lower environmental impact than fungicides either alone or as part of integrated pest management in reducing synthetic fungicide application . Several strains of *Pantoea agglomerans* have been reported as effective in suppressing diseases of fruit crops, such as fire blight of apple and pear , brown spot of pear , and cranberry cotton ball caused by *Monilinia oxycocci* . *P. agglomerans* was reported as an antagonist of *P. expansum*, *Botrytis cinerea* and *Rhizopus stolonifer* on apple cv. Golden Delicious and on pear . However, biocontrol agents do not generally posses a broad spectrum of activity and they are not as effective as fungicides. Simultaneous application of several physical and chemical methods could provide more effective means of control and consistent results than that of one approach alone. Some physical treatments and exogenous substances, such

as chitosan, amino acids, antibiotics, calcium salts, and carbohydrates have also been used to enhance biocontrol of antagonists against fungal pathogens . Pre–storage hot water dips of fruit at temperatures above 40ºC have been shown to be effective in controlling storage decay, not only by reducing the pathogen but also by enhancing the resistance of fruit tissue, influencing host metabolism and ripening . Postharvest dips are applied for a few minutes at high temperatures, because fungal spores and latent infections of the pathogen are either on the surface or in the first few cell layers under the peel of the fruit **(Zamani, *et al.*, 2009) .**

Their beneficial effects in controlling *Penicillium* rots on citrus fruit have been documented . Hot water treatment may eliminate incipient infections by removing spores from wounds and acting directly on spore viability and/or inducing defence mechanisms in the outer layers of the epicarp which inhibit pathogen growth . Heat treatment can also provide further advantage of enhancing fruit coloration, but does not lead to softening. It inhibits the activities of cell wall hydrolytic enzymes in apple fruit and reduces ethylene production . Sodium bicarbonate (NaHCO3), commonly known as baking soda, was selected for integration with the biocontrol agent . It is a common food additive for pH adjustment, taste, texture modification, and spoilage control, and has been shown to have antimicrobial activity against *P. digitatum* on citrus fruit . It is inexpensive, readily available, and could be used with minimal risk of injury to the fruit. However, it is a poor eradicant unable to kill spores and its inhibitory effect is not very persistent. Its inhibitory activity depends on the presence of salt residues within the wound infection sites occupied by the fungus and on interactions between this residue and constituents of the peel . Biocontrol agents which can persist for long periods may protect fruit from post-treatment infection . Combining heat treatment and chemical compounds with an antagonist, could possibly be synergistic . The antagonist is applied after hot water treatment as it cannot survive at 50ºC. Thus the problem with application of the biocontrol agent before hot water treatment is that it must be heat tolerant . The objective of this study was to determine if green mould on oranges could be reduced by combination of the biocontrol agent *P. agglomerans* along with sodium bicarbonate and hot water treatment. The experiments were designed to develop an integrated strategy to control postharvest decay on oranges caused by *P. digitatum* that would be as effective as chemical control **(Zamani, *et al.*, 2009) .**

Penicillium digitatum the cause of citrus green moldrespect is important postharvest pathogens and cause serious losses annually . The disease is currently managed with synthetic fungicides. However, these chemicals become pose a significant risk is widely, with continued use of fungicides chemicals on food agriculture crops due of their potential effects on human and the environment . Pathogen resistance is another factor militating against the continuous use of synthetic fungicides . The extensive use of agrochemicals especially fungicides, with more . carcinogenic risk than other pesticides . may give rise to undesirable effects on animals and human . This damage increased significantly with the improper use and randomly led to then grow to reduce the use of these chemicals that accumulate in fruits, vegetables. Plant's extracts are one of several non-chemical control options that have recently received attention. However, actual use of these extracts to control post-harvest pathogens of fruits and citrus pathogens in particular is still limited **(Ghassan, *et al.*, 2013) .**

Plan extracts from plant species *Withania somnifera* and *Acacia seyal* led to the inhibition of the growth of fungus *Penicillium digitatum* by up (70%) when used for 21 days under the conditions of storage of natural citrus . Glucosinolates from mustard and horseradish also showed antimicrobial activity against *P. digitatum* . Showed study in vitro for evaluate (fenugreek seeds, harmal seeds, garlic cloves, cinnamon bark, sticky fleabane leaves and nightshade leaves and fruits) against *P. digitatum* that crude extracts of nightshade fruits cinnamon bark have completely inhibited the growth of tested fungal isolates and reached values LC50 to = 57.5 µg ml . The plant extracts reported effective against the fungi *Penicillium digitatum* include *Allium sativum*, *Azadirachta indica*, *Withania somnifera* and *Acacia seyal* . The objective of this study is to evaluate using of botanical pesticides as means to protect crops and their products and the environment from fungicides . Antifungal activity of crude extracts of five plants against green mould rot of citrus caused by *P. digitatum* under conditions *vitro* and *vivo* during storage **(Ghassan, *et al.*, 2013) .**

Postharvest green mould, caused by *Penicillium digitatum* Sacc. and blue mould, caused by *Penicillium italicum* Weh. , are the most economically important postharvest diseases of citrus fruit in Mediterranean climates . Currently, these diseases are primarily controlled with chemical fungicides, such as Imazalil or

Tiabendazol. The increasing public concern to pesticides applied in fresh fruit, the development of resistant fungal populations to fungicides and the possibility of a high number of fungicides may be withdraw for use, has increased the interest on alternative treatments to chemicals. Physical methods, such as heat treatment has a great potential to substitute postharvest fungicides **(Carla nunes, *et al.*, 2007) .**

Heat treatment can be applied to fruits by immersion in hot water, vapour heat, hot air, or short hot water brushing. Heat treatment is a practice used not only to control postharvest disease but also to reduce the sensitivity of the fruits to cold storage temperatures, thus reducing chilling injury . The exact mechanism of action of heat in controlling decay of citrus fruit is not clear, although has a direct and indirect inhibition on the pathogen. The possible direct effect is reported as one or more of the following mechanisms: pectic enzyme inactivation or denaturation of other proteins, lipid liberation, destruction of hormones, metabolic injury . The indirect effect is the stimulation of host-defence responses, by production of antifungal materials that act as a first defence against invading pathogens, and followed by the induction of several additional mechanisms such as building of a passive barrier to the pathogen by the production of lignin-like polymers catalyzed by phenyl ammonialyase (PAL) , and synthesis of phytoalexins . Curing citrus fruit (holding fruits at relative high temperature and humidity for two or three days) has demonstrated to be a very effective physical treatment to avoid *P. digitatum* and *P. italicum* decay . Other study using an intermittent curing treatment of 2 cycles of 18 h at 38ºC with an intermediate period of 6 h at 20ºC, allowed a total control decay of *P. italicum* on inoculated 'Clementina de Nules' mandarins . However, using curing treatments in a commercially scale is not easy, since stock fruits for 1-3 days is required. Also, a prolonged heat treatment could induce high weight losses, which are directly responsible of a reduction of the postharvest life of the produce, that more rapidly suffer wrinkling and softening **(Carla nunes, *et al.*, 2007) .**

In the present work we studied the development of a curing treatment adequate to the standard management used in the packing houses, by reducing curing time and their effects on postharvest quality parameters.

Carla nunes, *et al.* (2007) evaluated the effect of temperature and time on oranges decay development of *Penicillium digitatum* and *Penicillium italicum*, the most

postharvest diseases of citrus fruit. The assays carried out in inoculated 'Valencia late' oranges showed in both seasons an excellent control of both pathogens, when fruits were exposed to treatments at 40ºC for 18 h, and stores for 5 days at 5ºC plus 7 days at 20ºC. Concerning quality changes slightly effects were observed on fruits submitted to curing treatment. These results suggest that this treatment could be an environmental friendly alternative to chemical fungicides in oranges packinghouses.

Orange is majorly consumed for their vitamins and mineral contents . These vitamins promote a healthy immune system and prevent heart disease and high blood pressure. Orange also contains a significant amount of important minerals such as calcium, magnesium and potassium. However, the major postharvest problem of citruses is decay by fungi. Specifically, thedisease of sweet orange (*Citrus sinensis*) includes green rot by *Penicillium digitatum*, blue rot by *Penicillium italicum* especially at refrigeration temperatures, sour rot by *Geotrichum citriauranti*, anthracnose by *Colletotrichum gloeosporioides*, stem end rots by *Diploidia natalensis* and *Phomopsis citri* **(Oladele and Owolabi 2016) .**

In Nigeria, adequate cooling storage facilities are lacking and orange fruits are mostly transported, stored and marketed at ambient temperature. Typically shelf life of fruit in such circumstances is about 7 days and this limit the country's participation in international trade of commodity. Methods of extending shelf life of oranges include inoculum reduction by pre harvest farm sanitation and fruit washing, waxing, pre harvest heat treatment and controlled or modified atmosphere storage. Postharvest disease in *Citrus sinensis* is commonly controlled by applying chemical and hot water treatment. Heat treatment, apart from reducing decay, was also reported to alleviate some physiological disorder such as chilling injury in orange fruits and other citruses . However, problems with mould growth on fruits and food, concern for human health and environment and development of fungicide resistance prompt the use of sodium carbonate to control storage mould on fruits . This study therefore seeks to assess the efficacy of integrated control of postharvest decay on sweet orange fruits by hot water and sodium carbonate (Na2CO3) applications during subsequent storage at 28°C. It also seeks to determine the effect of such treatment on internal quality attributes of orange fruits **(Oladele and Owolabi 2016) .**

Zahra Ibrahim El-Gali , (2014) noticed citrus is one of the most widely grown plants in the world; however, it is affected by many types of fruit rot diseases after harvesting. Green mold disease of orange, caused by Penicillium digitatum Sacc, can cause extensive postharvest losses. The goal of this research was to use postharvest calcium applications to reduce fruit rot disease by soaking in solutions of CaCl2 and to test the effect of calcium on mycelial growth. Application of calcium chloride at 0.2%, 0.5% and 1.0% concentrations significantly decreased mycelial growth. In vivo studies showed fruit treatment by calcium soaking at 2% , 4% and 6% concentrations significantly reduced rots incidence of fruits. The optimal conditions of fungal growth were 25ºC and 87% relative humidity .

The objectives of this research studied by **Henik, *et al.*, (2012)** . in which to evaluate the effectiveness of the combination of plant crude extracts and yeasts as an alternative to replace the synthetic fungicide to control green mould rot caused by *Penicillium digitatum* on citrus fruit. This pathogen caused 90% of citrus losses during the storage. Control of this pathogen mainly with chemicals, but concerned with environmental contamination, human health, and pathogen resistance, chemical treatment is frequently decreased. *Eugenia caryophylata* crude extracts and *Candica utilis* showed to be the best combination to attain a reduction in green mould incidence by 90.3% and disease severity by 96.26%. Furthermore, the combination of *E. caryophylata* crude extracts and *C. utilis* had a more potent antifungal activity than imazalil. The effectiveness of the combination of plant crude extracts and yeasts can be an alternative treatment to replace the synthetic fungicide to control *P.digitatum* on citrus fruit, but the application in the packaging line needs further investigation .

Plant extracts could be useful in the management of fungal decay in postharvest conditions. This study investigated the phytochemical profile and effects of Plumeria latex against the postharvest fungal pathogens of sweet oranges (*Citrus sinensis L Osbeck*). Polar and non polar solvent extractions revealed alkaloids, glycosides and terpenoids as the major phytoconstituents present in Plumeria latex. Postharvest fungal pathogens of oranges such as *Aspergillus niger, A. fumigatus, A. terreus, Penicillium digitatum and Rhizopus arrhizus* were tested against various extracts of Plumeria latex. Antifungal assay of the extracts recorded significant inhibitory activity against *Aspergillus terreus* and *Penicillium digitatum* by the petroleum ether

extract. Being the most effective on all species, Plumeria obtuse was found to have potential antifungal properties followed by P. rubra after five days of incubation. Based on these results, application of Plumeria latex can be considered a useful strategy to be included in an integrated approach for controlling postharvest fungal disease of oranges **(Sibi, *et al.*, 2012) .**

Heat treatment can be applied to fruits by immersion in hot water, vapour heat, hot air, or short hot water brushing. Heat treatment is a practice used not only to control postharvest disease but also to reduce the sensitivity of the fruits to cold storage temperatures, thus reducing chilling injury **(Porat *et al.*, 2000 , Sanchez-Ballesta *et al.*, 2000 and Ben-Yehoshua, 2005).** The exact mechanism of action of heat in controlling decay of citrus fruit is not clear, although has a direct and indirect inhibition on the pathogen. The possible direct effect is reported as one or more of the following mechanisms: pectic enzyme inactivation or denaturation of other proteins, lipid liberation, destruction of hormones, metabolic injury **(Barkai-Golan and Philips 1991 and Barkai-Golan , 2001).** The indirect effect is the stimulation of host-defence responses, by production of antifungal materials that act as a first defence against invading pathogens, and followed by the induction of several additional mechanisms such as building of a passive barrier to the pathogen by the production of lignin-like polymers catalyzed by phenyl ammonialyase (PAL) **(Golomb, *et al.*, 1984),** and synthesis of phytoalexins **(Kim, *et al.*, 1991 and Ferguson, *et al.*, 2000)** Curing citrus fruit (holding fruits at relative high temperature and humidity for two or three days) has demonstrated to be a very effective physical treatment to avoid *P. digitatum* and *P. italicum* decay **(Stange and Eckert 1994 , Tuset, *et al.*, 1996 and Plaza, *et al.* 2003) .** Other study using an intermittent curing treatment of 2 cycles of 18 h at 38ºC with an intermediate period of 6 h at 20ºC, allowed a total control decay of *P. italicum* on inoculated 'Clementina de Nules' mandarins **(Pérez, *et al.*, 2005) .** However, using curing treatments in a commercially scale is not easy, since stock fruits for 1-3 days is required. Also, a prolonged heat treatment could induce high weight losses, which are directly responsible of a reduction of the postharvest life of the produce, that more rapidly suffer wrinkling and softening **(Carla, *et al.*, 2007) .**

Plant essential oils have the potential to replace the synthetic fungicides in the management of postharvest diseases of fruit and vegetables . The aim of this study was to access the *in vitro* and *in vivo* activity of essential oil obtained from oregano (*Origanum vulgare* L. ssp. *hirtum*), thyme (*Thymus vulgaris* L.) and lemon (*Citrus limon* L.) plants, against some important postharvest pathogens (*Botrytis cinerea*, *Penicillium italicum* and *P. digitatum*) . *In vitro* experiments indicated that *P. italicum* did not show any mycelium growth in presence of thyme essential oils at concentration of 0.13 µl/ml **(Andrew, *et al.*, 2013) .**

Green mould (*Penicillium digitatum*) and sour rot (*Geotrichum citri-aurantii*) in early season, thin-skinned navel oranges (*Citrus sinensis*) are of serious concern to the Australian citrus export industry. In this study, was examined possible alternative treatments of pathogen including the use of non-restricted fungicides in combination with gibberellic acid (GA3), generally regarded as safe (GRAS) compounds, and/or elevated temperature. When inoculated fruit were dipped in solutions containing the fungicide imazalil alone and in combination with GA3, thiabendazole, or carbendazim, the infection rate was reduced. GRAS compounds, sodium carbonate, and mineral oil, were also effective in reducing infection rates when mixed with imazalil, as was sodium carbonate alone and when mixed with GA3. GA3 was not effective at the rate tested (50 mg litre-1) **(Cunningham and Taverner , 2007)** .

This study was carry out to test chemical and biological agent to control green mold caused by *Penicillium digitatum* on orange fruit. In vitro test three natural chemical compound used for inhibition linear growth for *P. digitatum* , acetic acid, vinegar and neem extract. Acetic acid was most effective to inhibition pathogen in concentration 0.5% it was 100% comparing to neem extract 5% and vinegar 2% it was 62% and 50% respectively. The biological agent yeast *Saccharomyces cerevisiae* 25 ×107 and *Rhodotorula spp* 35 ×106 give a good result for inhibition linear growth of pathogen it was 84.60% and 70.23% **(Oadi, *et al.*, 2012).**

Sweet oranges are prone to spoilage by filamentous fungi as a result of their high levels of sugars and low PH values. These fungi are known to produce toxins which are deleterious to human health. This study was therefore conducted to isolate, characterize and identify the filamentous fungi associated with the spoilage of sweet oranges sold in major Awka Markets, Nigeria. The fungi were identified as

Aspergillus niger, *Rhizopus stolonifer*, Mucor mucedo, *Penicillium digitatum*, *Fusarium oxysporum* and *Aspergillus flavus*. The percentage distribution of the fungi was 27.5%, 22.5%, 15.0%, 10.0%, 7.5 and 17.5% for *Aspergillus* niger, *Rhizopus stolonifer*, Mucor mucedo, *Penicillium digitatum*, *Fusarium oxysporum* and *Aspergillus flavus* respectively. Aspergillus niger caused the highest degree of spoilage. Good agricultural practices, adequate storage facilities and good handling practices must be put in place to reduce the incidence of these fungi in sweet oranges thereby minimizing their spoilage **(Onuorah, *et al.*, 2015) .**

An increasing need to find and implement alternative antifungal postharvest treatments as part of integrated management programs for disease control. Among alternative physical decay control methods, heat treatments are the most common and popular because they are relatively effective, simple, cheap, and easy to apply and combine with other control systems. In this article, research work based on the evaluation of heat treatments used alone or in combination with other physical, chemical, or biological methods for the control of citrus green mold . The most important postharvest heat treatments that have been tested against *P. digitatum* on fresh citrus fruits are curing, hot water dips (HWD), and hot water rinsing and brushing (HWRB). Typical citrus curing employs exposure of fruit for 2-3 days to an air atmosphere heated to temperatures higher than 30°C at relative humidity higher than 90%. HWD are generally applied as relatively brief immersions (1-5 min) in water heated to 40-55 °C. HWRB consists basically in packingline machinery that treats the fruit by the application of hot water over rotating brushes at a relatively high temperature (55-65 °C) for a short time (10-60 s). Efficacy results, general performance, modes of action, limitations, advantages and disadvantages, and commercial feasibility of these heat treatments are discussed **(Palou, 2013) .**

Erminawati Wuryatmo, (2011) . Showed that citral contains isomers and oxidation/reduction products volatile phase appeared to have potential as a commercial antifungal treatment of citrus fruit . Therefore , the activity of the Citral isomers as well as their related compounds in the vapour phase against spores of *P. digitatum*, *P. italicum* and *G. citri-aurantii* was examined in *vitro* to determine if differences in Citral composition are likely to influence its antifungal activity .

In vitro results suggested that volatile citral has potential to control citrus postharvest fungal spoilage, and application of Citral in the vapour phase as a fumigant may minimize its phytotoxic effect on the fruit . In addition , fumigation can easily be applied for relatively short periods in storage chambers or continuously over the long term within packages **(Williams *et al.*, 2000)** . Several fumigants have been used to control postharvest disease of citrus due to *P. digitatum* **(Chu *et al.*, 2000) .**

Bouzerda, *et al.*, (2003) . studied that a total of 46 yeast isolates, antagonistic to *Penicillium digitatum* Sacc., the causal agent of green mold of citrus, were isolated from the fruit surface of three citrus cultivars 'Clementine', 'Salustiana' and 'Valencia-late' from different orchards in the Souss Valley, Agadir, Morocco. The selection of antagonistic yeasts was based on their efficacy to protect artificial wounds inoculated by *P.digitatum.* Nineteen strains reduce the incidence of the disease by 50% or more compared to the untreated control and two of them (L13 and L22) limited the percentage of infection to 2%. The isolate L22 significantly reduced green mold on citrus during 30 days of storage at 4°C followed by 7 days at 25°C. The population of this isolate increased in wounds of citrus fruits at 25°C and 4°C and remained viable over a period of 30 days .

Edward Ntui O., (2015) showed that Post-harvest deterioration is a major problem of sweet orange (C. sinensis) production in Akwa Ibom State, Nigeria. Miicrobial infection of the fruits is mainly responsible. The present study was therefore, carried out to identify and biologically control the micro-organisms responsible for orange fruit rot during storage. Aqueous leaf extracts of Azadirachta indica and Chromolaena odorata were used as biological agents against fungal isolates. Samples of rotten orange fruits were collected from different markets across the state. Four fungal isolates (*Penicillium digitatum, Aspergillus niger, Aspergillus flavus and Cladosporium herbarum*) obtained from naturally infected fruits were confirmed to be causal agents through pathogenicity testing. Phytochemical analysis of the extracts revealed higher amounts of polyphenols, flavonoids, saponin, tannin and alkaloids in A. indica compared to C. odorata. In-vitro investigations showed that 30% concentration of A. indica leaf extracts caused highest mycelial growth inhibition of the four pathogens (70, 75, 83 and 88% respectively) compared

to the control, while extracts of C. odorata caused relatively lower inhibition of mycelial growth (50, 61, 61, 62% respectively) at the same concentration. Percentage inhibition increased with increase in extract concentration. These results indicate that aqueous leaf extract of A. indica is a better biocontrol agent of post-harvest orange fruit fungal diseases. Further studies are ongoing to test the validity of these results in the field .

CHAPTER THREE

3. MATERIAL AND METHODS

3.1 The site of study :

The Benghazi city was selected for site of study , local vegetables and fruits markets at different location have been selected inside and outside (suburb) Benghazi city e.g. Bohady , Sedikalifa markets , and Bodesera .

3.2 Sample collection :

At the begnin total 40 kg from different citrus varieties have been randomly collected , starting from first of December 2016 until the end of May 2017 . 10 kg of each citrus varity have been taken including Orange (Sweet , Navel , Sour and Blood) , Mandarine , Lemon , and Grapefruits . Immediately the collect fruits samples were kept in sterile plastic bag and sealed to prevent contamination and loss of moisture . All collect fruits were storaged at room temperature 27 ±1 until running the experiments .

3.3 Screen the fruits samples :

After weighted of all citrus fruits samples of the above previous varities (3.2) visioual screening and respection have been done to separate the infected and injuried fruits from the healthy one .

3.4 Isolation , cultivation and Identification

3.4.1 Source of pathogen :

Spores of growing Penicillium species was isolated from the surface of infected citrus fruits which collected previously (3.2) by using sterilized needle few spores have been transfered from infected site (blue and green colored) and placed in the center of the 9 cm petri dish . Four different selective media were chosen : (Sabroid Agar "SDA" , Potato Dextrose Agar " PDA" , Molar Agar , and Nutrient Agar) to test the suitable substrata for Penicillium growth . The used media was supplemented with chloramphenicol (250mg per liter) as a bacteriostatic agent **(Dawson and Ito , 2001)** .

Three replicates were used from each previous media after inoculated with Penicillium spores . All plates were covered with cellophane to prevent contamination with another microorganism and kept at 27°C for one week after that the petri dishes were transferred to the reifridge to stop fungal growth **(Kreuawab, *et al.*, 2007)**.

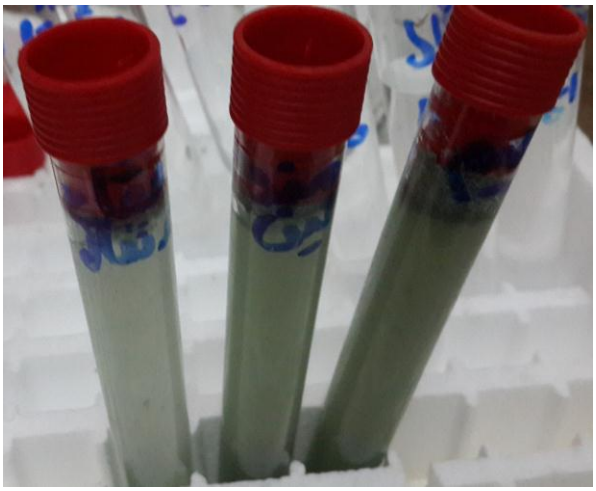
(Fig. 3.1) Grapefruits infected with green mould

3.4.2 Inoculum preparation :

After one week of incubation growing of *Penicillium spp.* was growing on Sabroid Agar and forming small separate colonies. Almost harvested all the spores were harvested from the on the surface of the media using sterile glass rod and putted it in a clean test tube contain 10 ml of sterile distilled water then closed and mixed well (Original inoculum) . The density of spores suspension was equivalent to approximately 10^{-9} spores / ml **(Palou, *et al.*, 2001) .** From original inoculum three serious dilution have been preparated (10^{-1} , 10^{-2} , 10^{-3}) **(Edward Ntui , 2015)** number of spores suspension have been determined for each dilution , by putted 1 ml from each dilution on clean slides and calculated under the microscope .

e.g : The preparation of first dilution (10^{-1} spores/ml) **:-** Putted 9 ml sterile distill water in clean test tube and added 1 ml from original inoculum then closed and mixed well . In our study each group from samples injected with different inoculum (isolate) , some groups injected with inoculum from lemon , another groups injected with inoculum

with orange , grapefruits respectively to investigate all of these inoculum given same symptoms or non (Fig. 3.2) .



(Fig. 3.2) Three types of serial dilutions

3.4.3 Pathogenicity test and artificial inoculation :

A total 320 matured healthy citrus fruits which have been collected (3.2) from studied fruits were by washing 160 fruit samples with chlorox 1% in 10 liter sterile distal water and 160 non washed 80 samples from each one saved in reifrigeter at (5°C) for (10) hour and another 80 samples keeped at room temperatures for 10 hour and all of these samples were wounded by sterile metal cork borer , 3 holes with 1cm deep on 40 samples have been made and another 40 samples bored 2cm deep in juice sacs in 3 different sites in middle and lower part of each single fruit and all of 320 samples injuried in 2 sites in the peel upper and middle **(Downes and Ito , 2001) .** All injured or wounded fruits were artificially inoculated by three methods (Fig. 3.3) .

3.4.3.1 First method (direct injection of inoculum) :

107 samples inoculated with first dilution (**10^{-1}**) and another 107 samples inoculated with second dilution (**10^{-2}**) and last 107 samples inoculated with third dilution (**10^{-3}**) , **(Edward Ntui O., 2015)** injected 1.5 ml from each dilution in to the holes and peel injuries by sterile syringe , the point of inoculation was sealed with petroleum jelly (sterile Vaseline) to prevent contamination . The inoculated fruits were then placed in carton boxes to create a humid environment to indice rots and incubated at 27°C for 7

days with daily observation for symptoms . A control was also setup for each citrus fruits

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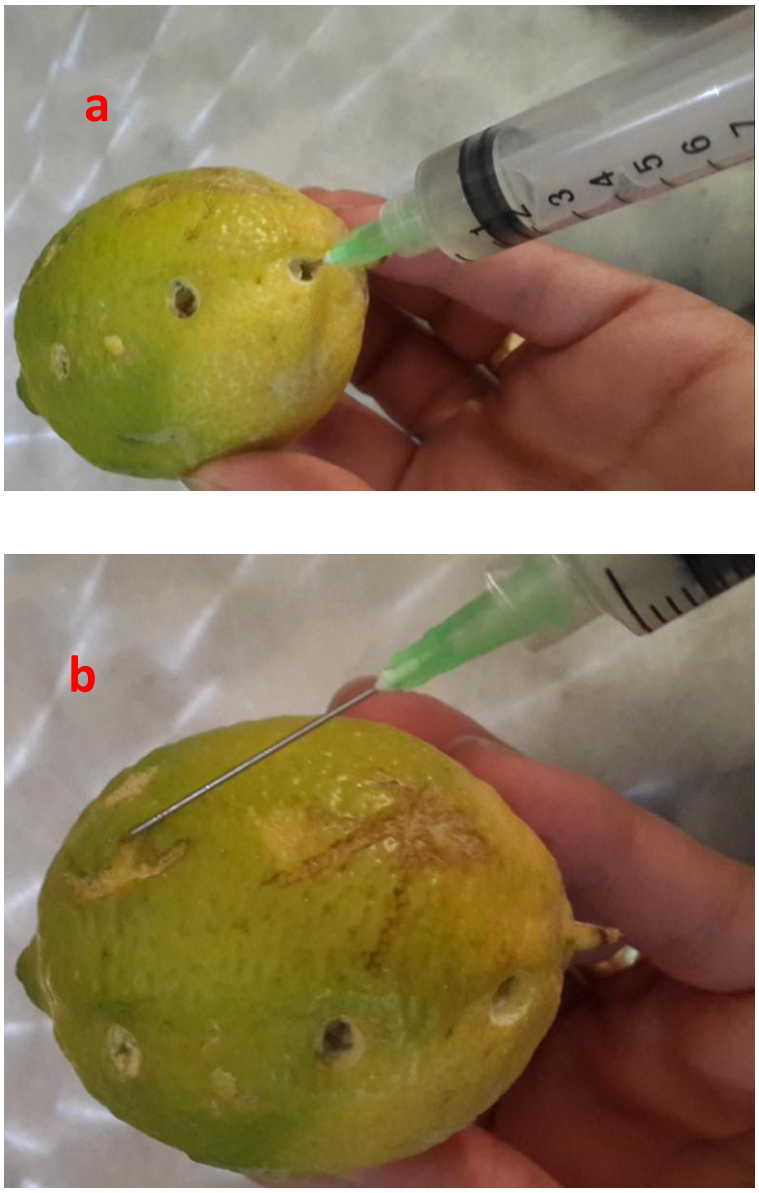


(Fig. 3.3) Artificial inoculation with direct injection by sterile syringe
a : In the hole and b : In peel surface of the healthy lemon fruit

3.4.3.2 **Second method** (spray the inoculum):

Take 4 samples from each types of fruits studies , 2 samples injuried by sterile knife in all direction in peel and juice Sacc and another 2 samples wounded with the same way which maintained previous then spray all samples with the first dilution (**10^{-1}**) conidia / ml the point of the inoculation sealed with sterile vaseline then put the samples in carton boxes to create a humid environment to stimulate moulds and keep it to incubated at 27°C for 7 days with daily observation for symptoms . A control was also setup for each samples without injuried and spray with the dilution (Fig. 3.4) .

(Fig. 3.4) a : Artificial inoculation by spray dilution
b : Injuires by sterile knife , c : Injuires by sterile metal borer .

3.4.3.3 **Third method** (contaminated by spore of *Penicillium spp.* directly)**:**

Take 2 samples for each type from studied fruits and wounded with the same way which maintained previous then used sterile needle with cotton to take spores from *Penicillium* growth on petri dish directly and putted these spores in the wounded then sealed point of inoculation with sterile vaseline and put the samples in carton boxes to create a humid environment to stimulate rots and keeped to inoculated at 27°C for 7 days with daily observation for symptoms . A control was also setup for each citrus fruits .

In each method used different source of *Penicillium* isolated from different type of citrus fruits to preparation inoculum sources (Fig. 3.5) .

(Fig. 3.5) Artificial inoculation by direct put of spores .

3.4.4 Test the affectivity of different artificial inoculation methods :

In this experiment three methods of artificial inoculation were applied on some healthy samples of citrus fruits after wounded (3.4.3) as following : firstly direct injection with three type of *Penicillium* spore suspension , Secondly spray fruit surface with first dilution (**10^{-1}** spores/ml) , Thirdly putted *Penicillium* spores directly from old *Penicillium* growth in petri dish . This study to known what the best and quick method causing infection or all of these experiment will appositely in the infection mechanism , to test the arrival way of inoculum and detect the amount of spores needed to cause initial infection.

3.4.5 Identification of fungal genera and species :

The pure culture of Penicillium isolated were identified following the most recent text book and documented keys in fungal classification **(Raper and Fennell , 1965 ; Pitt , 1979 and Domsch, *et al.*, 1980) .**

3.4.6 Morphology and microscopic characteristics of Penicillium isolates on culture .

The obtained isolation of Penicillium after growing on Sabroid agar was confirmed by morphological and cultural characters including : Colony , colour , shape , margins and pigmentation , while microscopic texture was Mycelium colour , shape , septation , branching , the conidia colour , shape , size and septation by stained with methyl cotton blue **(Pitt , 1979 and Fallik, *et al.*, 2000) .**

3.4.7 Effect of cooling period – room temperatures – air atmosphere and infection severity on growth of *P. spp* :

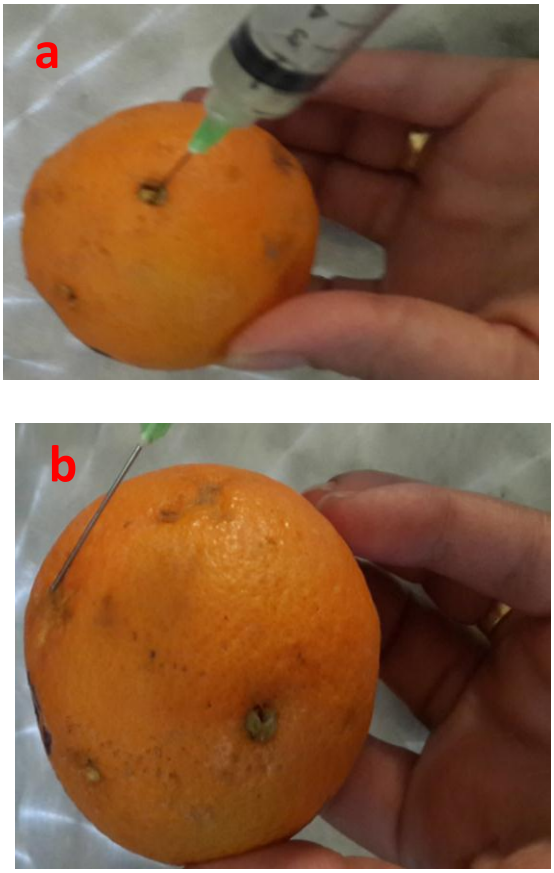
Around sample of fruits were studied after putted in reifridge at 5°C for 10 hours and another samples kept at a humid and room temperature for 10 hour then all of these samples injuried and injected with 3 different dilutions of *Penicillium* spore suspension 10^{-1} , 10^{-2} and 10^{-3} spores/ml , which explained before (3.4.2) , and another samples from each type of studied fruits putted in air atmosphere after injuried only without injection (artificial inoculation) with pervious dilutions . This experiment will research

the effect of different temperatures degrees on growth of *Penicillium* and infection severity (Fig 3.6) . In all experiments in our study used samples from each citrus varities as control (without injuries and artificial inoculation) .

(Fig. 3.6) a: Storage of studied citrus fruits at 5°C for 10 hours
b: Storage of studied citrus fruits at room temperature for 10 hours .

3.4.8 Test the different wounds depth and location on Penicillium growth :

This experiment was carried out by making five wounds on tested all citrus fruit surface by done two wounds in upper and middle part of peel and 3 holes in the upper , middle and lower part of juice sacs . With two depths 1cm and 2cm by using sterile metal borer , in this experiments measured the thickness of peels for all studied fruits .This test was objected to investigate the wound depth in juice sac and location on fruit peel are appositeness and susceptible for grown and activity of inoculated *Penicillium* spores . Moreover , to detect *Penicillium spp*. become more active when reached and close contact to the peel or near to the central axis on the juice sacs , also to known which thickness of peel more suitable for growth and activity *Penicillium spp*. (Fig. 3.7) .



(Fig. 3.7) Artificial inoculation by direct injection of inoculum .
a : In the hole of juice sac and b : In peel surface of the healthy orange fruit

3.4.9 Study the natural micro flora found on fruit peel surface of fruits and how the effect on Penicillium growth .

In this study 10 samples from each citrus fruit were selected randomly from each citrus varities (3.2) , five fruits were washed with 1% chlorox in 10 liter sterile distile water and the another five samples unwashed . All samples were injuried and inoculated with the same methods which maintained previously (3.4.3) all fruits were placed in clean carton boxes to create a humid conditions and kept at room temperature 27°C for 7 days .

To identification unknown microflora found on the peel of tested fruit samples .Washed 3 samples from all studied fruits with sterile distile water only , the washed suspension from all types of studied fruits with S.D.W kept in closed sterile test tubes , seven petri dishes of Nutrient Agar were prepared containing 1 ml of extracted microflora suspension was added to the surface of solid Nutrient Agar media to each single plates and distributed in zigzag fashion . All inoculated petri dish were kept at 37°C for 24 hour . Waiting the growth for these unknown microflora . Reisolation was take place by using

selective media (Macconkey agar) and to distinct and recognize the growing isolated microflora . Identification was carried out by using phonex™ system , for bacteria and for fungi identification species which have been expected in natural flora as (3.4.5) .

This study aimed to explain the roles of wild natural microflora on growth and activity of *Penicillium spp*. either stop , stimulate or delaying the artificial infection processes .

3.4.10 Study effect of juice extract from citrus fruits on growth and activity of *Penicillium spp*.

Preparation of juice from peels , sacs and whole fruit were carried out from seven healthy fruit samples mention in (3.2) . Each fruit peels , sacs and whole fruit were separated and cutted to small peaces then putted in blender to obtainer extracts and transfered to sterile plastic container for each types of juice . By using sterile cork borer we transfer 1 piece from *Penicillium* colonies to each juices in plastic container to contaminated and kept the plastic container at room temperature for (7) days with daily observation (Fig. 3.8) . The aim of study to investigated which part of the fruit more suitable for growth the fungus isolated .

(Fig. 3.8) Contaminated three types of juice with *Penicillium* growth .
a :The Lemon , b : The Blood Orange .

3.4.11 Modification of hydrogen ion concentration (pH) in healthy tissue of citrus fruits to study infection density mechanism of *Penicillium spp.*

In this tested some compound were used to change and modify the natural pH of host tissues . Three types of compounds were used first by natural pure and solution of apple vinegar , second by solution of sodium bicarbonate and thirdly by solution of sodium chloride and finally used sterile distilled water as control, determination pH three materials by using strip detector .

3.4.11.1 Pure apple vinegar is done by two ways :

First way by direct injected 2 ml of concentration apple vinegar 4-5% pH (4.5) in each inject 0.2 ml by sterile syringe in all type of fruits studied , kept fruits for absorption the apple vinegar for 7-10 minute and then injuried and contaminated with methods mention in 3.4.3 .

Second way by prepared solution from 50 ml apple vinegar 4-5% pH (5) in 50 ml S.D.W in glass flask then mixed well and injected studied samples with 2 ml by sterile syringe in each inject 0.2 ml then repeat work maintained previous .

3.4.11.2 The second material solution of sodium bicarbonate (NA2HCO3) pH (8) by adding 13 gram sodium bicarbonate to 50 ml S.D.W in glass flask then mixed well and repeat work mention above .

3.4.11.3 The third material solution of sodium chloride (Nacl) "salt food" pH (7.5) by adding 10 gram Nacl to 50 ml S.D.W in glass flask then mixed well and repeat work mention above . A control for this study S.D.W pH (7) applied same work maintained previous .

3.4.12 Study the effect of rapaid lignin formation of injuried citrus fruit peel on Penicillium infection .

Injuried all 7 studied fruits in peels only with sterile metal borer then directly covering the injuried with sterile plastic and keeped these fruits for 12 hour then inject the injuried with first dilution (**10^{-1}**) conidia / ml from inoculum source and keeped to incubated in

carton boxes to create a humid environment at 27°C to stimulated the injuried cell to produce lignin before fungal entry to delayed or prevent infect (Fig. 3.9) , **(Eldon Brown and Ismail , 1980)** .

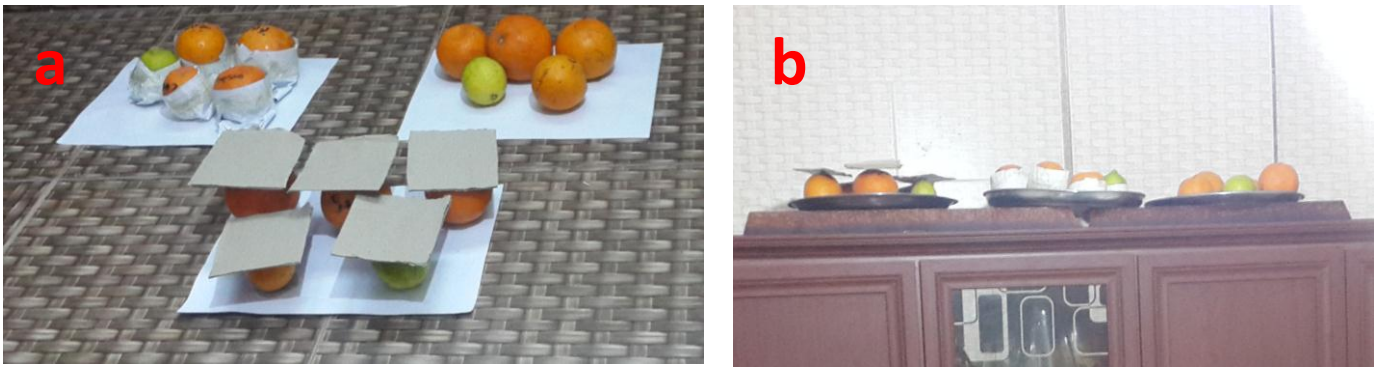
(Fig. 3.9) Covering point of injuries by sterile plastic cover for 12 hour.

3.4.13 Study epidemology and aerodynamic mechanisms of post-harvest green mold disease in vitro .

These experiment were applied through collecting 1kg of healthy citrus fruit varities (3.2) . Mechanical wounds were done by injuries the peel surface with sterile metal cork borer . Each type of these injuired fruits were covered from the upper surface with carton and the another covered from sides with clean paper cheet then kept in room temperatures . Half of fruit samples were placed at distance 2.5 meter high from room surface and the another half samples putted direct in the room surface on clean paper cheet . Inoculum source of *Penicillium* spores were putted as full open petri dishes containing full growing Penicillium colonies in the ground center surface exposured directly to the air current for 2days after that the exposure fruits will kept for 7 days with daily observation (Fig. 3.10 and Fig. 3.11) .

This current experiment was aimed to detect the aerodynamic of *Penicillium spores* and to know the effect of graphity on spore movement and distribution vertically or horizontally .

(Fig. 3.10) a : Cover fruits from sides in the initial experiment .
b : Cover fruits from upper surface .

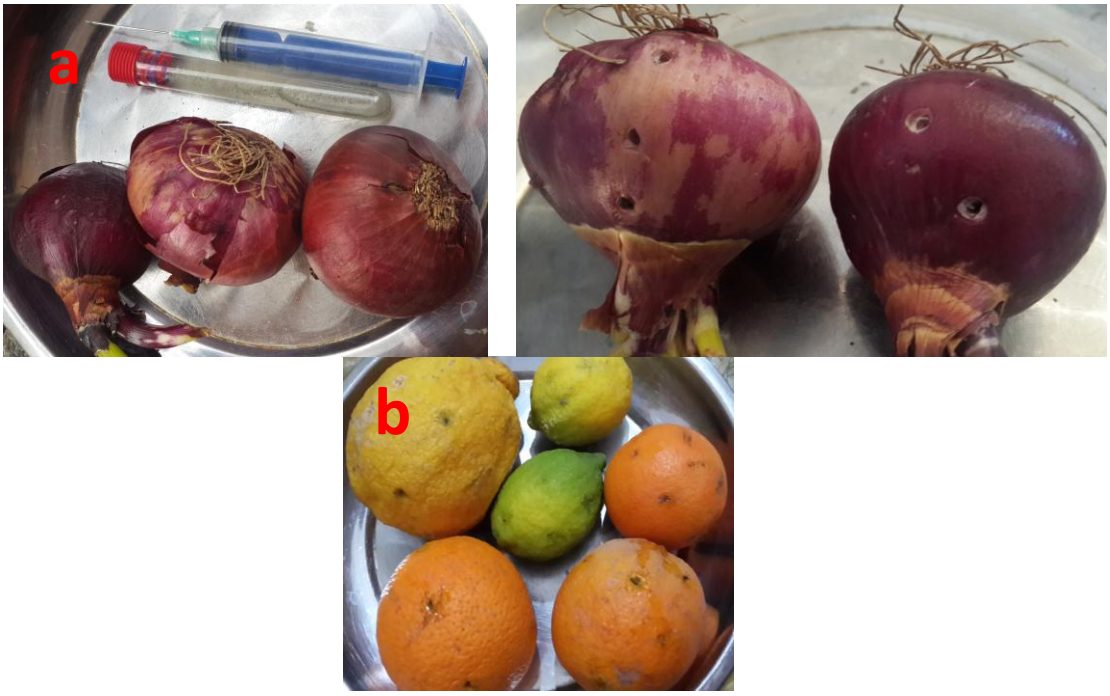


(Fig. 3.11) a : Samples in ground surface .
b : Samples putted in the distance about 2.5m high .

3.4.14 Study the specificity of different *Penicillium* isolates from infected vegetables fruits on citrus fruit infection .

Rotted onion (*Allium cepa*) bulbs were collected from vegetable Benghazi market infected with *Penicillium allii* , isolation of few spore by using sterile needle cultured on 9cm petri dishes containing Sabroid Agar then incubated at room temperature . Then three serious dilution have been done from *Penicillium .spores* (10^{-1} , 10^{-2} , 10^{-3} spores/ml) , three wounded samples for each type of studied fruits were injected with these prepared dilutions of *Penicillium spores* (10) with the same previous method (3.4) . The same procedures were repeated to the healthy onion bulbs through injected of onion bulbs with *Penicillium digitatum* spores isolated from infected citrus fruits . Inoculated onion and citrus fruits infected at 27°C for 7 days with daily observation . This

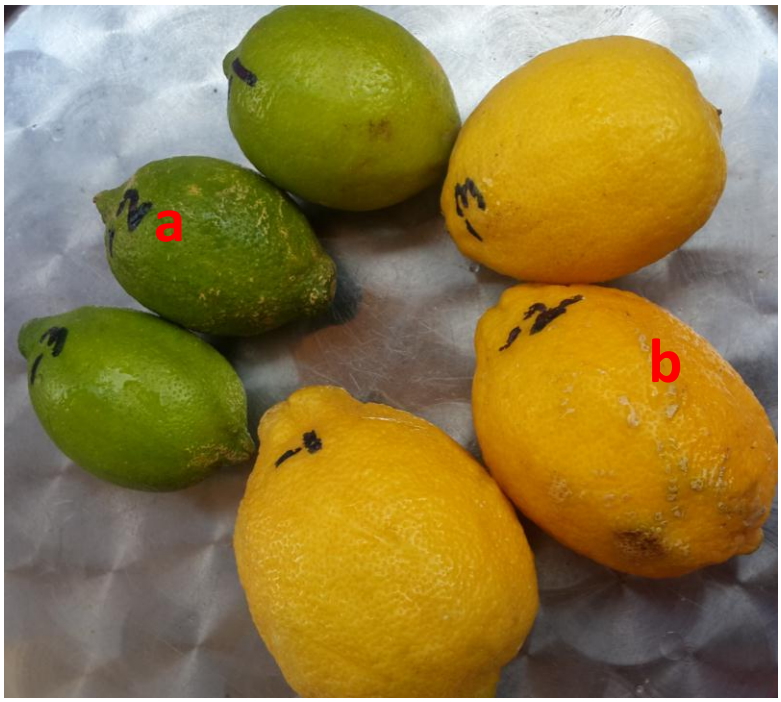
experiment done to improve the infection specification of *Penicillium* isolates to differs fruits samples (Fig. 3.12).



(Fig. 3.12) a : Samples of Onion injected by *Penicillium digitatum* .
b : Samples of citrus fruits injected by *Penicillium allii* .

3.4.15 Study effect of maturity stages of citrus fruits on infection severity .

In this study 1kg of lemon fruit samples were collected from vegetable market showing differences in maturity colour yellow and green colour . The same artificial inoculation methods are repeat as mentioned previously . The inoculated injuired lemon fruit samples were inoculated at room temperature 27°C with daily observation . The aim of this study to explain the best time of harvesting **(Lawal , *et al.*, 2013)** to avoide infection and prelonging shelf of life of citrus fruit to become healthy (Fig 3.13) .



(Fig. 3.13) a : Immature lemon fruit (green color) ,
b : Mature lemon fruit (yellow color) .

3.4.16 Study the interactions between different isolation of *Penicillium* species in *vitro* :

In this study different isolate *Penicillium spp.* were used growing on Sabroid Agar media by using sterile cork borer we take from colony margin of *Penicillium* isolate from navel orange , sour orange , mandarin and lemon isolate was used as competitor for reaction with the another previous isolates . The distance between two discs about 1.5cm . The same experiment was repeated by using grapefruits isolate as competitor which compared with fruits mention above , All plates were kept investigate at 27°C for 7 days with daily observation . The aim of study to investigate the interactions between different isolates on solid media (Sabroid Agar), to detect all these seven isolates dependently to the same or different genus and species (Fig. 3.14) .

(Fig. 3.14) Two different isolates of *Penicillium* species on Sabroid Agar .

3.4.17 Control of *Penicillium* green mold by using two physical methods (hot water and sea water) , two natural oils , mineral oil and wax .

At the beginning of this control experiment , media preparation from Sabroid Agar mixed with sea water instead of S.D.W , after sterilization media was pured in 9cm petri dishes . 7 plates were cultivated with 7 isolates from previous citrus fruits varities (3.2) . All inoculated plates were placed at room temperature 27°C for 7 days with daily observation .

3.4.17.1 Test of olive oil , Eucalyptus oil and mineral oil (Johnson's baby)

The total tested fruit samples were 48 fruits represented from sour , navel orange , lemon and grapefruit . These samples fruits were divided into two parts . The first part fruit samples injured and contaminated with method mention in 3.4.3 then anointed with oils : olive oil , Eucalyptus oil and mineral Johnson's baby oil .

The other part of fruit samples were anointed with previous oils then injuried , contaminated and inoculated for 7 days at 27°C with daily observation .

3.4.17.2 Test of hot water :

The total 16 samples from fruits mention in 3.4.17.1 divided into two parts , first part of fruits injuried , contaminated as 3.4.3 , then soaked in hot water for 5 minute at 45 – 50°C , then removed these fruits from hot water , the another parts soaked in hot water firstly for 5 minute at 45 – 50°C , then removed from the hot water and kept to dry then injuried , contaminated and kept for inoculated for 7 days at 27°C with daily observation .

3.4.17.3 Test of using sea water :

By used 16 samples from fruits mention in 3.4.17.1 divided into two parts , first part of fruits injuried , contaminated as 3.4.3 , then soaked in sea water for 3 hours , then removed these fruits from sea water and kept to dry , the another parts soaked in sea water firstly for 3 hours firstly , then removed the fruits from the sea water and kept to dry then injuried , contaminated and kept for inoculated for 7 days at 27°C with daily observation .

3.4.17.4 Test sterile wax :

By used 16 samples from fruits mention in 3.4.17.1 divided into two parts , first part of fruits injuried , contaminated as 3.4.3 , then covering with liquid sterile wax , second parts of fruits covering with liquid sterile wax firstly , then injuried , contaminated and kept for inoculated for 7 days at 27°C with daily observation .

CHAPTER FOUR

4. RESULTS

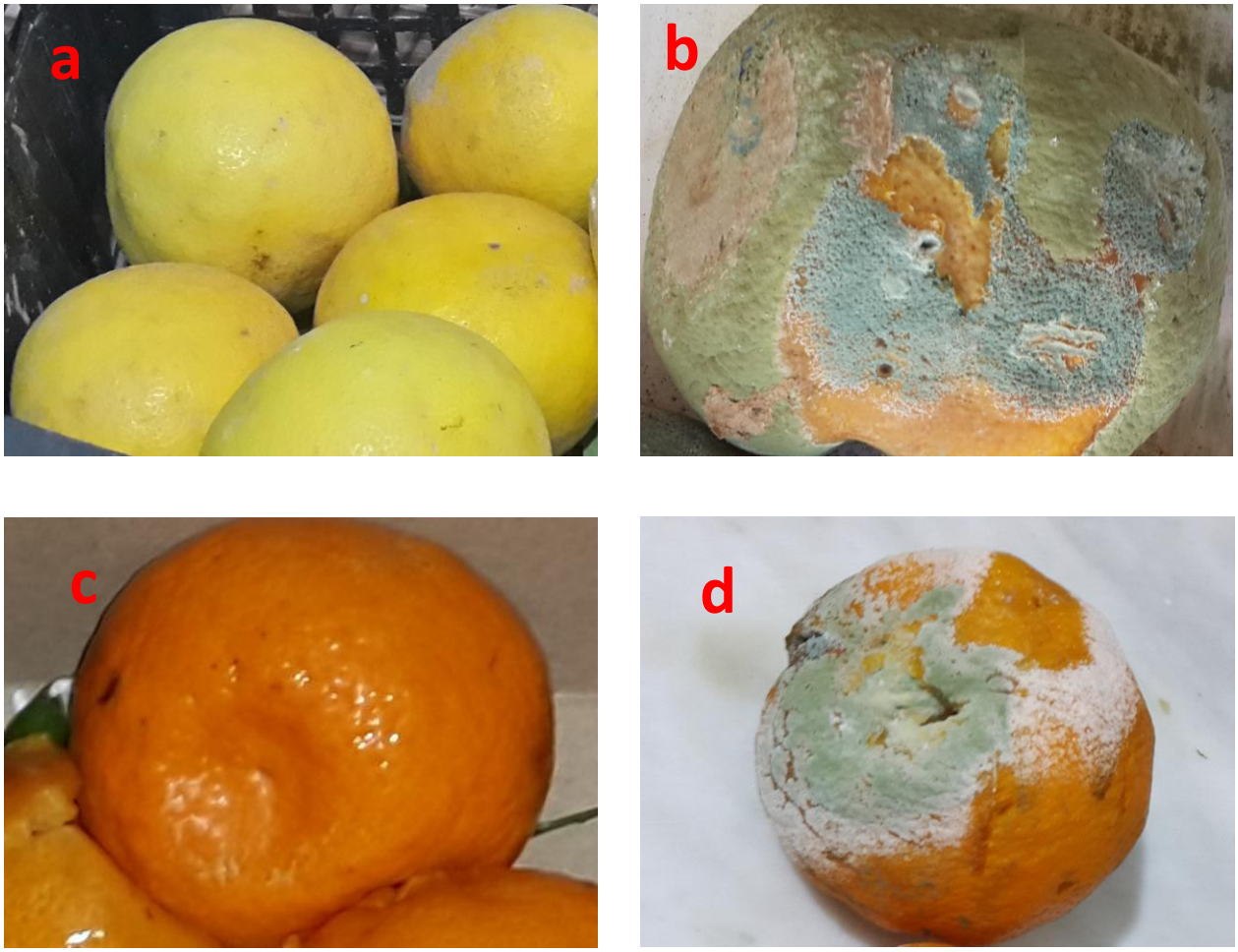
4.1 Visual screening of tested citrus fruit samples .

The total number of four citrus fruit varities which including : Orange varities (Blood , Sweet, Sour and Navel) , mandarine , lemon and grapefruits . From macroscopic investigation of the previous citrus varities Table (4.1) and Fig (4.1) show that the lowest infection of tested citrus fruits were (1/70) in sweet orange and the highest were in Navel and Blooder orange fruits (5/70) from the results of visual screening .

Table (4.1) Citrus varity , number of non infected and number of infected fruits .

No	Citrus varities	Number of Non infected fruits	Number of Infected fruits
1	**Orange (*Citrus sinensis*)**	**------**	**------**
1.1	**Blood orange (*Citrus sinensis*)**	**65**	**5**
1.2	**Sweet orange (*Citrus sinensis*)**	**69**	**1**
1.3	**Sour orange (*Citrus aurantium*)**	**68**	**2**
1.4	**Navel orange (*Citrus sinensis*)**	**65**	**5**
2	**Mandarin (*Citrus reticulata*)**	**67**	**3**
3	**Lemon (*Citrus Limon*)**	**66**	**4**
4	**Grapefruits (*Citrus Paradisi*)**	**66**	**4**

Total number of citrus fruit from (70) by weight 10kg



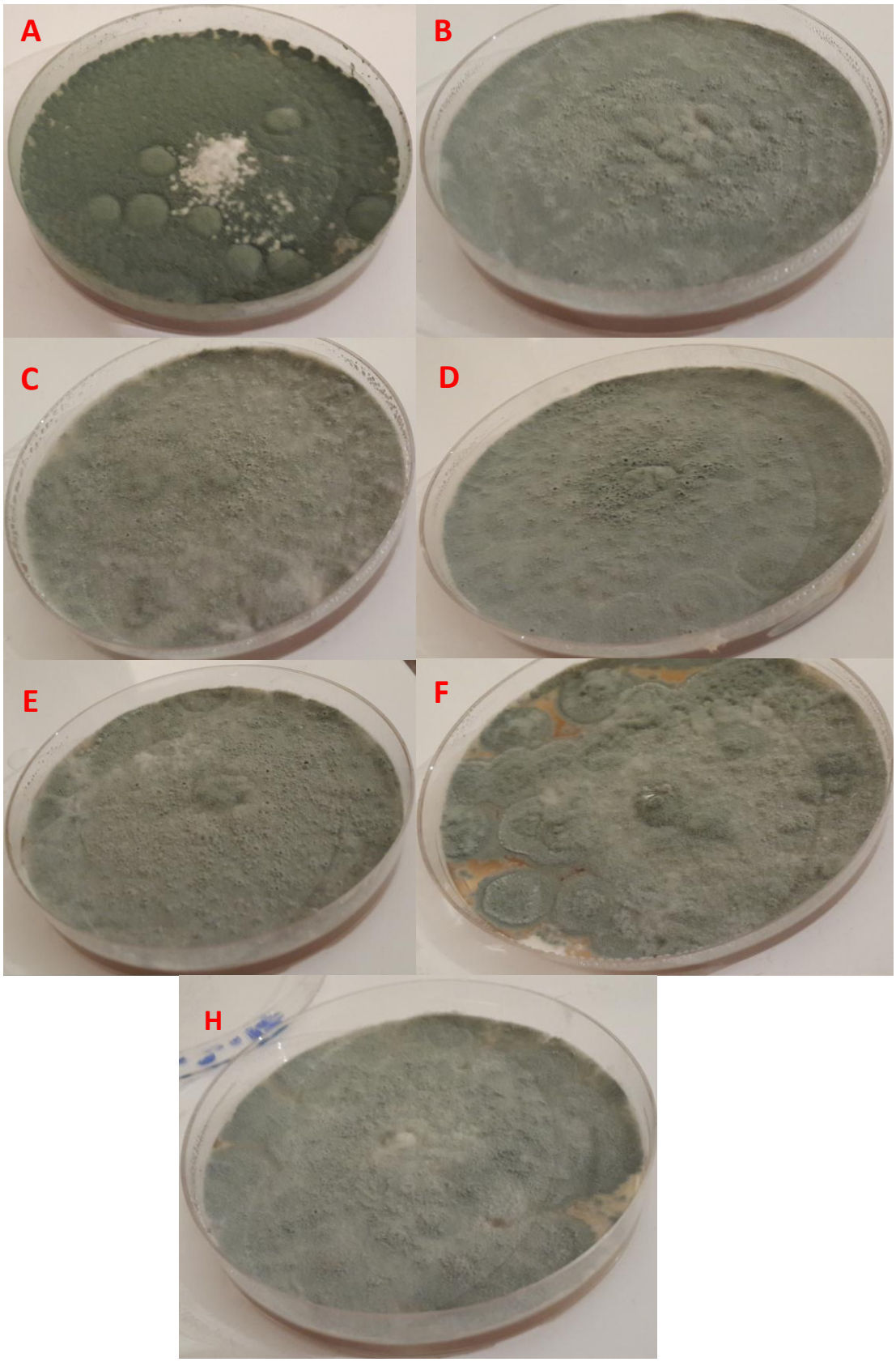
(Fig. 4.1) a: Non infected grapefruit , b: Infected grapefruits with *Penicillium spp.* , c: Non infected Mandarine , d: Infected Mandarine with *Penicillium spp.*

4.2 The result of cultivation of seven *Penicillium* isolates on four types of artificial media .

Four different types of artificial media were selected : Sabroid Agar , Molar Agar , Nutrient Agar and Potato dextrose Agar , to get the good of best growth of seven different isolated of *Penicillium spp.* inoculated under the same condition Table (4.2) and Fig (4.2)

Table (4.2) Four types of used media and resulted *Penicillium* growth .

No.	Type of media	Growth	Incubation time at 27°C
1	**Sabroid agar**	**Best growth**	**7 - 10**
2	**Nutrient agar**	**Slightly or Slow**	**7 - 10**
3	**Molar agar**	**Slightly or Slow**	**7 - 10**
4	**Potato Dextrose Agar**	**No growth**	**7 - 10**



(Fig. 4.2) Growth on Sabroid agar .
A : Grape fruits, B : Sour Orange, C : Sweet Orange,
D : Blood Orange, E : Lemon, F : Mandarine, H : Navel Orange .

4.3 The results of the artificial injection of healthy four citrus severities in *vitro* .

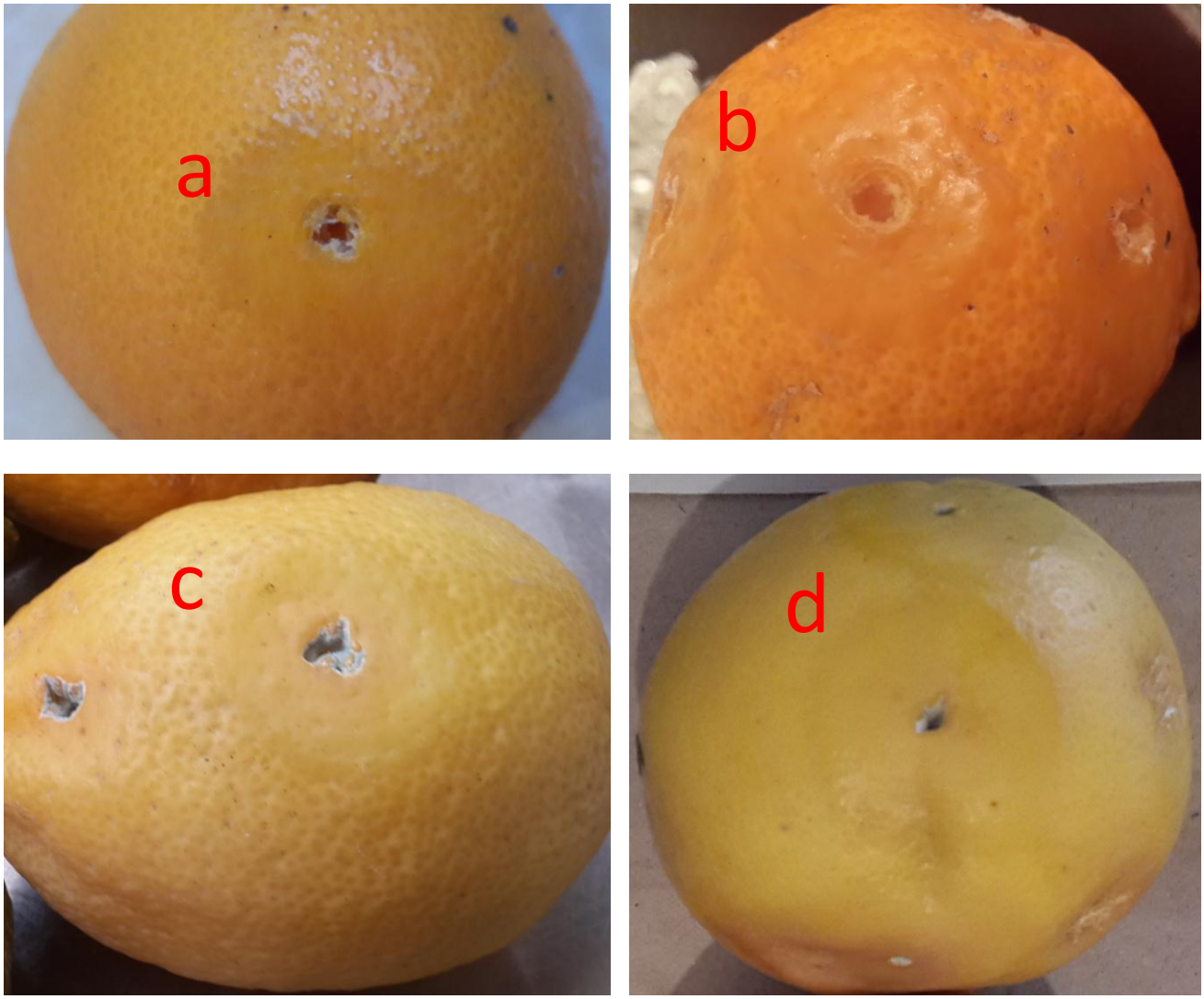
Three types of artificial contamination were used . The first method was direct injection of healthy citrus fruits , secondly by using spray of first dilution of spore suspension (**10^{-1}** spores/ml) , thirdly direct contamination of citrus fruits by fresh growing spores .

4.3.1 The results of the direct injection with different inoculum density of *Penicillium spp.* spores .

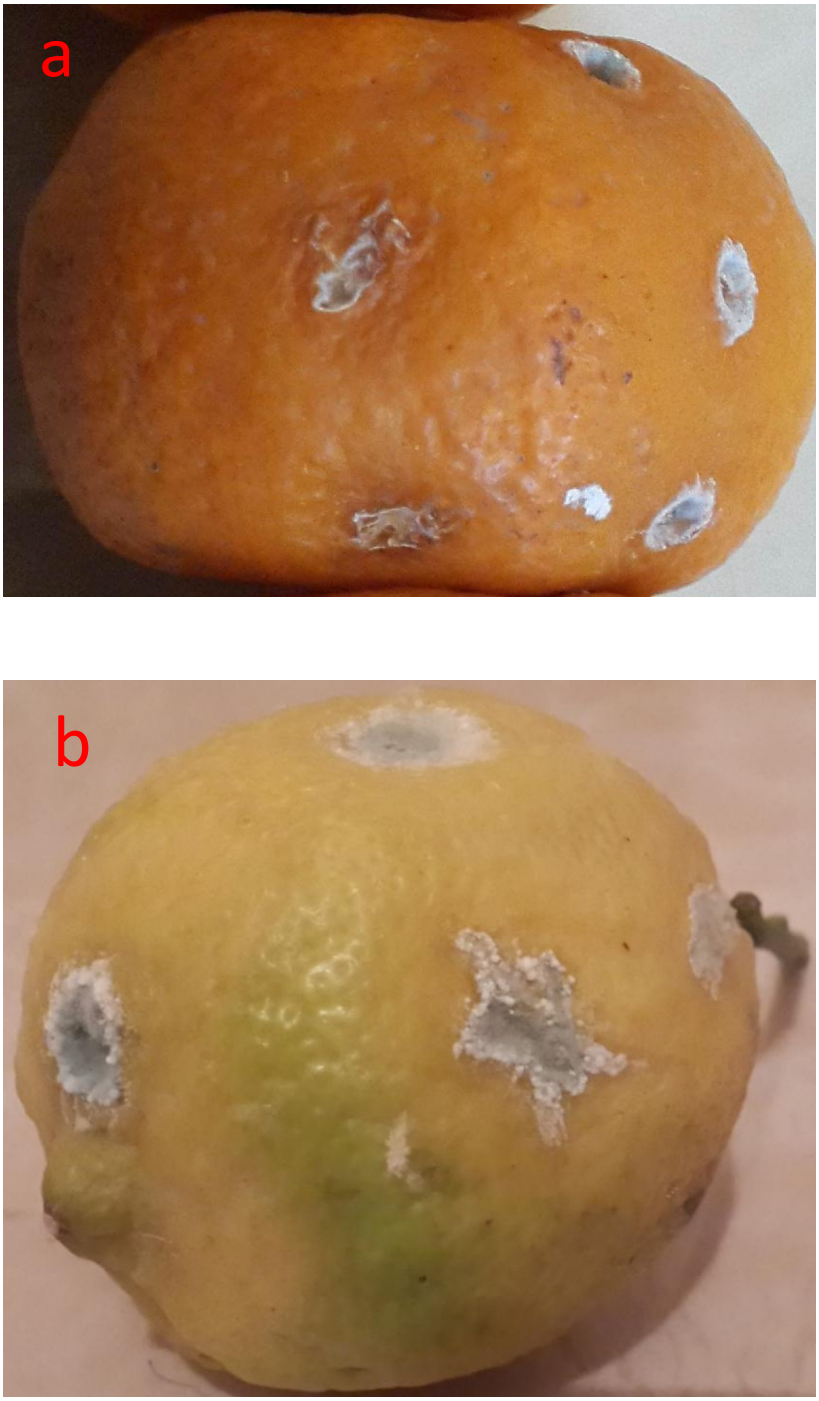
The results of the experiment (4.3) with three different types of artificial contamination as mention in 3.4.3.1 , 3.4.3.2 , and 3.4.3.3 appears the same obtained result on all the four citrus varities in the typical symptoms to infect by mould caused by *Penicillium spp.* and there is different appears in the time period to manifestness initial typical symptoms by three types inoculum source **10^{-1}** , **10^{-2}** , **10^{-3}** spores/ml with disperse 12 hour between them .

The early infection area (Initial symptoms) appears after 48 hour by first dilution and after 60 hours by second dilution and 72 hours by third dilution . By the second method of artificial contamination after 8 days (metal borer) 12 days (sterile Knife) and by third method of artificial contamination after 7 day , as a softening of the tissue about the point of injection after 48 hours in Mandarine and Grapefruit , in the third days in Blader , sweet , sour , navel and in fourth day in the lemon which turn in to water soaked area and slightly discolored spot from ¼ to ½ inch (2.5 cm) in diameter then the softening watery spot developed after 1 to 1.5 days at 27°C±1 to weight mycelia appears in the surface which produced olive green spores when the grown fungal is about 1 inch in diameter then developed after one day to green fungal growth but retains margin is white (10 – 20) mm , then increased the fungal growth area gradually on the fruits surface to spread the olive green spores to covered all fruits from 10 – 12 days then the spores developed to hypha growth from 13 – 19 days , then appears from 3 – 9 integrated white circles with cotton textures and the last symptoms in all infected fruits becomes

more softening and laxly with black color and they have bad smell , all of inoculum source used in our study given same symptoms (Fig. 4.3 to Fig. 4.11) .

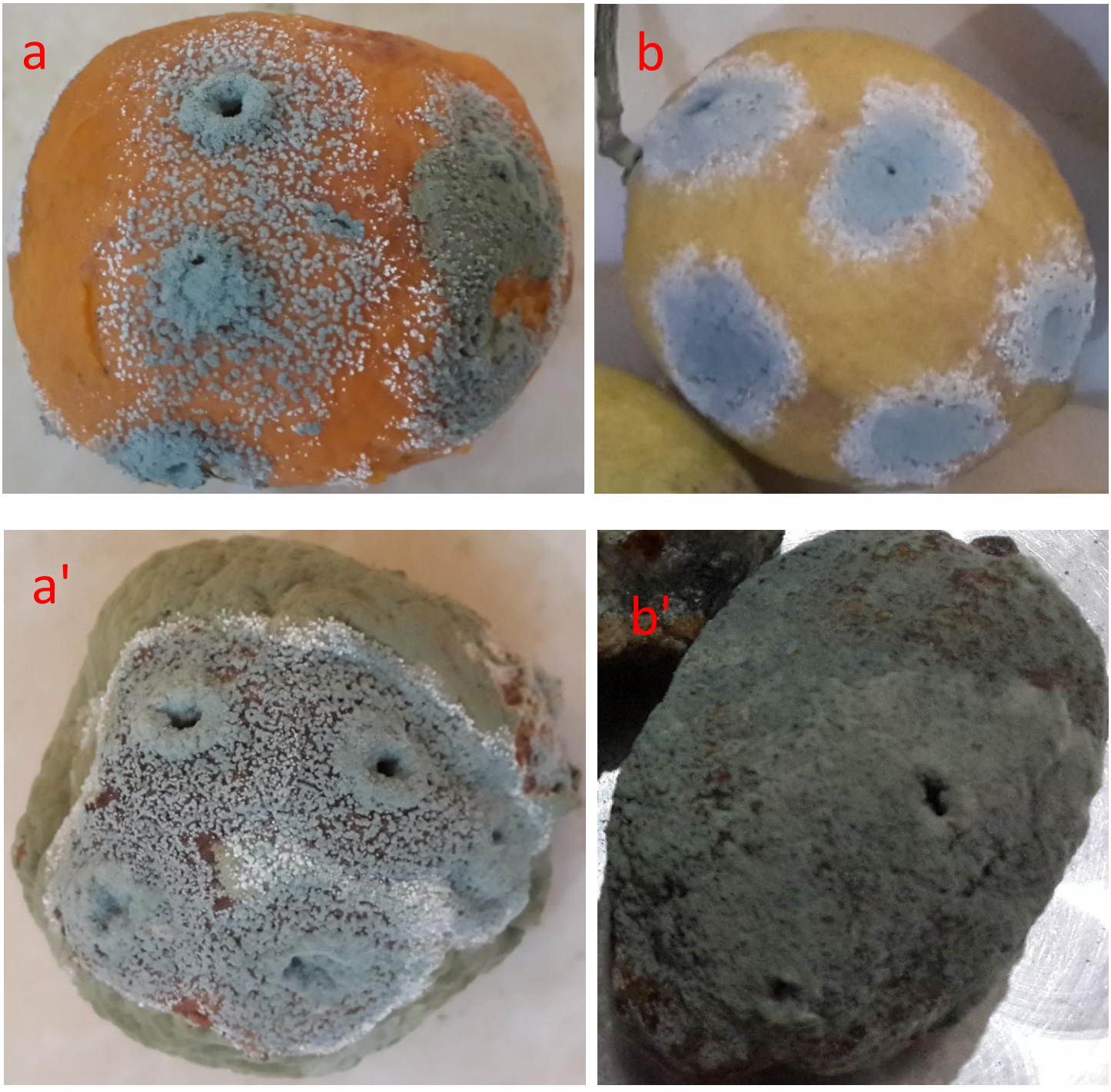


(Fig. 4.3) Water soaked area , a : In Orange , b : In Mandarine , c : In Lemon fruit and d : In Grapefruits .

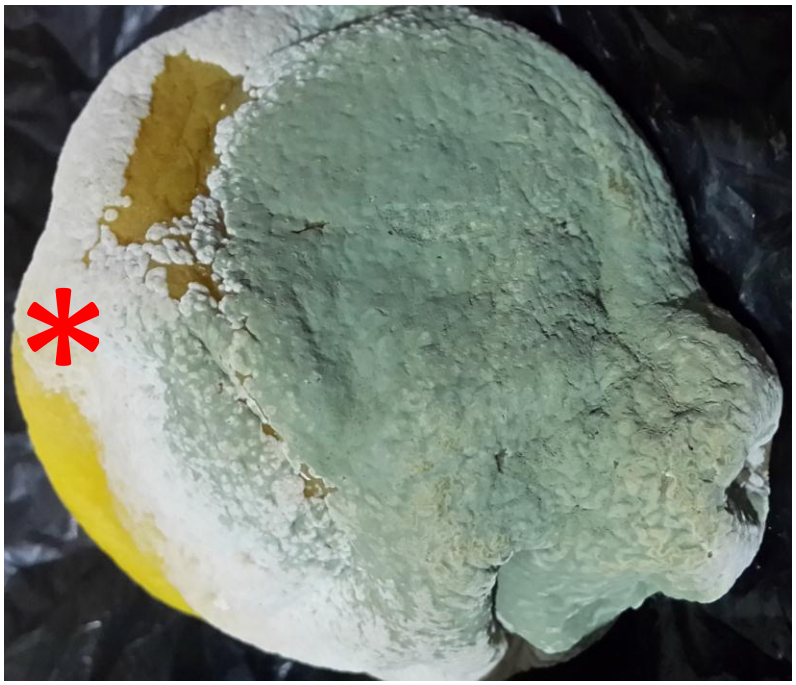


(Fig. 4.4) Appearing the white mycelia after inoculation with *Penicillium spp.*
a : After three day on Mandarine and b : After 5 days in Lemon .

(Fig. 4.5) Production of olive green spores by injected *Penicillium spp*. on a : Mandarine after 4 days and b : Lemon fruit after 6 days .

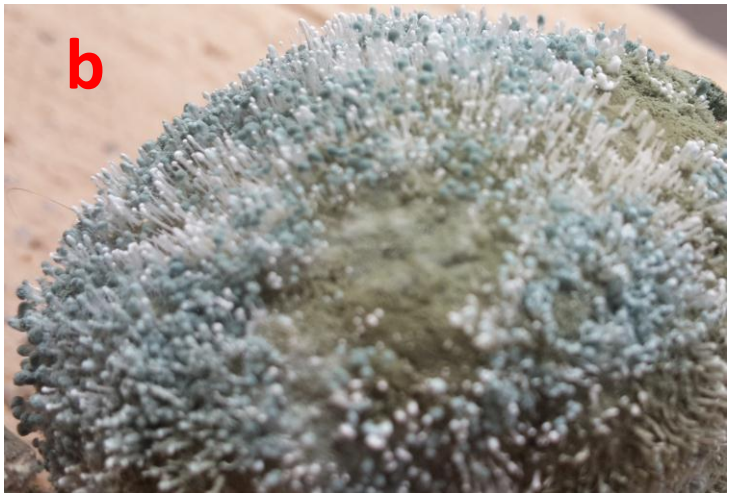


(Fig. 4.6) Gradually spread of *Penicillium spp.* growth with retain white margin
a , a' : In mandarine from (5 – 7 days) , b , b' : In lemon from (6 – 8 days)



(Fig. 4.7) Gradually spread *Penicillium spp.* growth with retain white margin (*) in Grape fruits after 7 days .

(Fig. 4.8) Gradually spread *Penicillium spp.* growth with retain white margin (*) in Navel orange after 7 days .



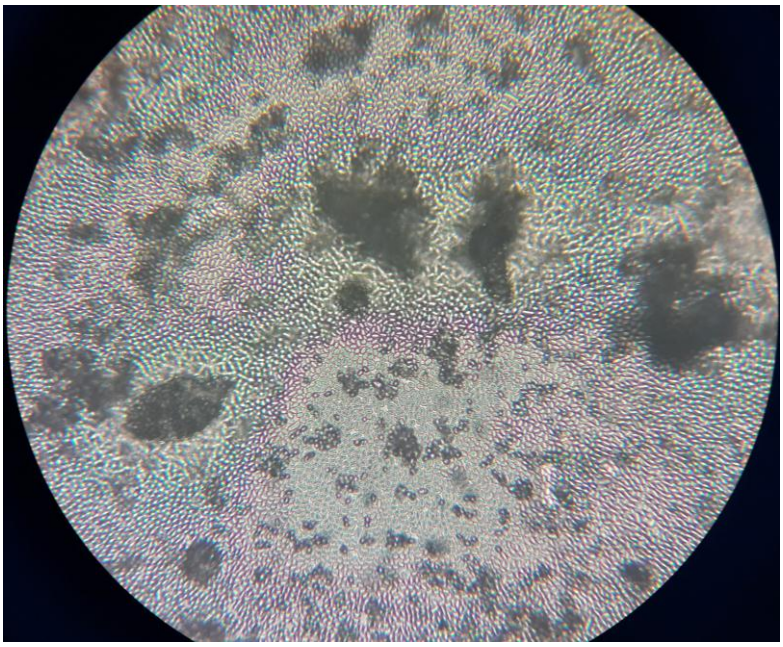
(Fig. 4.9) a : Initial manifestness of hypha growth after 11 days ,
b and c : Spread hypha growth on the peel surface after 16 days .

(Fig. 4.10) Integrated white circle with cotton texture on the fruit surface after 18 days .

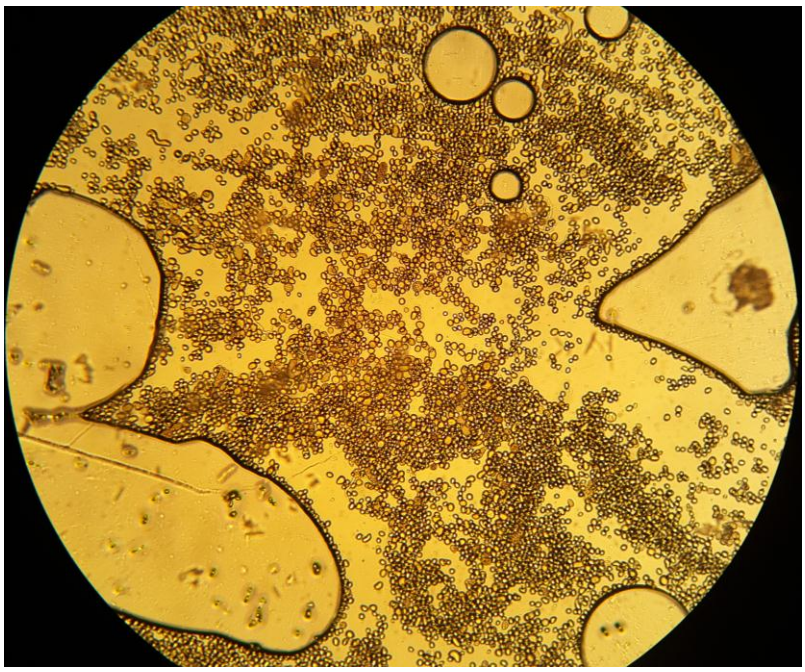
(Fig. 4.11) Some types of fruits after period of infection .

4.4 Calculation of inoculum density (Potential) of *Penicillium* spores suspension .

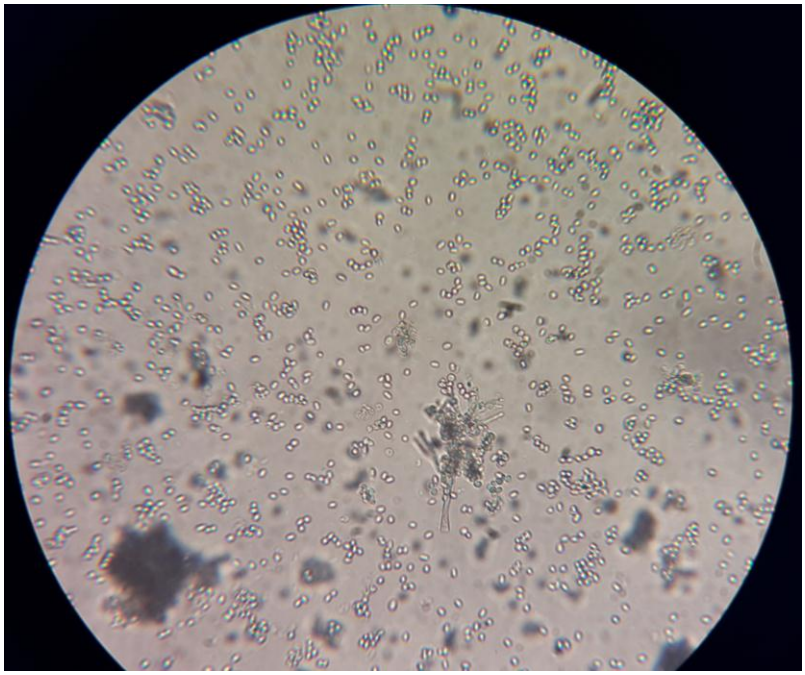
The result of three serial spore dilutions $10^{-1} = 10^{-9}$ spores/ml , $10^{-2} = 10^{-4}$, $10^{-2} = 10^{-3}$ approximately . The objectives of this experiment to capability of *Penicillium spp.* spore for causing infection and the longivity of symptom appearance the result on this experiment show differences in time period to exposure initial typical symptoms as following : after 48 hour by first dilution , after 60 hours by second dilution and 72 hours by third dilution (from Fig. 4.12 to Fig. 4.16) .



(Fig. 4.12) Density of *Penicillium* spores in first dilution .

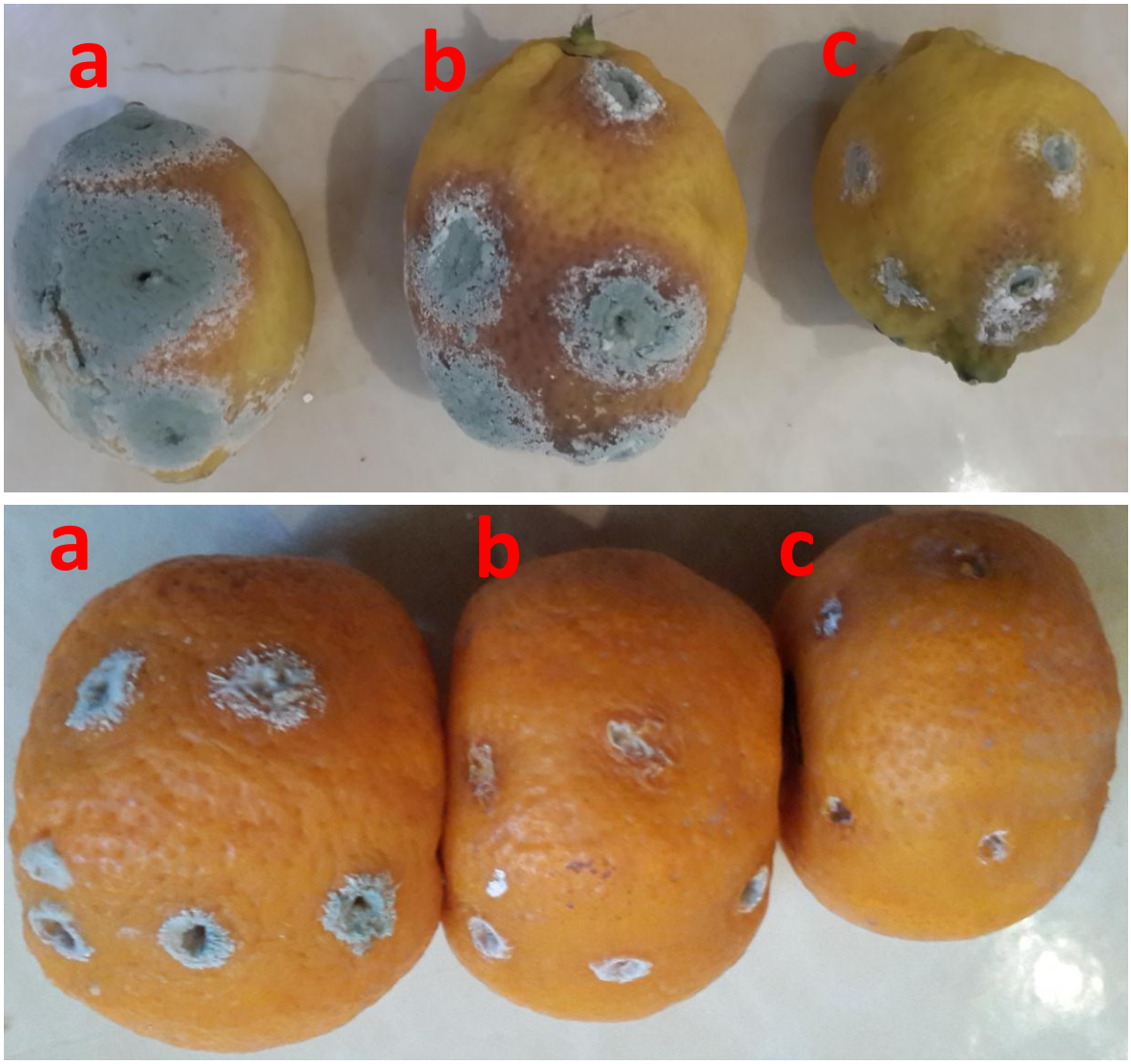


(Fig. 4.13) Density of *Penicillium* spores in second dilution .



(Fig. 4.14) Density of *Penicillium* spores in third dilution .

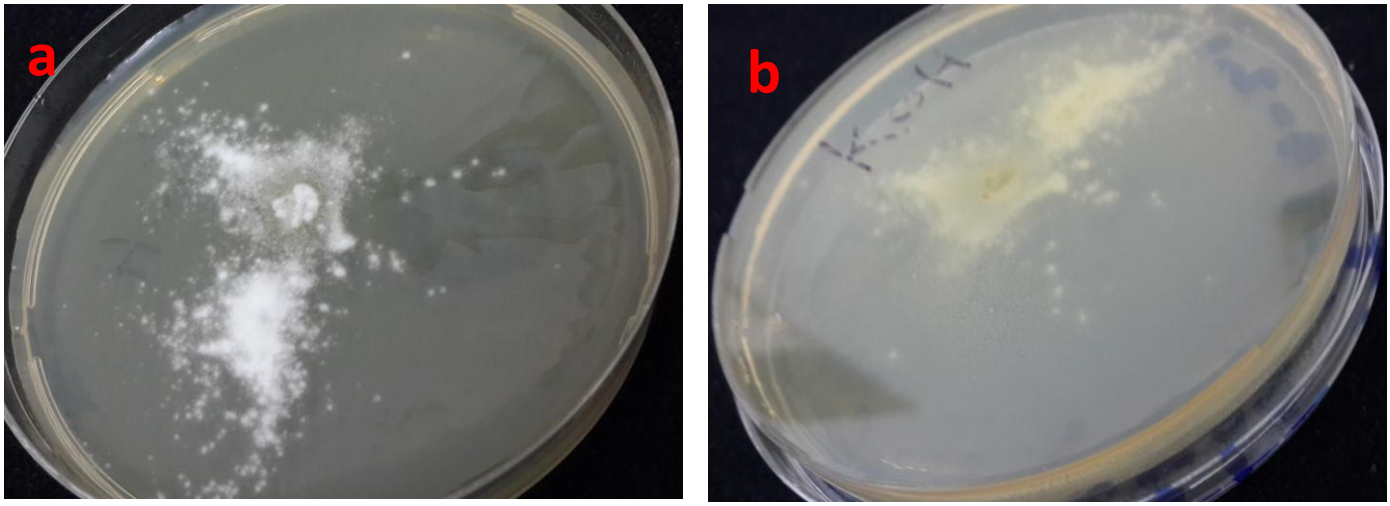
(Fig. 4.15) The differences between three types of inoculum density .



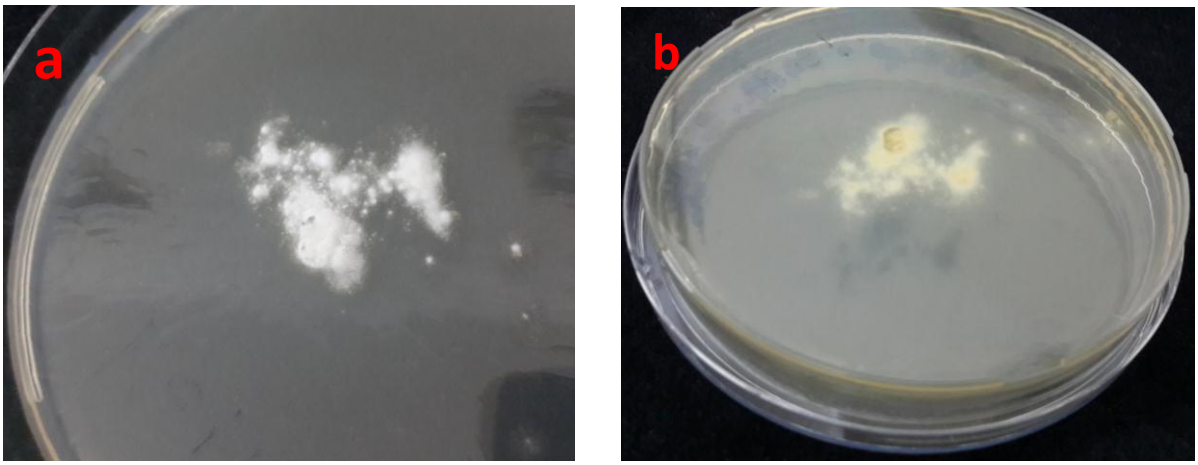
(Fig. 4.16) Different growth by different concentration of three dilution types from inoculum density , a : First dilution , b : Second dilution , c : Third dilution .

4.5 Cultural and morphological characteristics of Penicillium isolated and identification of causal agent (pathogen) .

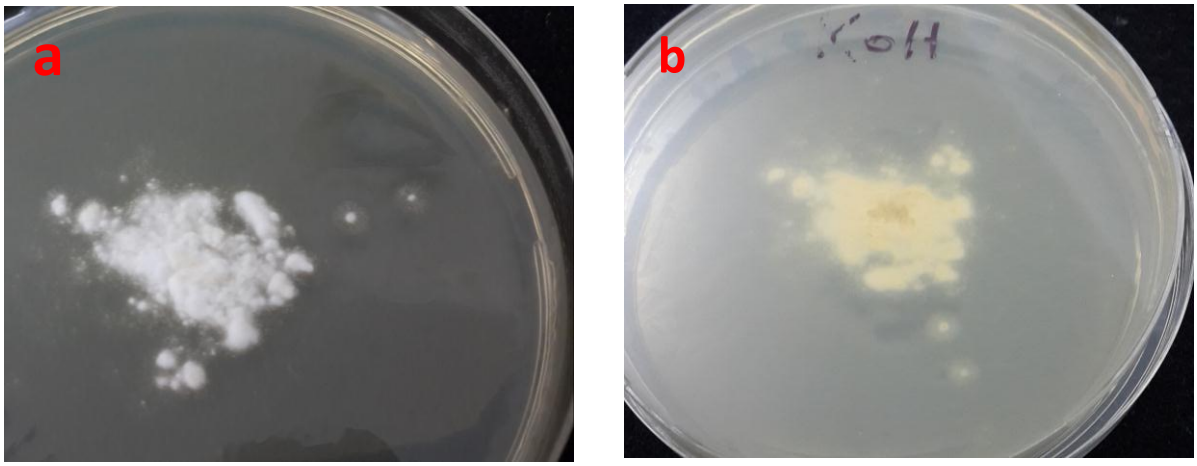
On Sabroid agar the fungus formed radial colonies , thin of white mycelia reaching 1cm dim in 10-14 days at 24-27°C with greenish color and the substrate mycelium was olive green with a clear zone observed (from Fig.4.17 to Fig. 4.43) . Conidia spores of the fungus are showing two – stage branching , typically cylindrical phialides 15-20µm with broadly truncate base and evenly rounded tip , smooth walled and chains of cylindrical single called conidia 3.5 - 5 x 3 - 3.35 µm . Based on the symptoms , the pigment produce by this fungus ranged between white , yellow and orange color , according to cultural and morphological description the fungus was identified as *P. digitatum* .



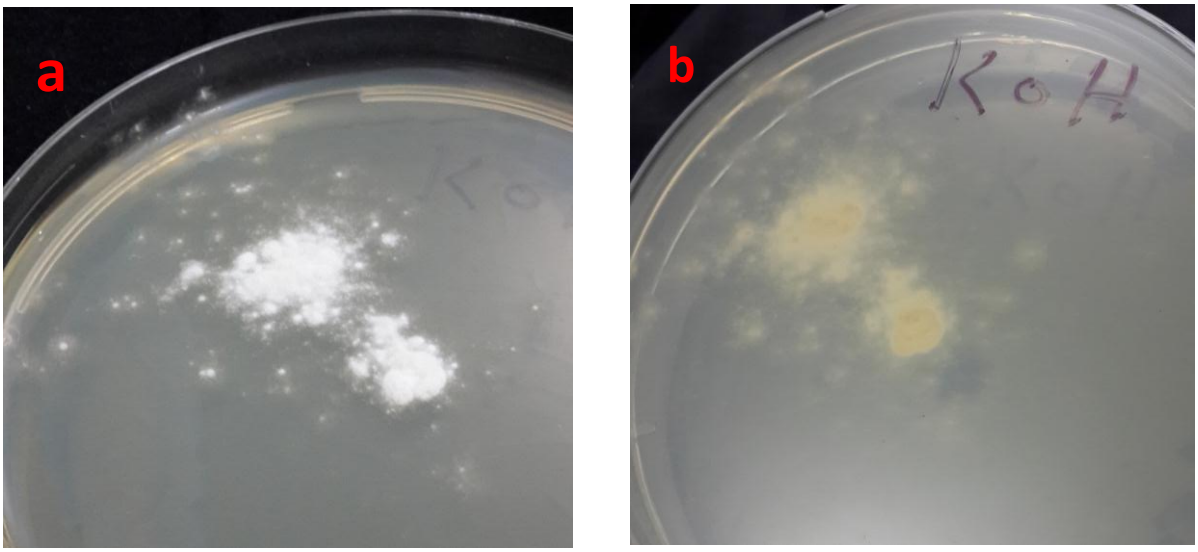
(Fig. 4.17) a : The growth of *Penicillium spp.* after 24 hours isolated from Grapefruits and b: color of pigment Produced by *Penicillium spp* .



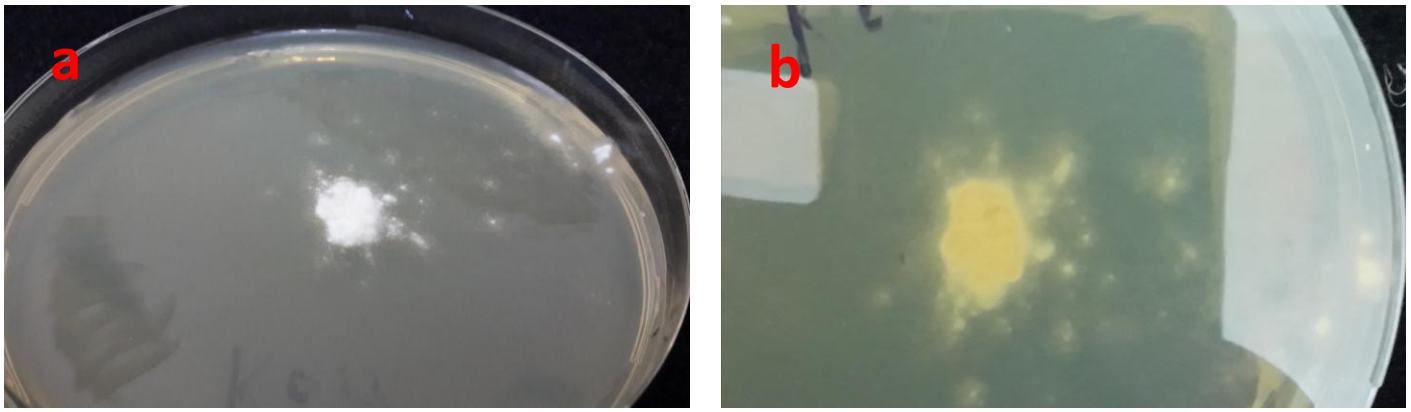
(Fig. 4.18) a : The growth of *Penicillium spp.* after 24 hour in Navel orange , b: color of pigment Produced by *Penicillium spp* .



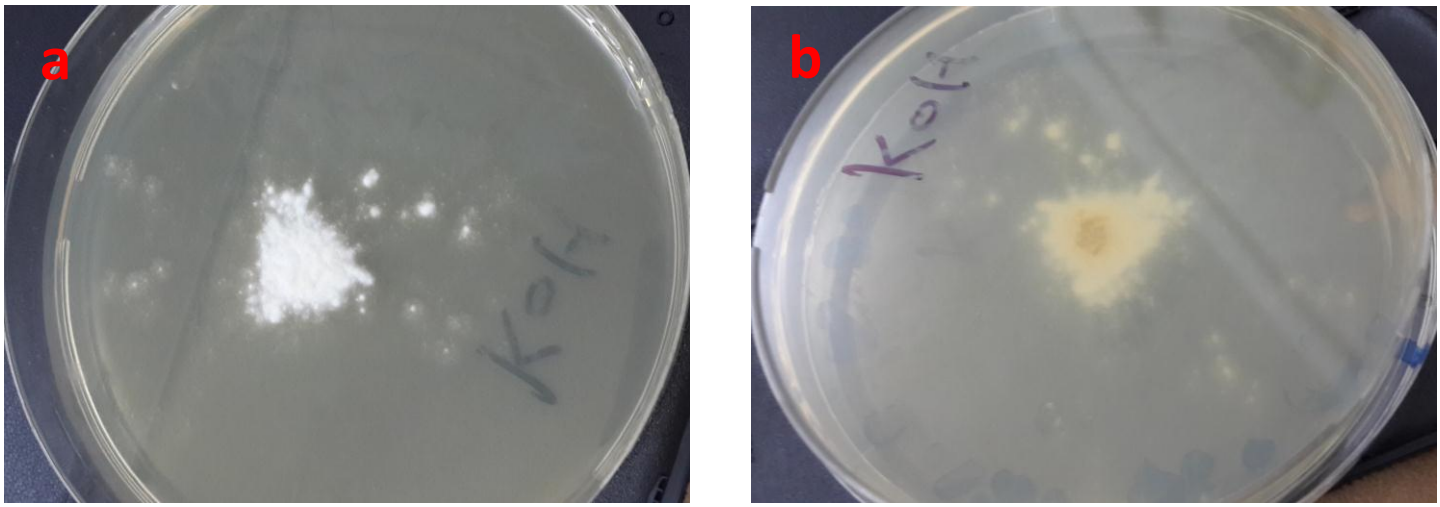
(Fig. 4.19) a : The growth of *Penicillium spp.* after 24 hour in sour orange , b: color of pigment Produced by *Penicillium spp*.



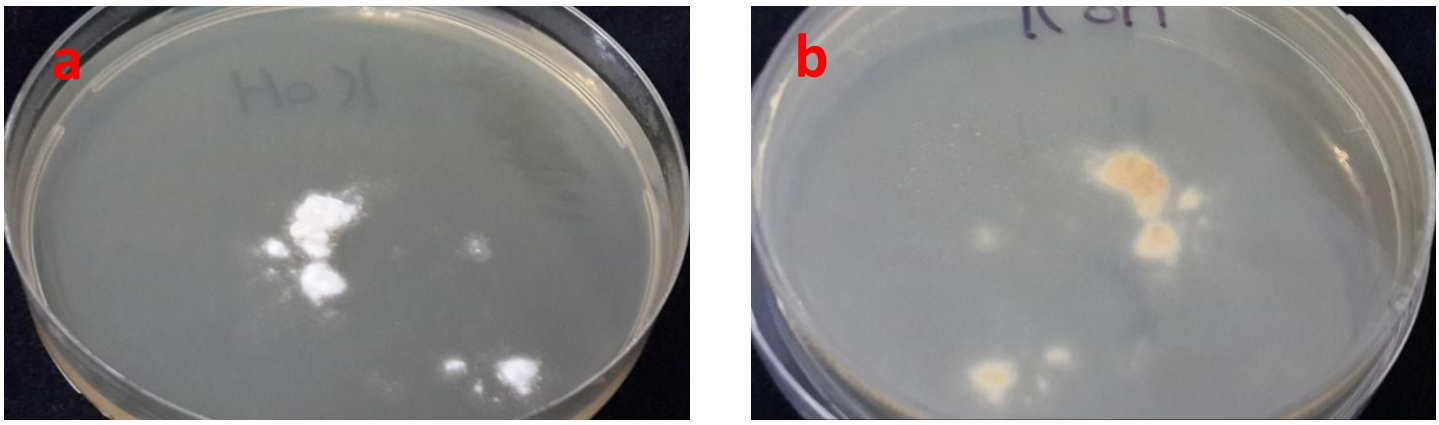
(Fig. 4.20) a : The growth of *Penicillium spp.* after 24 hour in Sweet orange , b: color of pigment produced by *Penicillium spp.*



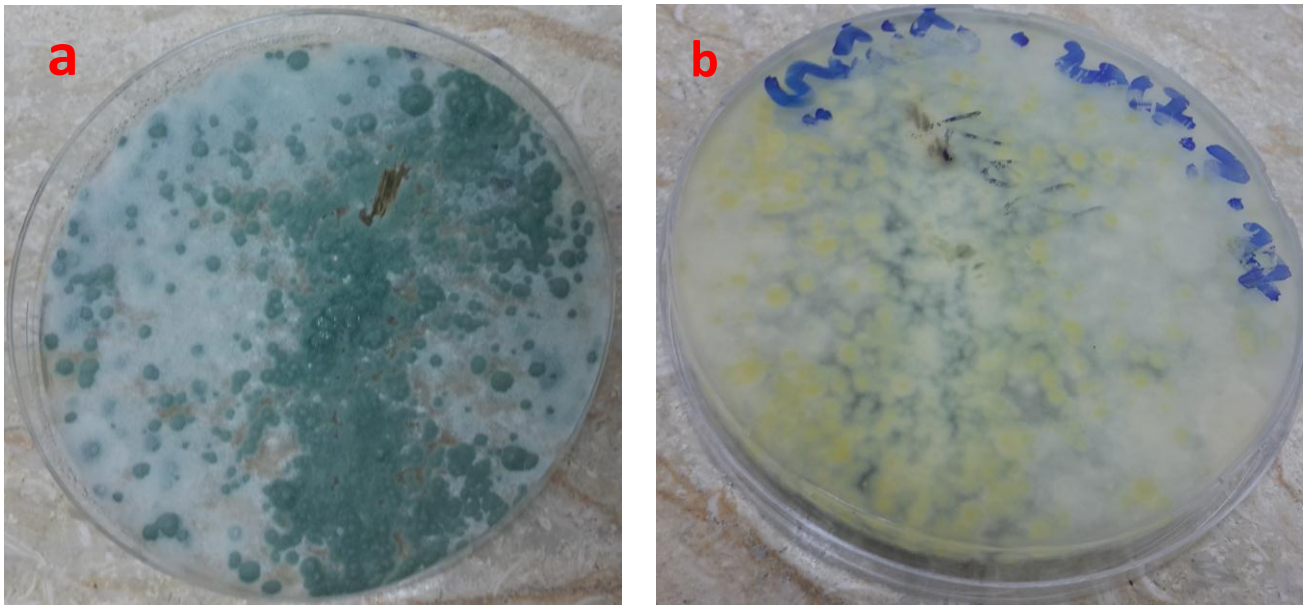
(Fig. 4.21) a : The growth of *Penicillium spp.* after 24 hour in Bladder orange , b: color of pigment Produced by *Penicillium spp.*



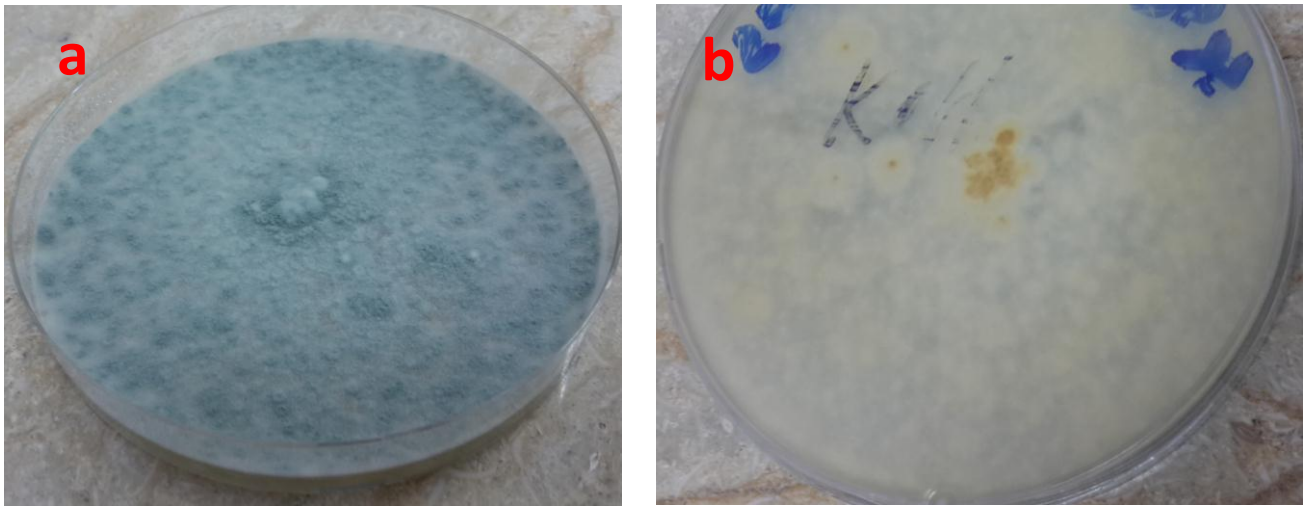
(Fig. 4.22) a : The growth of *Penicillium spp.* after 24 hour in Lemon , b: color of pigment Produced by *Penicillium spp.*



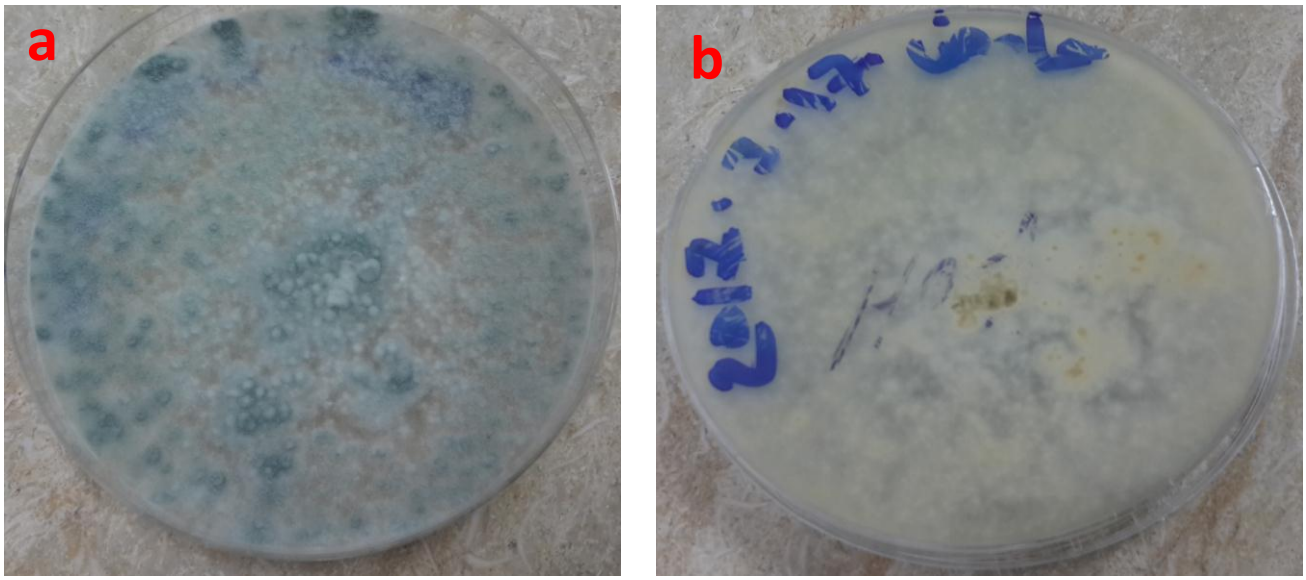
(Fig. 4.23) a : The growth of *Penicillium spp.* after 24 hour in Mandarine , b: color of pigment Produced by *Penicillium spp.*



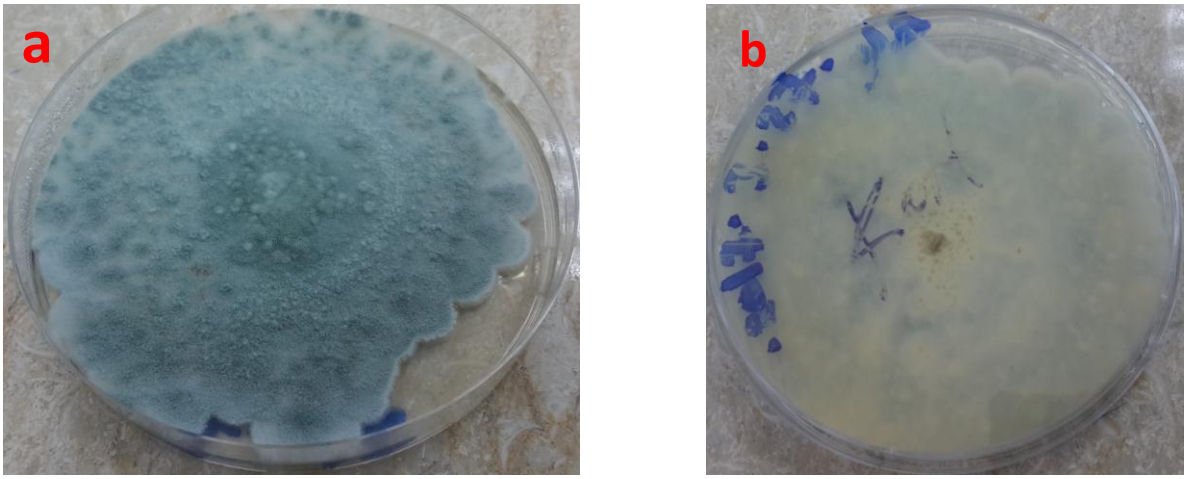
(Fig. 4.24) a : The growth of *Penicillium spp.* after 5 days in Grapefruits, b: color of pigment Produced by *Penicillium spp.*



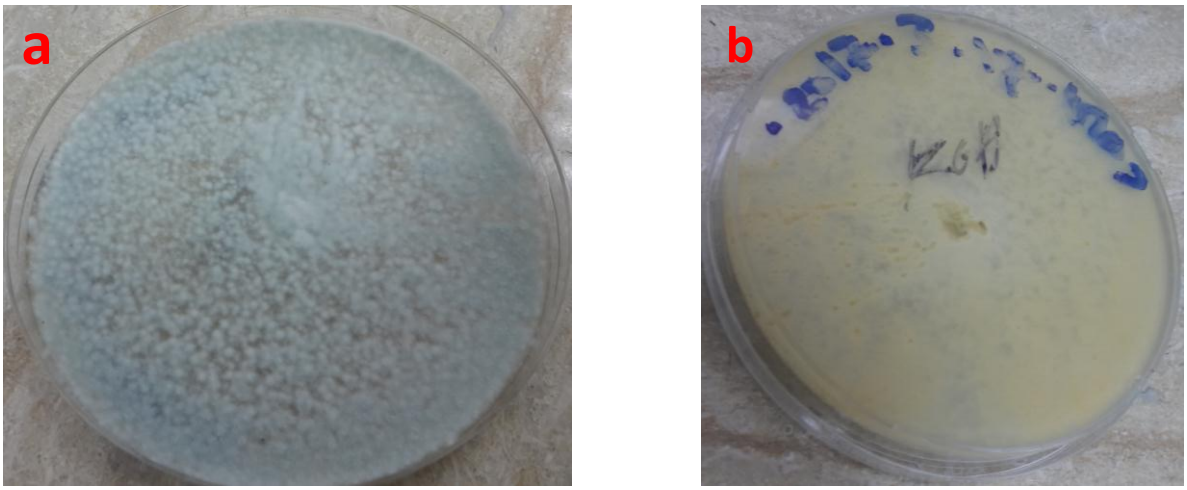
(Fig. 4.25) a : The growth of *Penicillium spp.* after 5 days in Navel orange, b: color of pigment Produced by *Penicillium spp.*



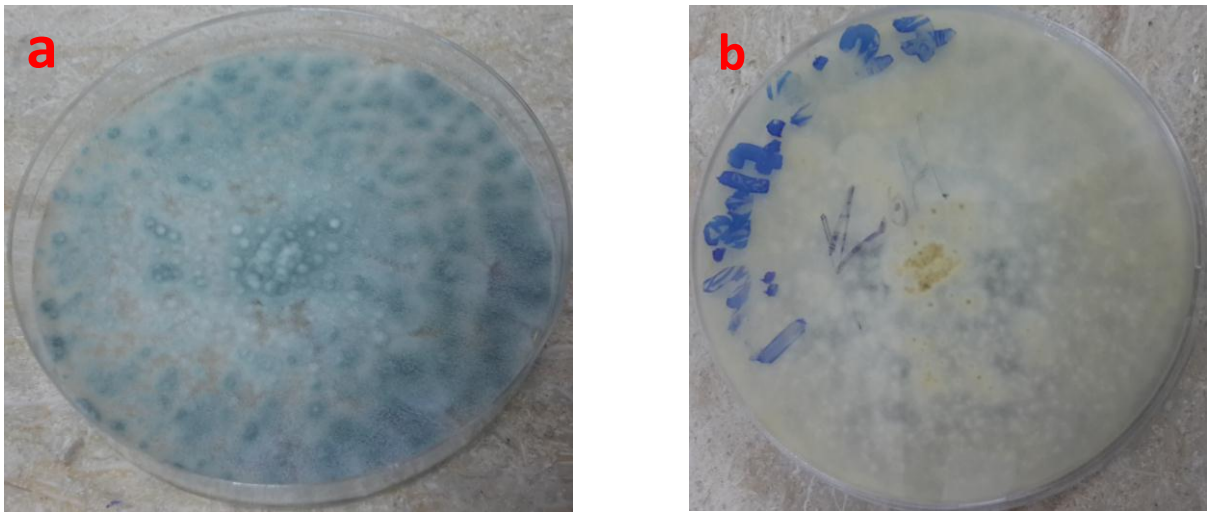
(Fig. 4.26) a : The growth of *Penicillium spp.* after 5 days in Sour orange, b: color of pigment Produced by *Penicillium spp.*



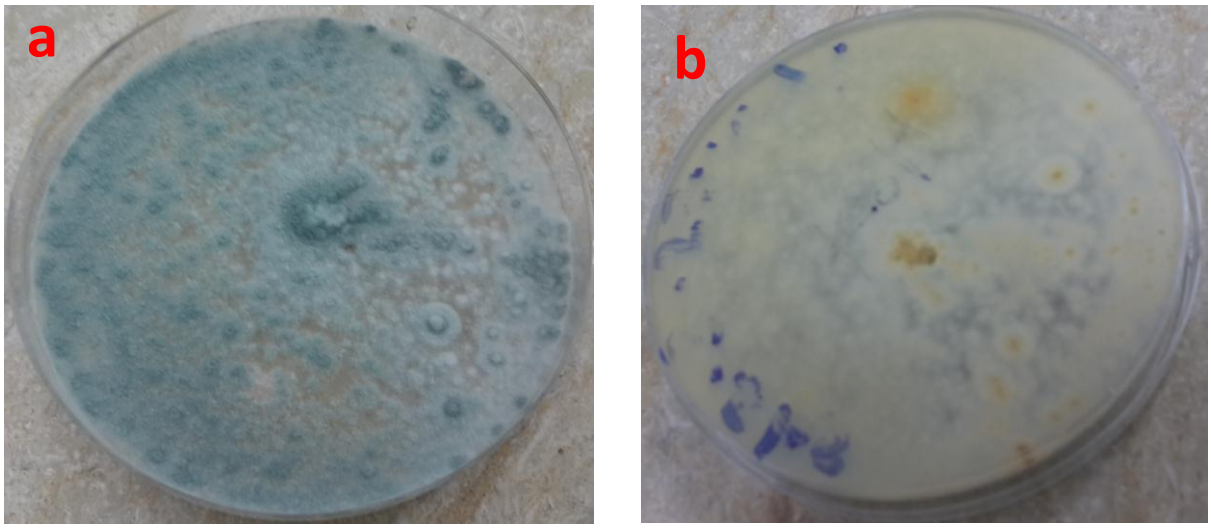
(Fig. 4.27) a : The growth of *Penicillium spp.* after 5 days in Sweet orange, b: color of pigment Produced by *Penicillium spp.*



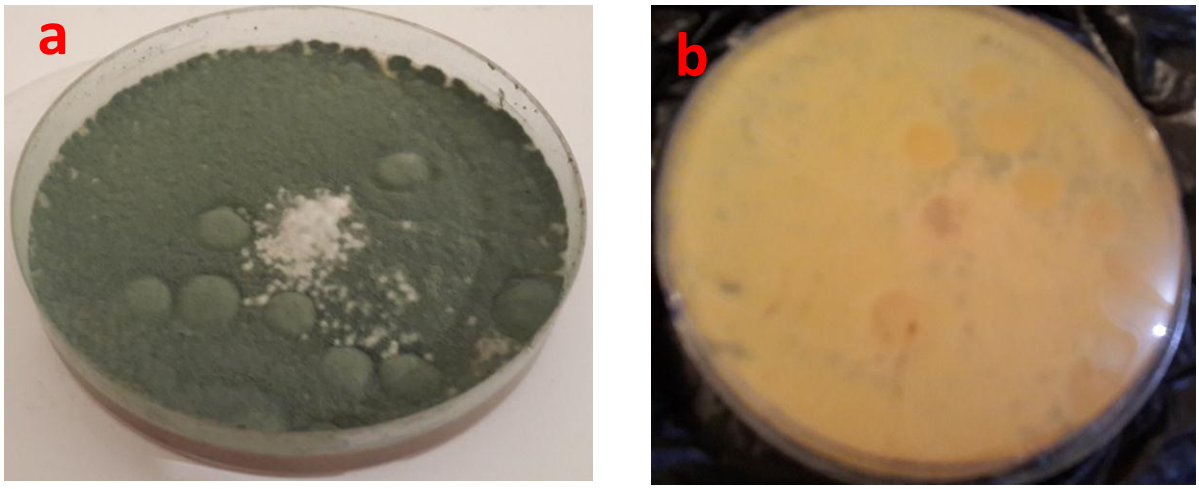
(Fig. 4.28) a : The growth of *Penicillium spp.* after 5 days in Bladder orange, b: color of pigment Produced by *Penicillium spp.*



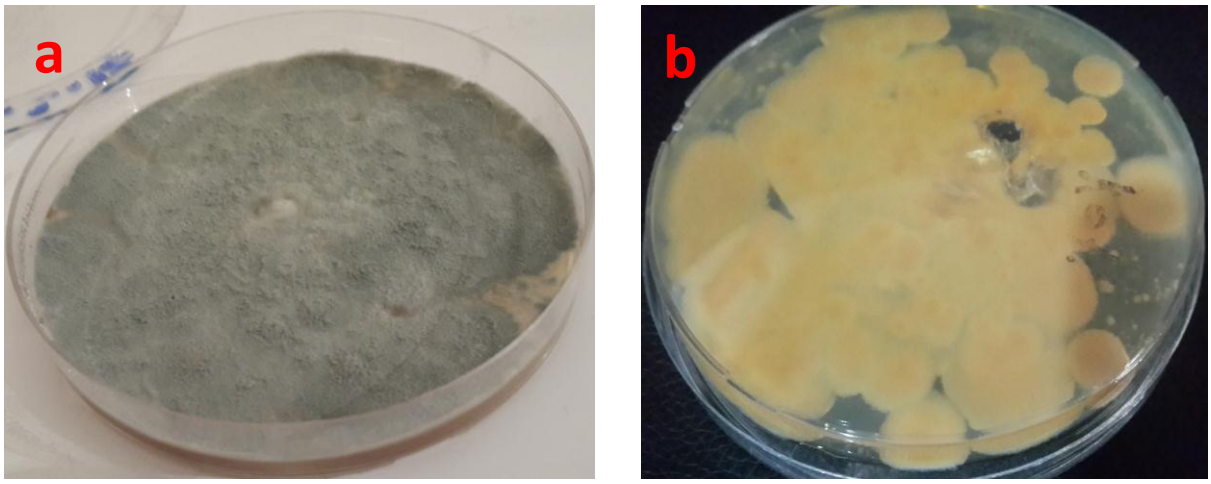
(Fig. 4.29) a : The growth of *Penicillium spp.* after 5 days in Lemon orange, b: color of pigment Produced by *Penicillium spp.*



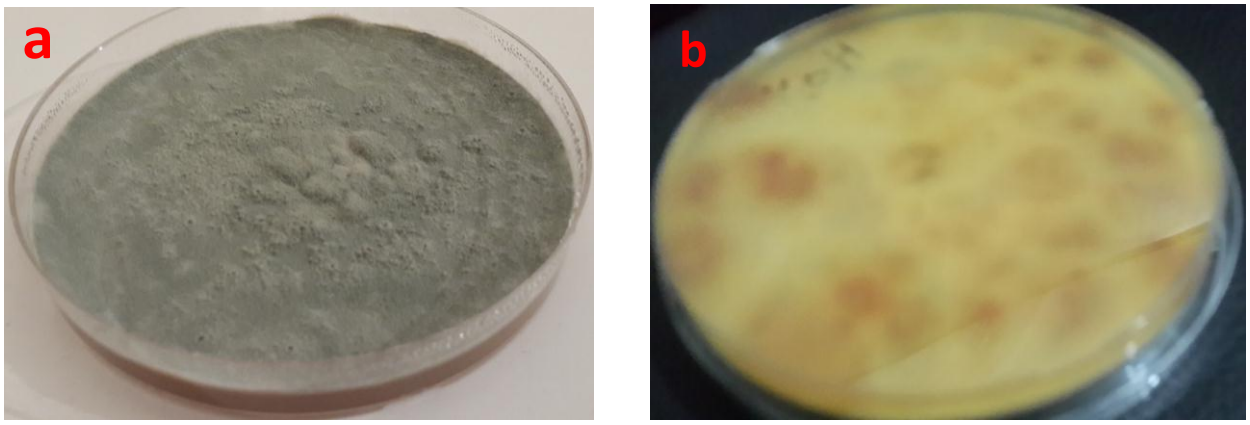
(Fig. 4.30) a : The growth of *Penicillium spp.* after 5 days in Mandarine orange, b: color of pigment Produced by *Penicillium spp.*



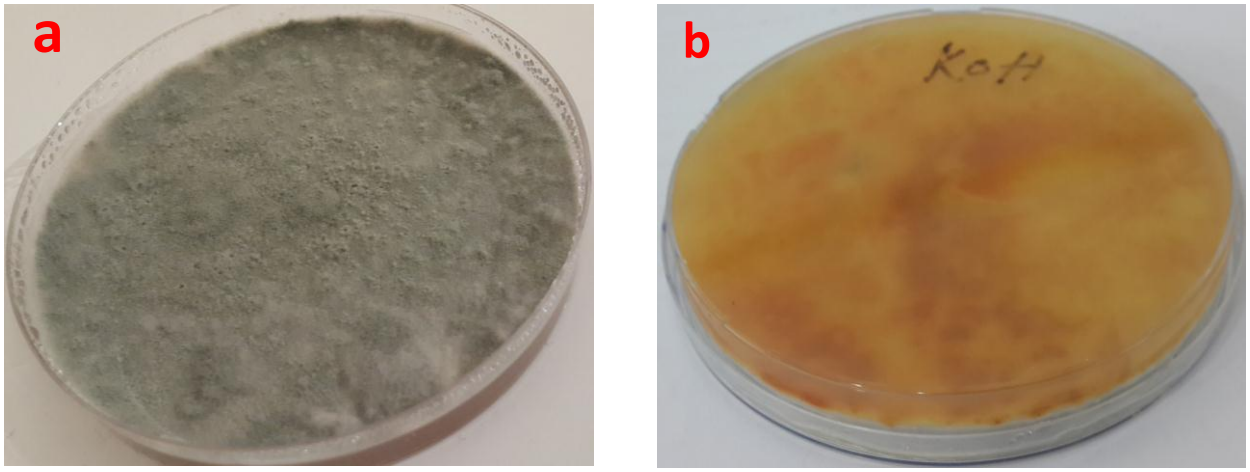
(Fig. 4.31) a : The growth of *Penicillium spp.* after 8 days in Grapefruits, b: color of pigment Produced by *Penicillium spp.*



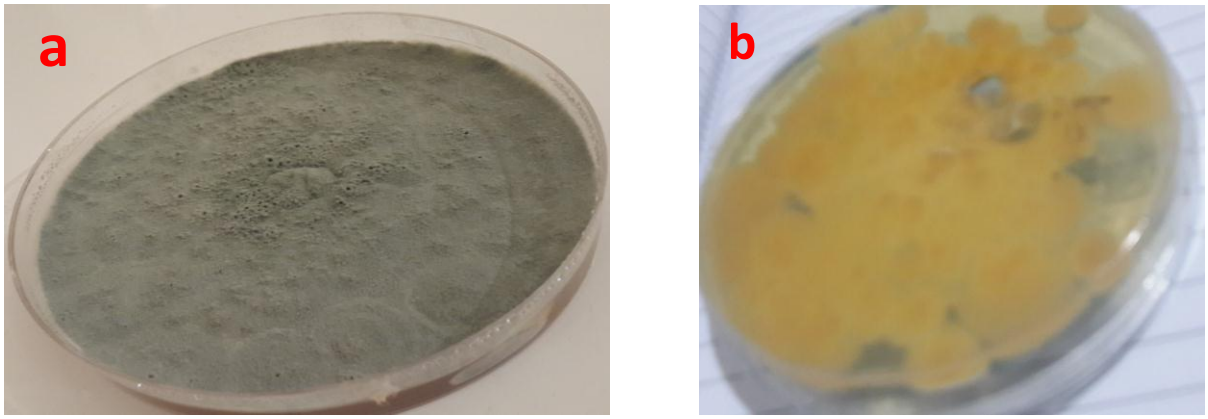
(Fig. 4.32) a : The growth of *Penicillium spp.* after 8 days in Navel orange, b: color of pigment Produced by *Penicillium spp.*



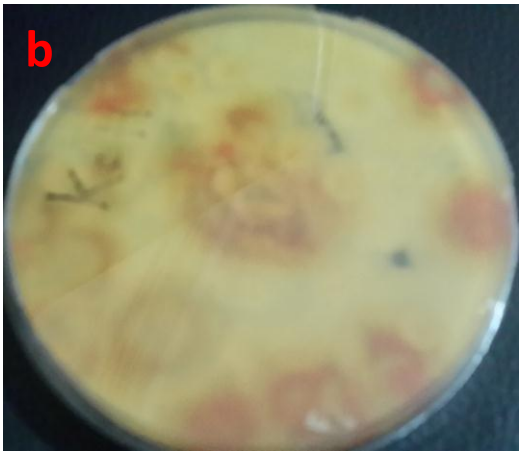
(Fig. 4.33) a : The growth of *Penicillium spp.* after 8 days in Sour orange, b: color of pigment Produced by *Penicillium spp.*



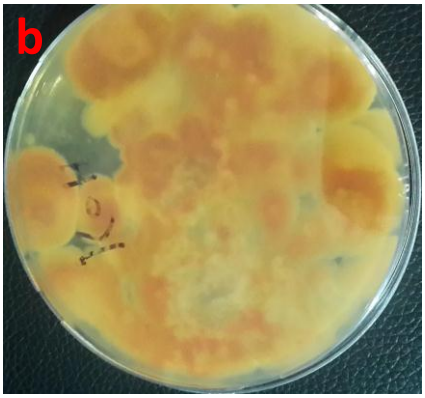
(Fig. 4.34) a : The growth of *Penicillium spp.* after 8 days in Sweet orange, b: color of pigment Produced by *Penicillium spp.*



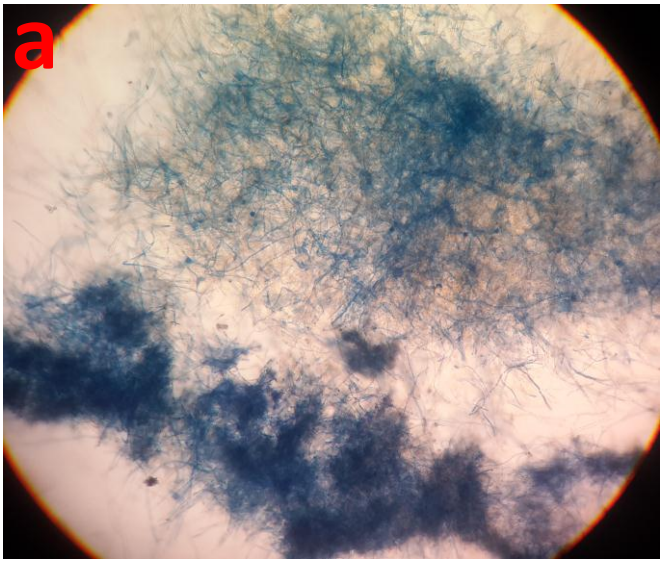
(Fig. 4.35) a : The growth of *Penicillium spp.* after 8 days in Bladder orange, b: color of pigment Produced by *Penicillium spp.*

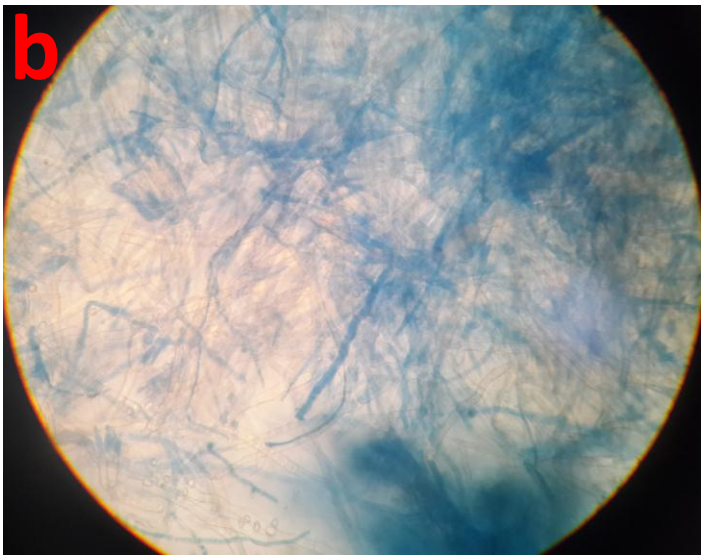


(Fig. 4.36) a : The growth of *Penicillium spp.* after 8 days in Lemon orange, b: color of pigment Produced by *Penicillium spp.*

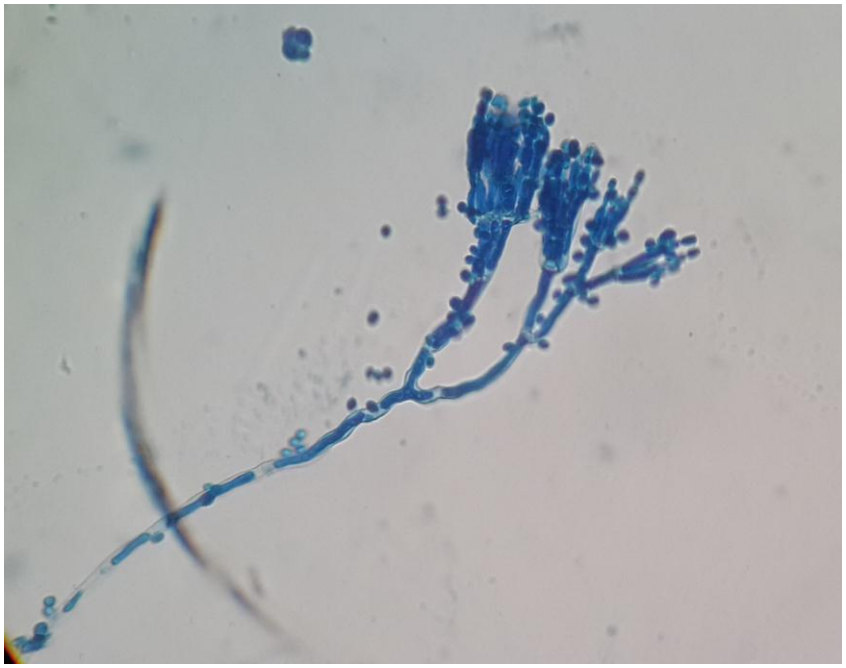


(Fig. 4.37) a : The growth of *Penicillium spp.* after 8 days in Mandarine orange, b: color of pigment Produced by *Penicillium spp.*

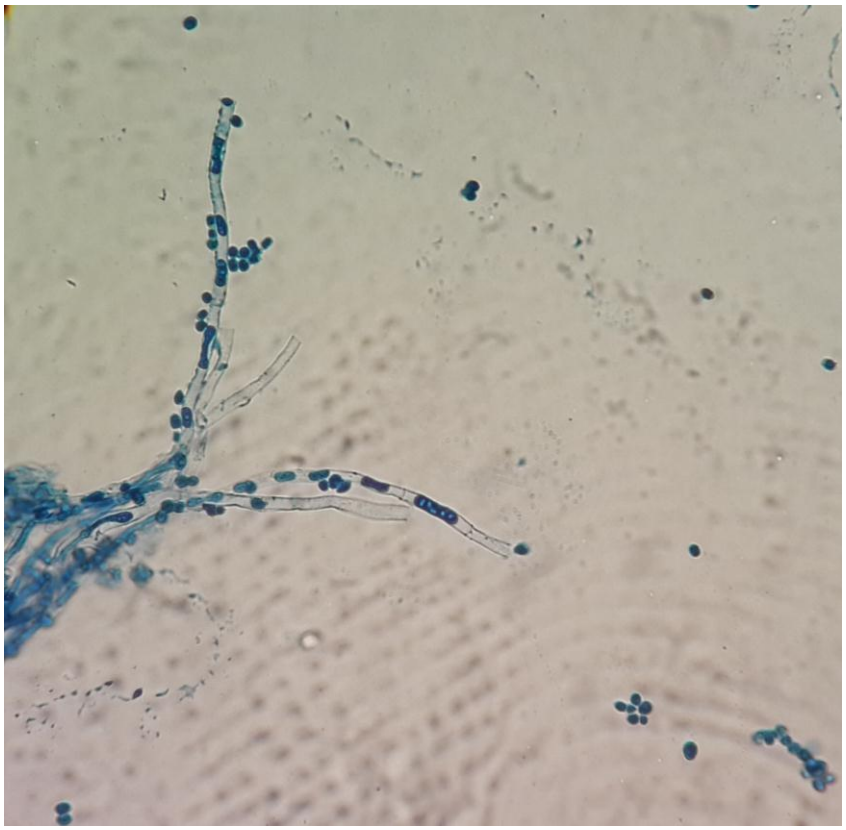




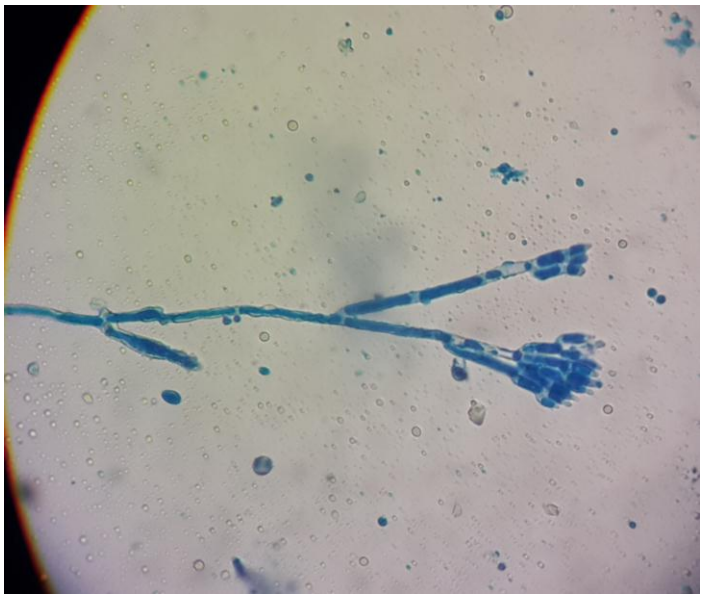
(Fig. 4.38) The field of *Penicillium digitatum* under lens a :10 , b : 40



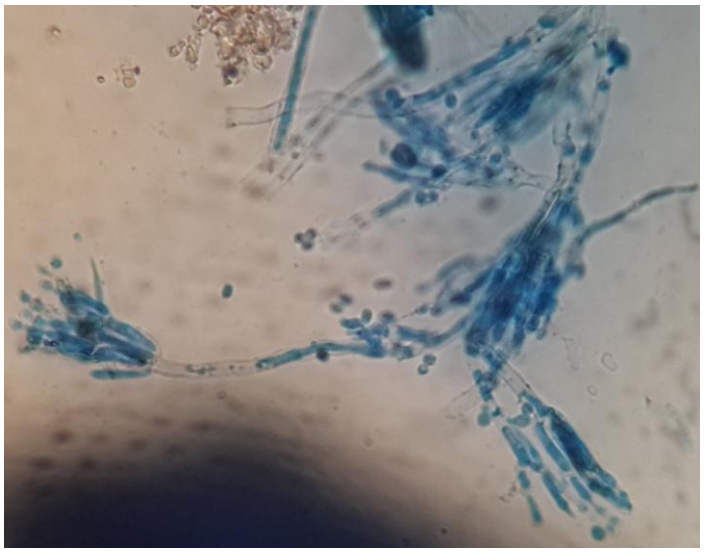
(Fig. 4.39) *Penicillium digitatum* stained with methyl cotton blue .



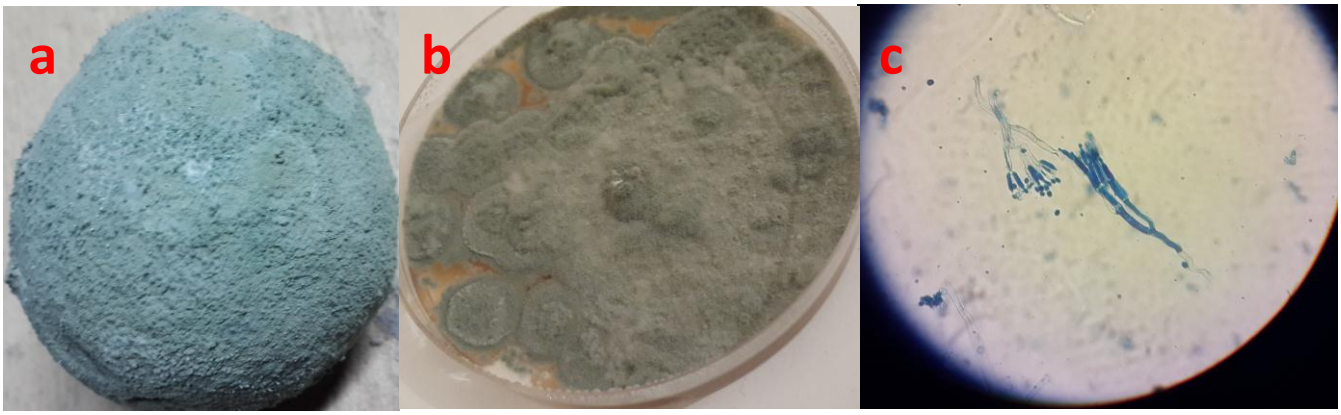
(Fig. 4.40) *Penicillium digitatum* .
The hypha and nucleii present inside it and septa between cells of hypha .



(Fig. 4.41) *Penicillium digitatum* .
The hypha branching and vertical phialides .



(Fig. 4.42) *Penicillium digitatum* .
Conidial chains and Conidia .



(Fig. 4.43) a : Green mold on orange , b : Culture of *Penicillium digitatum* and c : Conidiophores and conidia of *Penicillium digitatum* .

4.6 The role of refrigerate storage (cold storage) in infection :

The aims of these experiments to compare the effect cooling and non cooling period (time) to four citrus varities, the fruits which kept in reifridge under 5°C show early appearance of green mold symptoms compared with fruits storaged in room temperature 25 – 27°C show the delay appearance of symptoms green mold with dispersal time about 12 hour , while the samples kept in the outdoor which just injuried without injection with *Penicillium* spores and control samples (uncontaminated) do not show any appearance of green mold symptoms after kept for long time .

4.7 The result study of peel thickness of four types of studied fruits

The result obtained from studying peel thickness shoe appear that the *Penicillium digitatum* able to penetrate easily through thin or thick fruit peel but take different time so that the time factor is play importance role to appear the typical symptoms of green mold . Table 4.3 and Fig. (4.44) from the depth and location experiments which done or carried out on four citrus varities . The resulting growth was superficial not deep just only on surface (Aerobic area) . The best and rapid growth of *Penicillium* appears in thin peel in mandarine , grapefruits then blood , sweet , sour , navel , and the last in thick peel of lemon respectively (Fig. 4.45) . Although the lemon is last fruits infected with *Penicillium digitatum* . but it is the first fruit covered completely with growth of *Penicillium digitatum* before another studied fruits . Do not observational different effect in the sites of the five wounds on upper , middle and lower surface of the peel fruits which ensure any wounds on the peel suitable and facilitated entry and growth of *Penicillium digitatum* . Enhance , the holes in the juice sac it's the sites for absorb the water and nutrient materials . There is no different in the depth of wounds 1cm and 2cm .

Table 4.3 Peel thickness of citrus fruit varities **.**

Varities	Thickness of peel (cm)
Blood orange	**0.4 cm**
Sweet orange	**0.6 cm**
Sour orange	**0.3 cm**
Navel orange	**0.6 cm**
Mandarine	**0.3 cm**
Lemon	**0.7**
Grapefruits	**1.0 cm**

(Fig. 4.44) Cross section in the infected fruits explains growth of *Penicillium digitatum* in the studied citrus fruits after 26 days .

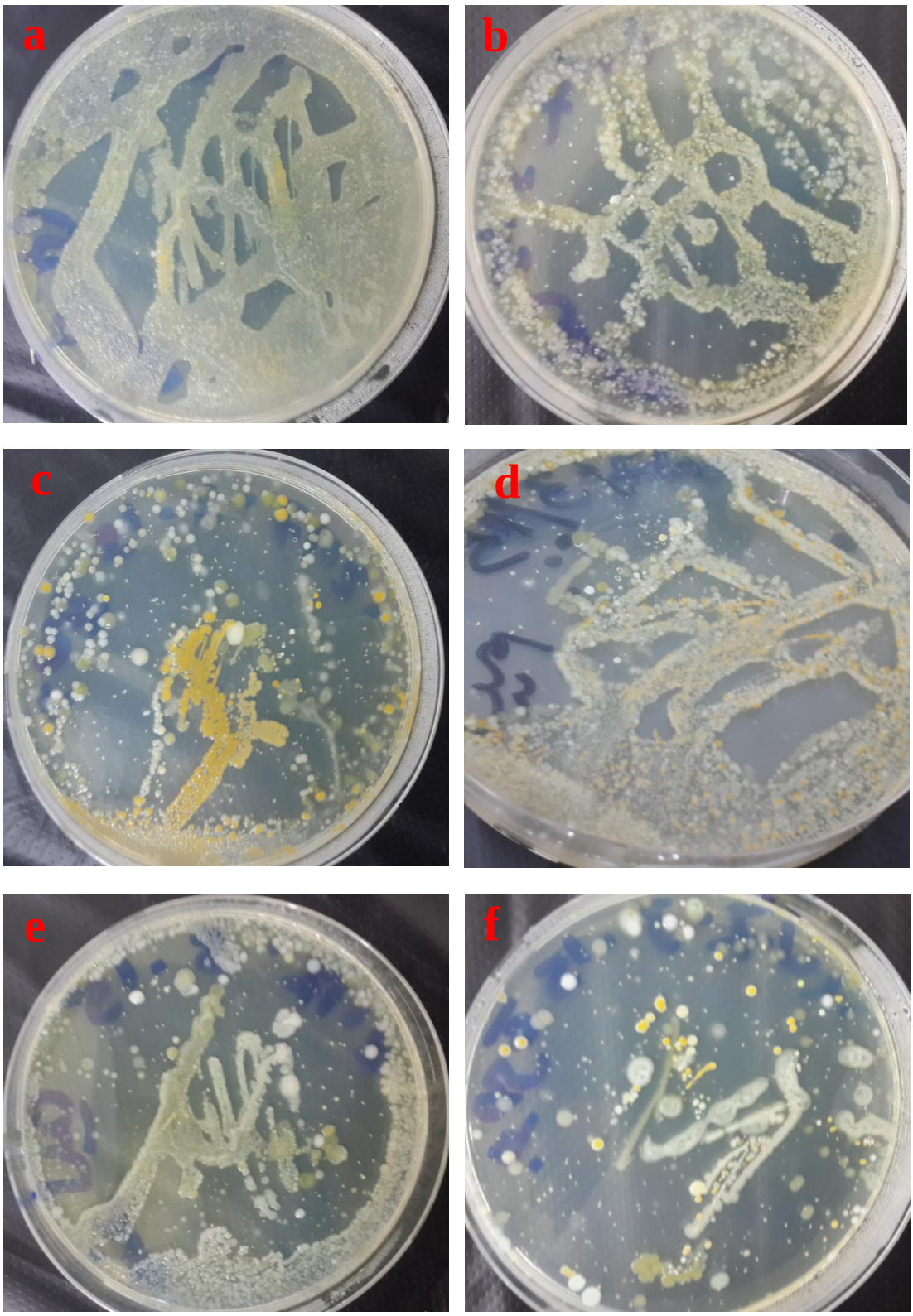
(Fig. 4.45) Cross section in studied fruits after 2 month .

4.8 Study natural surface microflora on the surface citrus fruit .

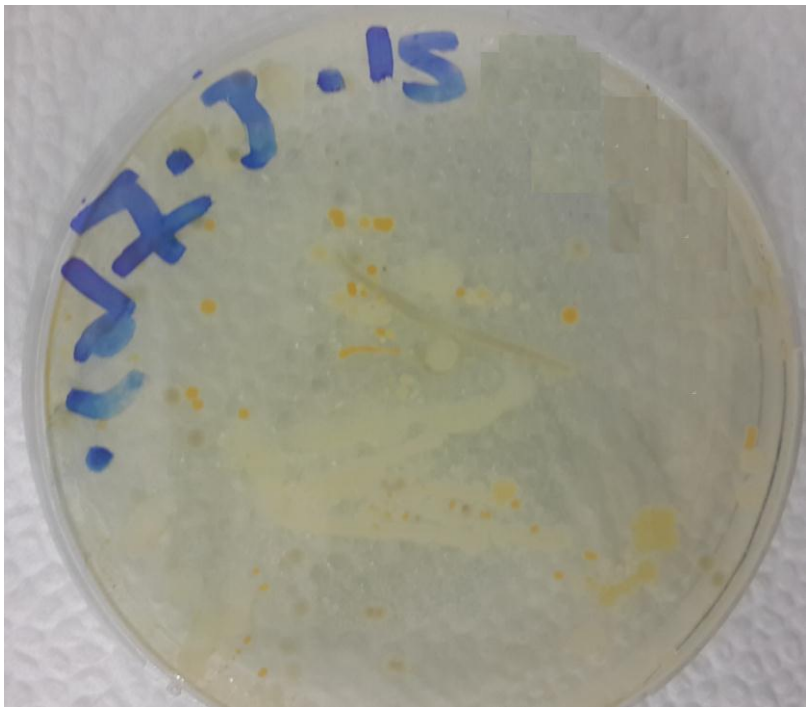
The conclusion obtained from study natural microflora which found on the surface of different citrus fruits by comparing washing and non-washing fruits collected from the same resource . The washing fruits (free of microflora) show rapaid growth than non-washing fruits (with microflora) with disperse time 12 hours . From the washing suspension of citrus fruits different type of bacteria was found and identified by using phonex system™ named as *Staphyloccous hominis* , *Staphyloccous haemolyticus* , *Staphyloccous warneri* . As mention in the table (4.4) which represent the natural microflora on surfaces studied citrus fruit (Fig. 4.46 and 4.47) .

Table (4.4) citrus fruit varities , types of bacteria , confidence value and inoculum density :

Varities	Type of Bacteria	Confidence value	Inoculum density
Blood	***Staphyloccous hominis***	**98 %**	**0.5**
Sweet	***Staphyloccous hominis***	**98 %**	**0.5**
Sour	***Staphyloccous hominis***	**99 %**	**0.5**
Navel	***Staphyloccous hominis***	**99 %**	**0.5**
Mandarine	***Staphyloccous hominis***	**90 %**	**0.5**
Lemon	***Staphyloccous haemolyticus***	**99 %**	**0.5**
Grapefruits	***Staphyloccous warneri***	**90 %**	**0.5**



(Fig. 4.46) Bacterial growth isolated from surface studied fruits in nutrient agar for a: Grapefruits , b: Sour orange , c: Blood orange , d: Lemon ,e: Mandarine and f : Sweet orange .



(Fig. 4.47) Bacterial growth isolated from surface of Navel orange in the nutrient agar.

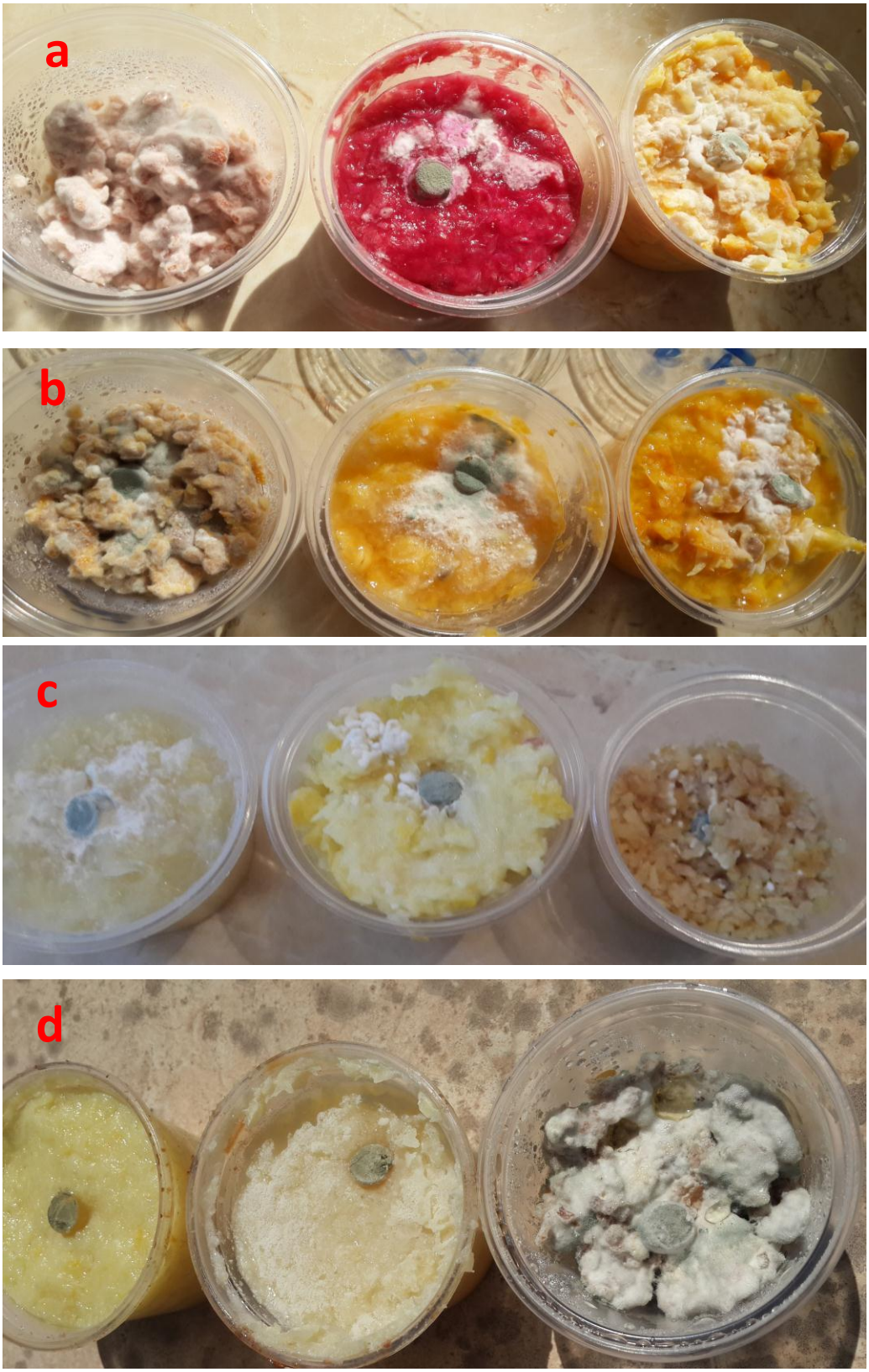
4.9 The observation *Penicillium digitatum* growth for three types of peel juice , sac juice and whole fruit juice (peel + sac).

The observed results of peel juice , sac juice and whole fruit juice (peel + sac) after contented with *P. digitatum* spore and kept at room temperature 27°C , the first initiation *P. digitatum* growth was observed after 48 hours in all peel citrus varities except blood orange which does not show any growth , table (4.5) . While in the sac juice does not show growth in sour orange , mandarine and grapefruits , observed growth in sour orange and lemon only in the third types of juice . After ten days obtained completely growth in peel juice on all types of studied fruits , while sacs juice and whole fruits juice have been spottily growth except sac juice of grapefruit do not show any growth for long time (Fig. 4.48 , 4.49 and 4.50).

Table (4.5) citrus fruit varities , Peel juice , Sac juice , and whole (Peel + Sac juice) .

Varities	Peel juice	Sac juice	Whole fruit juice (Peel + Sac juice)
Blood orange	-	+	-
Sweet orange	+	+	-
Sour orange	+	-	+
Navel orange	+	+	-
Mandarine	+	-	-
Lemon	+	+	+
Grapefruits	+	-	-

(+) Growth , and (-) Non growth .



(Fig. 4.48) Growth of *P. digitatum* spores on three types of juice after 8 days from contamination e.g : a : In Blood Orange , b : In Mandarine , c : In Lemon fruit and d : In Grapefruit .

(Fig. 4.49) Growth of *P. digitatum* spores on three types of juice lemon fruit and effect of time factor on growth of *P. digitatum* after 10 days .

(Fig. 4.50) Infected peel juice of studied fruits with *Penicillium* spores after 10 days .

4.10 The effect of modulation pH tissue of hosts by some materials :

After injection solution of sodium bicarbonate the initial symptoms was appears after 5 days at room temperature . while after injected the others chemical materials solution sodium chloride , concentration and solution of apple vinegar and sterile distill water the clear symptoms of *Penicillium* green mold have been observed after 48 hours with observation black color only in samples injected with apple vinegar (Fig. 4.51).

Table 4.6 The measurement of pH peel juice , pH sac juice and pH whole fruit juice .

Varites	Peel pH	Juice pH	Whole pH
Blood	**6**	**5**	**6**
Sweet	**5**	**6**	**6**
Sour	**5**	**5**	**5**
Navel	**5**	**5**	**6**
Mandarine	**5**	**6**	**6**
Lemon	**6**	**6**	**6.5**
Grapefruits	**5.5**	**6**	**6**

(Fig. 4.51) The measurement of pH peel juice , pH sac juice and pH whole fruit juice .

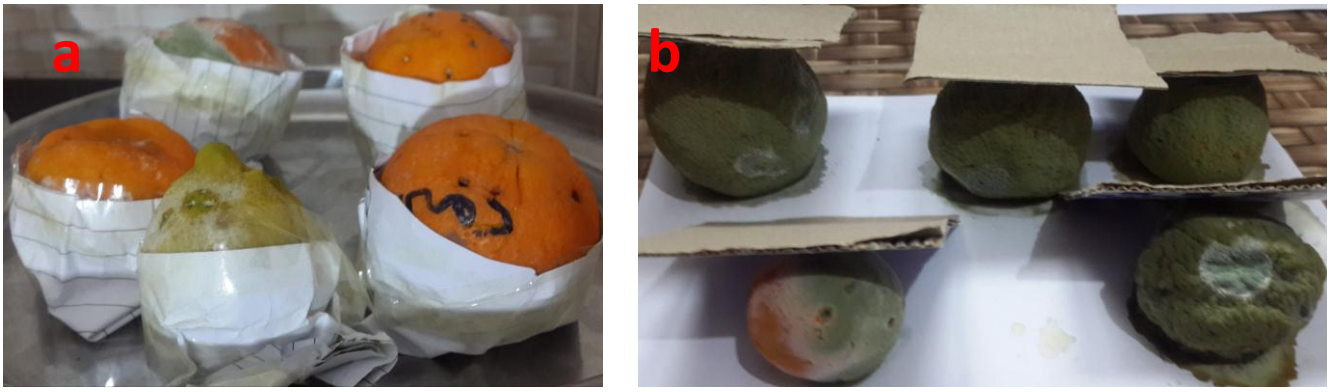
4.11 The result of lignification experiment .

The results obtained in injuired peels of all studied fruits which kept for 12 hours injected inoculum density 10^{-1} spores / ml caused delaying the symptoms of green mold about 6 days from initial fruit injection , but does not prevent the growth of *Penicillium digitatum* green mold (Fig. 4.52) .

(Fig. 4.52) a: Appear the initial symptoms of green mold after 6 days from injection with *Penicillium digitatum* spores .
b : Spread olive green spores with retain white margin after 9 days in the grapefruit .

4.12 In the study of the epidermology of disease and aerodynamic characteristic of *Penicillium digitatum* spores which maintained previous appears the following results :

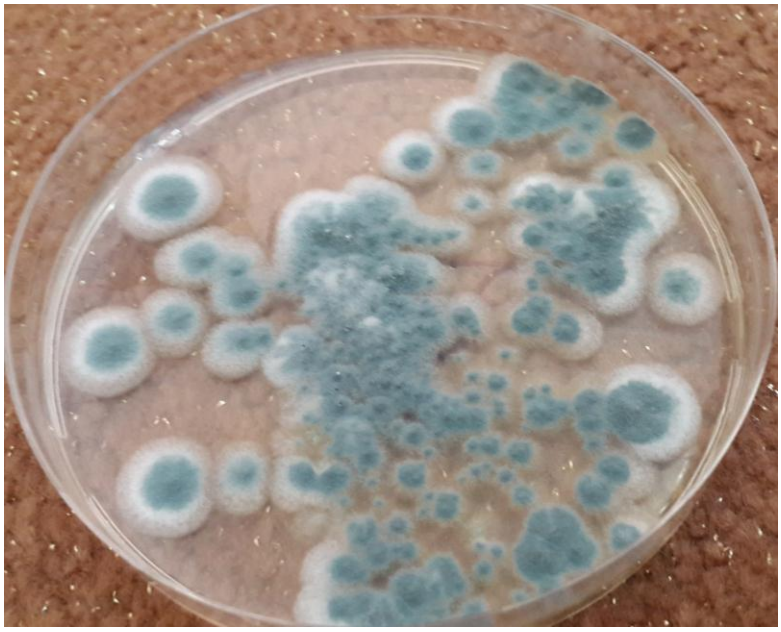
After 48 hours first early symptoms appears in samples putted in the upper surface with distance 2.5 meters high from the surface of the ground (Fig. 4.53) . While the other samples in the ground surface appears the symptoms after 5 days this affirmation the spores becomes suspended in the air before fall-down on the ground surface . In addition to that the samples which have been covered from the sides only was infected early after passing 48 hours . While the other samples covered from upper side the fruits was infected after 6 days . This affirmation the movement of spores *Penicillium* mold are vertically faster than horizontally in the same room condition .



(Fig. 4.53) a: Early infect in the samples covered from sides after 48 hours , b: Samples infected after 6 days .

4.13 The result of host specification of different *Penicillium* isolates .

Isolated *Penicillium allii* from different infected plant material (vegetable) chosen onion . After inoculation onion bulb with *Penicillium digitatum* isolated from citrus fruit showed there is no infection exposure for long time and the vice versa (Fig. 4.54 , 4.55 , and 4.56).



(Fig. 4.54) Growth *Penicillium allii* on Sabroid agar .

(Fig. 4.55) Onion after 5 days from injection by *Penicillium digitatum* .

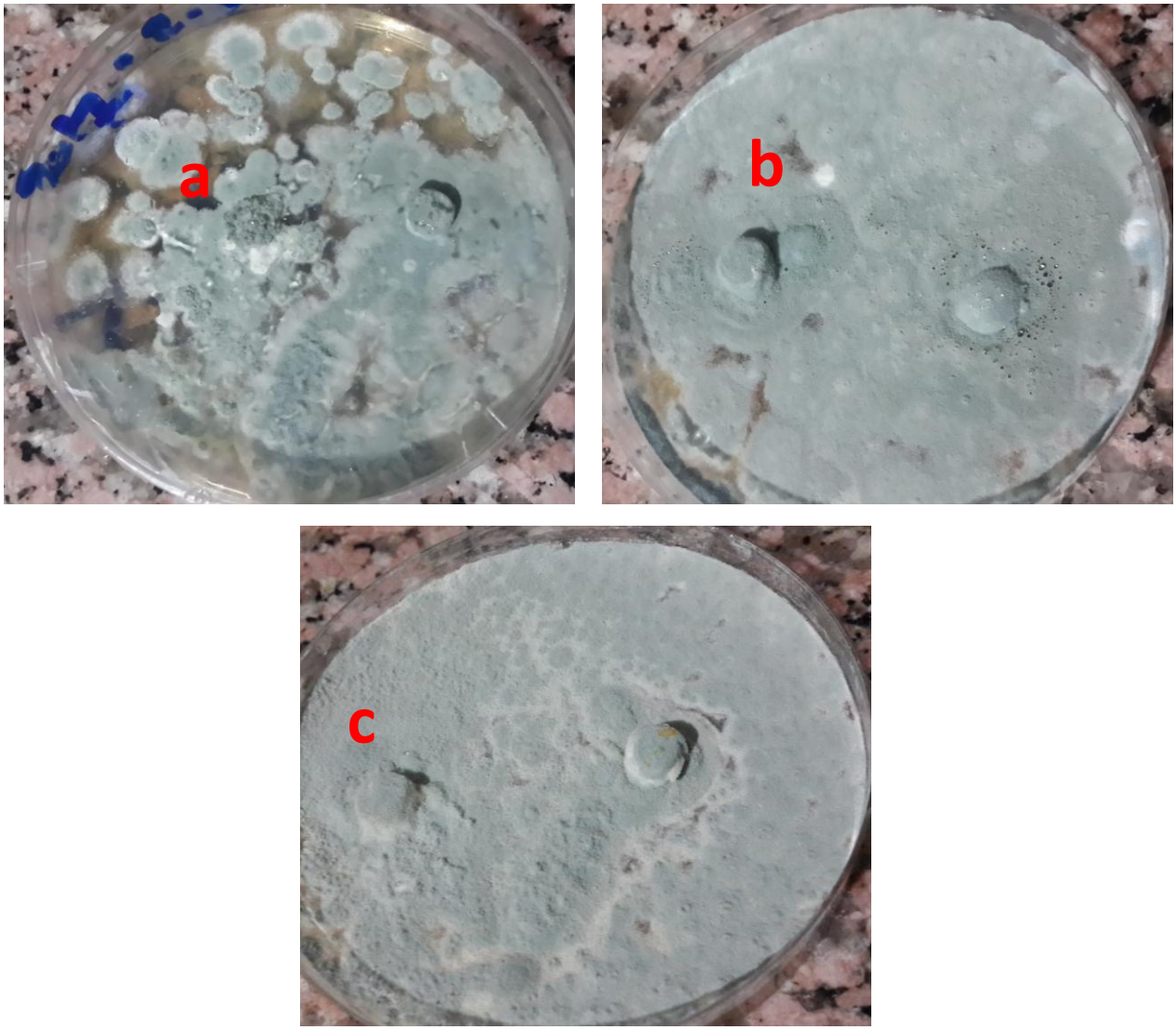
(Fig. 4.56) Samples of orange and lemon after 5 days from injected by *Penicillium allii*

4.14 The effect of maturity stage of lemon fruits .

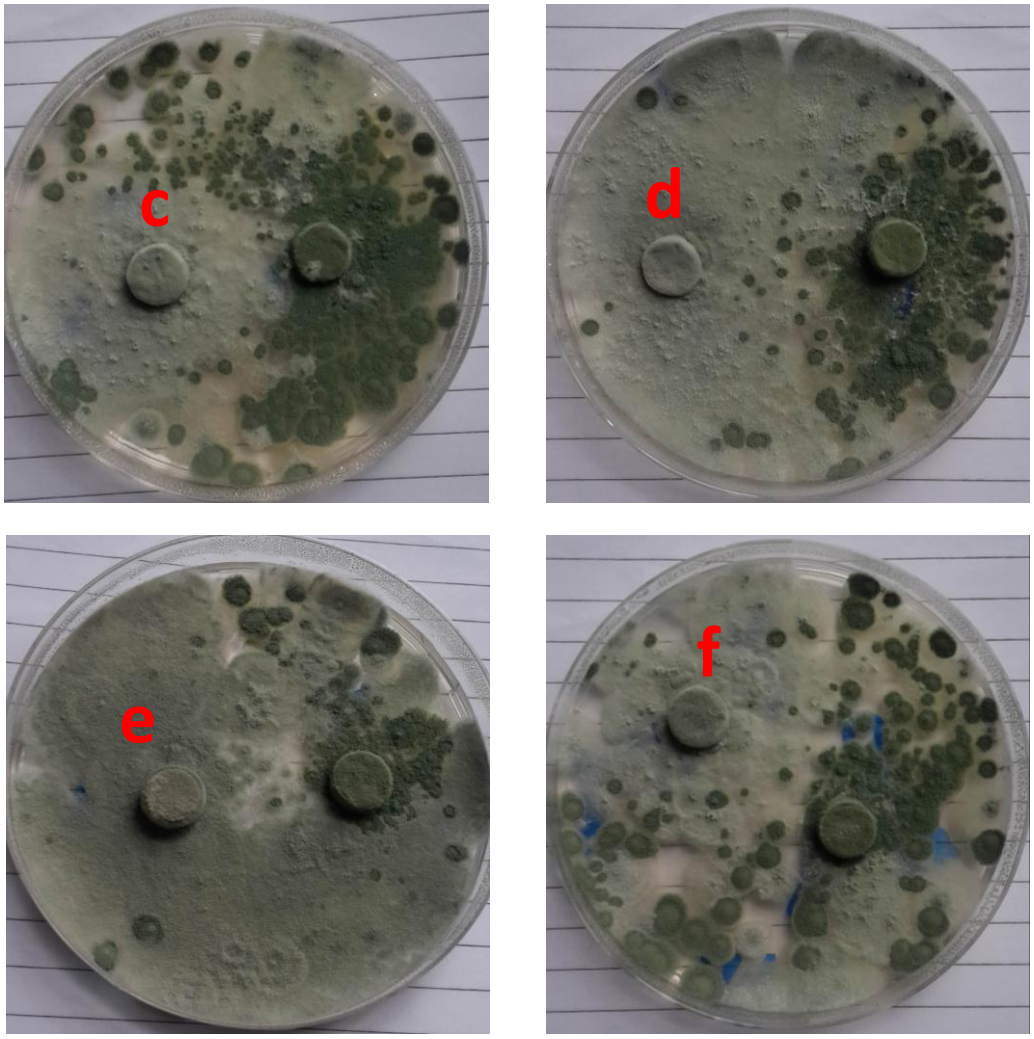
The experiments of testing the fruit maturity stages effect on enhance or delay infection by *Penicillium digitatum* . After selection mature and immature lemon fruits samples , inoculation both samples with similar (density) potential (10^{-9} spores / ml) and kept under the same condition . The result obtained from this experiment show that the effect of maturity of inoculated immature lemon fruits was delaying the infection about 6 days , while the inoculated mature lemon fruits was infect earlier after 4 days from inoculation time .

4.15 Study the interaction in vitro between different isolates of Penicillium species isolated from different fruit varities .

The results showed that there's no any significant differences between the sever *Penicillium spp*. isolated on Sabroid Agar . Both each two tested strain (isolate) was intermingling between each other which indicate that both tested isolates are from the same genus and species (Fig. 4.57 , and 4.58) .



(Fig. 4.57) The result interaction of different *Penicillium* species on Sabroid Agar after 8 days by using isolates from Lemon as mean compare with a: sour orange , b : Navel orange and c : Mandarine .



(Fig. 4.58) The result interaction of different *Penicillium* species on Sabroid Agar after 8 days by using isolates from Grapefruits as mean compare with
a : Blood orange , b : Sour orange , c : Mandarine ,
d : Navel orange , e : Lemon and f : Sweet orange .

4.16 Control of post-harvest citrus green mould disease .

No.	Treatments	Varities		Time of applied treatment	Appearance of symptoms
1	Hot water	1	Blood	5 minutes At 45 – 50 °C	After 3 day from inoculated with two methods of treatment
		2	Sweet		
		3	Sour		
		4	Navel		
		5	Mandarine		
		6	Lemon		
		7	Grapefruits		
2	Sea water	1	Blood	3 hours At ± 27 °C	After 3 day from inoculated with two methods of treatment
		2	Sweet		
		3	Sour		
		4	Navel		
		5	Mandarine		
		6	Lemon		
		7	Grapefruits		

3	**Khafour oil (Eucalyptus oil) Olive oil Johnson's baby oil**	**1**	**Blood**	**directly**	**After 3 day from inoculated with two methods of treatment**
		2	**Sweet**		
		3	**Sour**		
		4	**Navel**		
		5	**Mandarine**		
		6	**Lemon**		
		7	**Grapefruits**		
4	**Wax**	**1**	**Blood**	**directly**	**After 5 day from inoculated with two methods of treatment**
		2	**Sweet**		
		3	**Sour**		
		4	**Navel**		
		5	**Mandarine**		
		6	**Lemon**		
		7	**Grapefruits**		

CHAPTER FIVE

5. DISCUSSION

Penicillium digitatum the cause of citrus green molds respect is important post-harvest pathogen and cause serious losses reached 50 % of the total yield marketing quality and citrus industry **(Palou , *et al.,* 2001)**. The result of the current research was to study and investigate the pathogenicity , mechanism of infection and conformation of the identification of Penicillium species which cause green mold on local citrus fruit varities in vitro . The first experiment in this study was the isolation and identification of the causal agent of citrus green moulds *Penicillium digitatum* and blue moulds *Penicillium italicum* .

By using four types of artificial media to test the best growth of *Penicillium digitatum* give full growing colonies on Sabroid agar after 9 – 12 days of inoculation at 27°C . Typical colonies reaching 1.2 cm in diameter mostly greenish colour , growth very poor and restricted on nutrient ager , molar agar and no growth observed on Potato Dextrose Agar . Conidia typically cylindrical partly subglobose with broadly truncate base and evenly rounded tip , smooth – walled commonly 3.5µm . It produces an odour of decaying citrus fruits in vitro . The finding *Penicillium* have septate conidiophores the ultimate branches of which are vertically phialides (sterigmata) the phialides form basipetal chains of dry conidia , the identification comfer and indicate the presences of *P. digitatum* not *P. italicum* **(Domsch, K. H., *et al.*, 1980) .**

The results come similar with that reached by **(Raper and Fennell , 1965)** from this investigations of fungal identify , the result of the present study provide clear evidence the *p. digitatum* the mean causal agent of green molds on orange variates , Mandarine , lemon and grapefruit this pathogen occur in all most all citrus growing regions of the world and no anymore a new isolates of strain are found and the resultant of pathogenicity test , which include the change of wound location and depth on fruit surface will investigates strong and quick ability of *Penicillium* to reach the wound position in short time followed by successful penetration through citrus fruit peel , in this

study show there is no different in two depth 1cm , 2cm and any wounded in peel suitable and facilitate entry , growth of *Penicillium digitatum* and investigate this fungus growth in peel surface of fruit and absorb the nutrient material , water , from tissue of juice sacs which ensure this fungus from aerobic fungi . In addition to that , this experiment show abundance of inoculum potential of vital *Penicillium* spores in air .

These finding are in agreement with the results reported by **(Tarabih and El-Metwall , 2013)** . Both *P. digitatum* and *P. italicum* are sever wound pathogens that can infect the citrus fruits in the orchard the packing house and during transportation and marketing , the optimum temperature for mould growth is 27°C , no growth occurs above 30°C and growth is slow below 10°C , the cooling fruits for 10 hours at 5°C enhance the activity of growth enzymes inside fungal cell and sometimes break the dormancy **(Fu-Wen Liu , 2010)** . They reproduce very quickly and their spores are ubiquitous in the atmosphere and on fruit exterior and are simple and easily distributed by air currents due to dry and small size of spores . Therefore , the source of fungal inoculum in citrus orchards and packing houses is virtually continuous during the season **(Kanetis *et al.*, 2007)** . Citrus fruits can become soiled with conidia of the *P. digitatum* that are loosened in handling of diseased fruits .The conidia located in damage that laceration oil glands or penetrate into the albedo of the peel usually bring irreversible infection within 48 h at 20 – 25°C . The germination of conidia of *P. digitatum* inside rind wounds requires free water and nutrients **(Eckert and Eaks, 1989)** .

The prolongation of the marketing stage resulted in increased fruit weight loss and physiological changes of fruit tissues . The mean of penetration by post-harvest pathogens (Penicillium) in involved in the opportunistic type of infection in which the pathogens penetrate through a natural wound or one that occurs mainly after harvest or following storage stresses . This type of penetration may be exploited by the same pathogens that penetrate directly as well as others that require a breached or weak ened cuticle . However although pathogens may differ in their initial mechanisms of penetration . The colonization mechanism of the pathogens that penetration through wounds or directly are the same , in this study appears theirs no different between

methods of wounded by sterile knife or sterile metal borer which both facilitate the penetration of *P. digitatum* to cause infection . penetration by post-harvest pathogens through wounds in fruits resulted in earlier appearance of symptoms than direct penetration . This may indicate the Penicillium species are able penetrate wax layers of citrus fruits surface there layers do not seen to pose a serious barriers to penetration . In this case Penicillium well increase the incidence of infection increased thickness of the citrus fruits cuticle layer modulated the susceptibility to fungal attack **(Domsch et al., 1980) .** Since most of fruits and vegetables have thick cuticles it has been suggested that pathogens might secrete surfacetants in the form of proteins that reduce surface hydrophobicity and dissolve the wax layer pathogenesis related genes have long been known to be expressed only when the pathogen is inside the host once the host barriers have been overcome and the initial penetration has taken place , the pathogen e,g Penicillium switches from the biotrophic to the nerotrophic stage . The changes in due the transformation from a quiescent to an active infection in which cell death occurs and initial symptoms (Green mold) are observed .

The modulation of the citrus fruits tissues environment and the activation of mechanism of cell death induction . This implies that diffusible factors that have a direct or indirect phytotoxic activity are released by the pathogen . This conclusion by **Oladele and Owolabi (2016)** was similar to our experiment of pathogenicity . The explanation of successful infection of *P. digitatum* to all citrus varieties after artificial inoculation through mechanical wounds on peel surface , may due to the differences of peel thickness between orange types , Mandarine , Lemon , and grapefruits , which clear the thickness of peel studied fruits have important role in delay or enhance the infection citrus fruit with *P. digitatum* this differences may be attributed the presence of chemical compounds e.g volatile compounds , ascorbic and citric acids which change the acidity of peel substrate . The thickness of peel is considered to be a character of importance in many citrus fruits . It was noticed that peel thickness of Naval , Sour , Sweet and Bloody oranges decreased with increasing storage period , this decrease was significantly affect the defense mechanism and concentration of chemical compounds as phenol , citric acid , ascorbic acids and lingenine , some physical , nutritional and functional properties of the

fruit have play dramatic roles in facilitation of spore germination , penetration invasion and infection . Increase respiration level , sugar content and the antioxidant capacity , play major indirect roles in the enzymatic peeling because they affect the possibilities of shelf life time , (storage) and the quality of fruit juice , this conclusion was proved by **(Perez *et al.*, 2005)**.

From the molecular point of view pectin cellulose and hemicelluloses are mean chemical compounds of living cells will enhance *P. digitatum* spore to produce large amount of extra cellular enzymes to degreed and decompose the previous compounds in virulence manner , avoiding depriving any other microbic competitor specially at the beginning of invasion . Also the previous certain compounds are responsible for the harding and adherence of the peel (Skin) to the inside fruit tissues . These fore both pectinases and celluloses are needed for the enzymatic peeling . the celluloses are probably needed for the release of the pectinases the albedo and the pectinases contribute to the hydrolysis of the of the polysaccharides of the cell wall **(Ismail *et al.*, 2005)** . However the spore or the gape and the adherence of the peel to the fruit and it is thickness are different according to the citrus varities like Mandarine has thin peel with little inside space between peel and inside tissues will allow fungus to spread in quickly in short time compared with lemon or orange . Atmospheric temperature and change in pH to acidity level will drastically affect the Penicillium invasion **(Abd El-Morsie *et al.*, 2008) .**

From the experiment of lignification of injuries to citrus fruits studied and susceptibility to green mold caused by *P. digitatum* , the result shows of the injuired fruit surface which kept for 12 hours the peel cells were produce lignin before fungal entry then inoculated with *Penicillium* spores and kept at room temperature resulted in delaying the infection about 6 days but does not prevent the infection with *P. digitatum* . Accumulation of lignin occurred most rapidly at 30°C and relative humidity above 90% under these conditions lignin developed within 12h following injuiry , we found disagree results with **Brown and Ismail (1980)** that lignification was delayed or enhanced by peel oil or desiccation which caused damage to cells near the injury . These injuries were

easily penetrated by *P. digitatum* . The emerging spread and severe damage mold diseases on citrus fruits caused by *P. digitatum* species are increasingly recognized as presenting a worldwide threat to citrus production , quality and citrus industry . This is not new problem and *Penicillium* fungus have long been known to constitute a wide spread threat to citrus fruits , despite different control measures were applied in vivo and vitro *Penicillium* will develop a new resistant strains for survival.

Saprophytic growth on different substrate ability of all species can lead to extinction of the host and even allow the pathogen to persist in the absence of its host . The absence and lack of genetic breeding programs in citrus trees to develop and improve fruit quality and quantity while still remaining on mother tree will increase the resistance ability of post-harvest fruits to combit the infection of virulent Penicillium strains . Virulence is associated with rapid intra-host growth rates ultimately leading to rapid inter-host transmission specially for fungi like Penicillium have a high reproductive potential in large plant host range **(Abd-Allah, *et al.*, 2012) .** Virulence is a measure of the relative capacity a microbe to cause damage to a host . And high virulence is associated with rapid intra host growth rots , virulence of *Penicillium digitatum* increased by acidity of the host tissue , when fruits exposured to high density of *Penicillium digitatum* spores the capacity of virulence is increase **(Joseph, *et al.*, 2007) .** Each pathogens require special nutrient (chemical component) for growth found in specific plant without others . Therefor each pathogen infect specific host plant without the other **(Erminawati Wuryatmo, 2011) .**

Lemons are nonclimacteric fruit and have low respiration rates . They are therefore able to be stored for long periods of time . In contrast to other citrus varieties there are significant changes in the internal quality of lemon fruit during storage . During storage the percentage of juice increases (by up to 16 %) primarily due to the water stored in the peel . The acid content of fruit also increases (by up to 24 %) during storage and the peel colour changes from green to yellow **(Fu-Wen Liu , 2010) .**Therefore the mature lemon fruits infected earlier after four days compared with immature lemon fruits infected after 6 days . In the experiment of the interaction between different isolates of

Penicillium species in the plates showed non taxonomical differences between hyphal tip fusion which indicate all isolates are related to the same genus and species , despite the different colour of *Penicillium digitatum* isolated from infected grapefruits on Sabroid Agar media which show more greenish colour compared with other Penicillium isolates does not indicate or (appear) different strain , the greenish colour is result of producing more pigment but morphological are similar under microscopic examination .

All post-harvest disease of vegetables and fruits are due to inadequate handling , transport , packing , and storage facilities and further lack of technical knowledge about 10 – 15 % of the fruits are wasted from tree , this ways of infection result in decay and growth of *Penicillium digitatum* , which become activated because of the changing physiological state of the fruits and vegetables . Fruits , due to their low pH higher moisture content and nutrient composition are very susceptible to attack by *Penicillium digitatum* , which in addition to causing rots , may also make them unfit for consumption by producing mycotoxins **(Pankaj Sharma, *et al.*, 2013)** . In order to find suitable method of self – life extension more experimental work on citrus post-harvest research was needed post-harvest Penicillium moulds are considered the main cause of losses of fresh citrus fruits at the post-harvest , distribution and consumption levels , while reports on the level of these losses are conflicting a report by the Food and Agriculture organization **(FAO , 2010)** indicated that global average loss in Europe North America is about 29% . In the study growth of *Penicillium digitatum* in juice extracts of three different types of citrus parts – show that the *Penicillium digitatum* can grow in all type of fruit juice depending on citrus varities which indicate differences in chemical composition , water content , acidity , volatile oils , aromatic compounds . **(Davide and Droby 2016)** .

Efforts have been made to mimize their losses through developing better understanding of this biology of post-harvest diseases as well as by developing adequate post-harvest handling technologies and control strategies **(Prusky and Gullino , 2010) .** The experiment of which have been done in this research about modulation of environment pH of inside healthy of citrus fruit tissues show that all of used compounds

(concentrate and solution of apple vinegar , solution of bicarbonate sodium , solution of sodium chloride and sterile distal water used as control) with different value does not prevent or reduce of *Penicillium digitatum* growth but just lated the infection by used solution of bicarbonate sodium for 5 days , while the other materials does not have any effect which exposured the typical symptoms . In all tested citrus fruits which indicate the virulence of *Penicillium digitatum* was related to gene characters for each *Penicillium* strains . The results indicate tissue pH is an important parameter in aqueous environments , sine it affects the activates of enzymes and determines the expression virulence genes inside the host .

Modulation of pathogenicity factor – These post-harvest pathogenicity fungi modulate the host pH as a basis for expression of virulence factor during the colonization of the target host tissues . Pathogens may modulate their virulence by local acidification or alkanization the host tissue e.g colonization of acidified citrus and apple tissues by *Penicillium spp.* was enchareed by low pH . Application of neutralizing solutions depending on the type of pathogen . this approach is important for the control of post-harvest disease because its directly effect the germination of conidia and fungal colonization **(Askarne L.,** ***et. al.,*** **2013) .**

The mechanisms of citrus fruit colonization by *Penicillium digitatum* , elicit one types of symptoms soft rots in case of *Alternaria alternate* attacking citrus fruits may cause either soft or dry rot . The plants cell are made of several types of polysaccharides . The primary cell wall consists of cellulose and hemi–cellulose whereas the middle lamella has a high concentration of pectin . *Penicillium* that effect the pectin and primary cell wall lead to cell wall maceration and result in soft rots , whereas fungi attack the cellulose layer tend to kill the host cells but preserve the structure of the tissues **(Erminawati Wuryatmo , 2011) .**

Modulation of environmental pH of host tissues is as important parameter in aqueous environments , since it affect the activities of enzymes and determinations the expression of virulence genes inside the host **(Prusky and Yakoby , 2003) .** At change in the ambient pH during fungal attack may be expression of pathogenicity factor **(Skaria,** ***et***

***al.,* 2003)** . The effect of the pathogen on ambient pH the pathogen itself can dynamically after the local pH to fit its enzymatic arsenal with level of pathogenicity being related to the efficiency of the pH change . This ability lies behind the terms " alkaline fungi " and " acidic fungi " in case of all Penicillium species infection of citrus fruits such as *P. expansum , P. digitatum , P. italicum* **(*Hong – Yin Zhang, et al.,* 2004)** , use tissue acidification in their attack realized by accumulation of organic acids and/or H+ excretion e.g *Botrytis cinerea* decrease the host pH by secreting significant amounts of oxalic acid , while gluconic and citric acids are mainly secreted by P. expansum acidifies the tissues to pH levels of 3.5 to 4.0 **(Goldbach, *et al.,* 1997)** oxalic citric and gluconic acids exhibited strong Ca2+ chelating activities that weaken the plant cell wall by altering its mineral balance and their by affect the stability of cell membrane and cell wall pectate polymers **(Cunningham and Taverner, 2007)** .

The aerobiology study of *Penicillium* spore in our research was a new approach in post-harvest disease epidemiology . There are three steps needed to transport the air spore through are source take off dispersal deposition and effect many study of spore dispersal have been made in out-door environments . But our study was conducted in indoor environments to identify sources of Penicillium contamination in post-harvest citrus fruits . The aerodynamic characteristics of fungal spores . As fungal spores are much denser than air they will naturally fall through the air under the force of gravity . The rate at which they fall plays an important role in the dispersal and deposition of air borne spores . Spores that are fall quickly will tend to be less efficiently dispersal and more readily deposited than those that fall slowly . Any object falling through the air will eventually reach a " steady speed " called the settling speed fall speed . The settling speed of a spore depends on its physical properties : mass , size , and shape . However , environmental factors such as temperature or humidity can have small effects by altering the density air or spore . It self the gravitational forces acting on a spore are determined by its mass , while the drag forces depend on the size and shape of spore .

Release of *Penicillium* spores in air has three distict phases , take off , flight and landing so spores can be passively or actively released into the air . In our experiments

the result show the aerodynamic mechanisms of Penicillium spore under indoor environment indicate the high capacity and movement of released spore to reach wounded orange , lemon and grapefruits quickly through change or alternation of spore direction vertically and *Penicillium* spores become long time suspended in the air before fall-down because these spores it's dry and have low weight , therefore fall slowly , the samples putted on high distance infected earlier after 48 hours while samples putted on the ground surface infected later after 5 days . *Penicillium* species produce conidia in chain so they are mainly most of the fungi that employ an active spore release mechanism are basidiomycetes and ascomycetes **(Goldbach, *et al.*, 1997)** .Which all the *Penicillium* spores are released passively by wind force and air current , unfortunately , natural winds (and many indoor air flows) are turbulent which caused the concentration of spores in a spore plume diluted as the plume expands downwind . Because of this it difficult to define the " dispersal distance " for wind born spores . The control measure in this experiments were carried out in the work by using hot water , sea water , natural oils , mineral oils and wax show no significant effect and are non-significant important to reduce (minimize) or aberuncate of citrus infection by inoculated and non inoculated citrus fruits samples in *vitro* . The results in this study just delaying the infection for 5 days by wax and 3 days by used natural oils, mineral oils , sea water and hot water . Does not significant effect on growth of treated of the 5 treatments this does not agree with the other results reported by **(Tarabih and El-Metwally, 2013 and Carla Nunes, *et al.*, 2007) .** The absent of plant breeding for disease resistance , improving cultural method measurement and proliferation of resistant strains of the pathogen are the major problems associated with the used of these natural oil (olive oil , Eucalyptus oil) , mineral oil , wax and physical treat (hot water – sea water)

There is no one way to control plant diseases . The ever increasing of fungal pathogens to fungicides and growing concern about environmental pollution required an integrated complex of control measures . It became evident that the problem of Penicillium attack on citrus fruits can't be solved by any one system . Now adayes , develop a unique technique strategy to control postharvest diseases on fruits and vegetables by using safety nontoxic and environment friendly materials are urgent

necessary . present study was aimed to test different physical and chemical methods natural plant extract oils which may reduce and minimize the Penicillium mould on citrus fruits .

All control measures (methods) are aimed at protecting plants from becoming diseased rather than of curing them after they have become diseased . As long as plants and pathogens can be kept away from one another , no disease will be develop . The effective control of citrus post-harvest decays could maintain fruit quality , enhance fruit shelf life reduces the losses and increase grower's economic returns . The control of one or more diseases by using a single treatment method is not always effective . More effective control could be achieved using an integrated approach to prevent , reduce , and/or eradicate pathogen infections and disease development during pre-and post-harvest stages .

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APPENDIX

1. Preparation of the culture media:

Potato Dextrose Agar :

Potato infusion 4.0 gr

Dextrose 20.0 gr

Bacteriological agar 15.0 gr

Suspend 39 gr in 1 liter of distilled water (OXOID LTD., BASINGSTOKE , HAMP SHIRE) .

Sabroid Dextrose Agar :

Mycological peptone 10.0 gr

Glucose 40.0 gr

Suspend 65 gr in 1 liter of distilled water (OXOID LTD., BASINGSTOKE , HAMP SHIRE) .

Nutrient Agar :

Beef extract 3.0 gr

Peptone 5.0 gr

Agar 15.0 gr

Distilled water 1000 ml .

Mueller Hinton Agar :

Beef extract 2.0 gr

Acid Digest of Casein 17.5 gr

Starch 1.5 gr

Agar 17 gr

Suspend 38 gr in 1 liter of cold distilled water.

2. Chemical composition in citrus fruits species

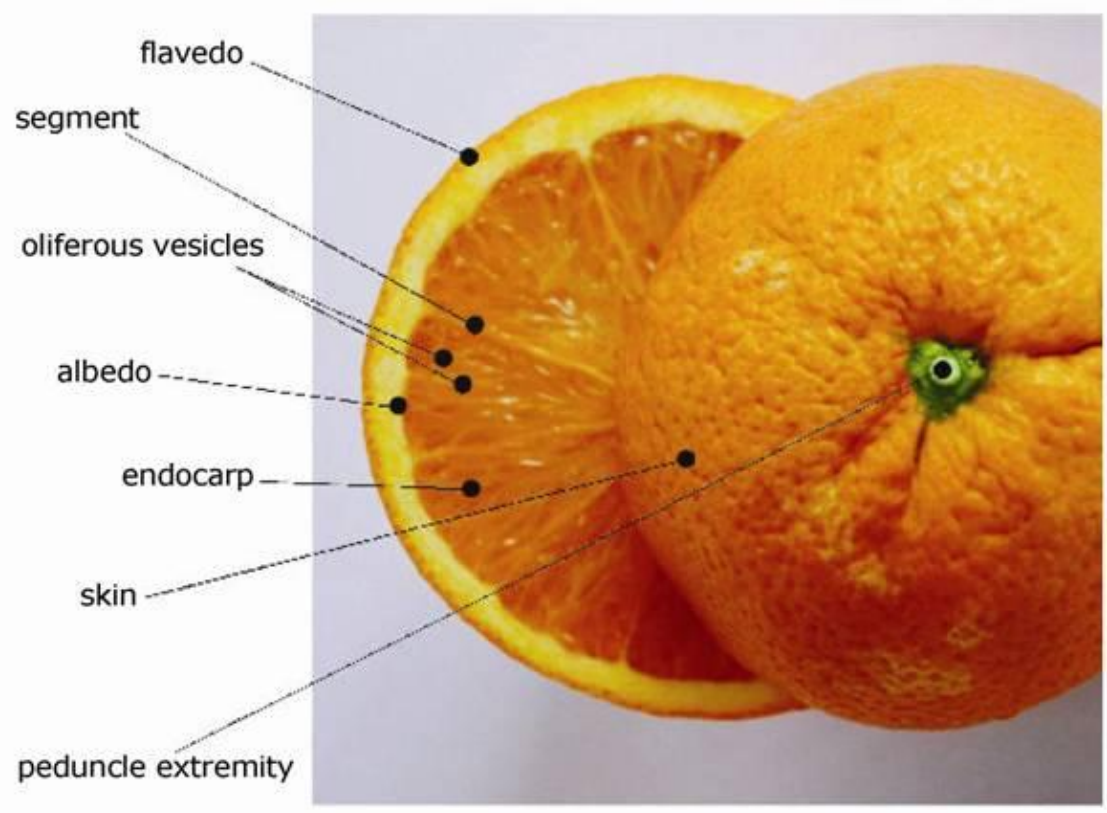
Citrus fruits species	Vitamin C mg/100 ml juice	Brix	TA % citric acid
Grapefruit	36.35 – 38.3	9.9 – 14.4	0.29 – 2.56
Mandarin	33 – 42	10.4 – 12.65	0.66 – 0.93
Orange	60.26	11.40	1.82
Lemon	45.9 – 56.66	9.1	6.7

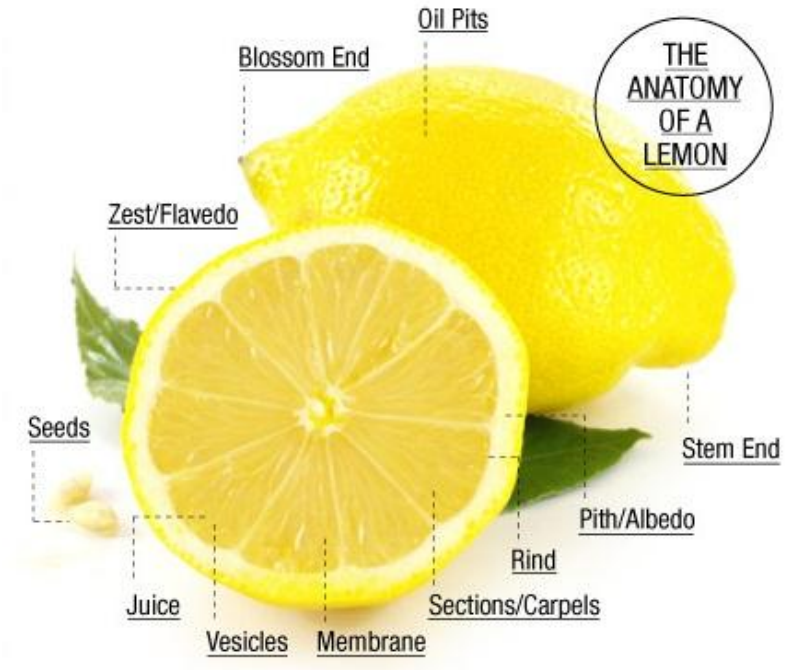
3. Nutritional facts about citrus fruit:

	Orange	Grapefruit	Mandarine
Weight (g)	131	236	84
Energy (kcal)	62	78	37
Fibre content (g)	3.1	2.5	1.7
Ascorbic acid(mg)	70	79	26
Folate (mcg)	40	24	17
Potassium (mg)	237	350	132

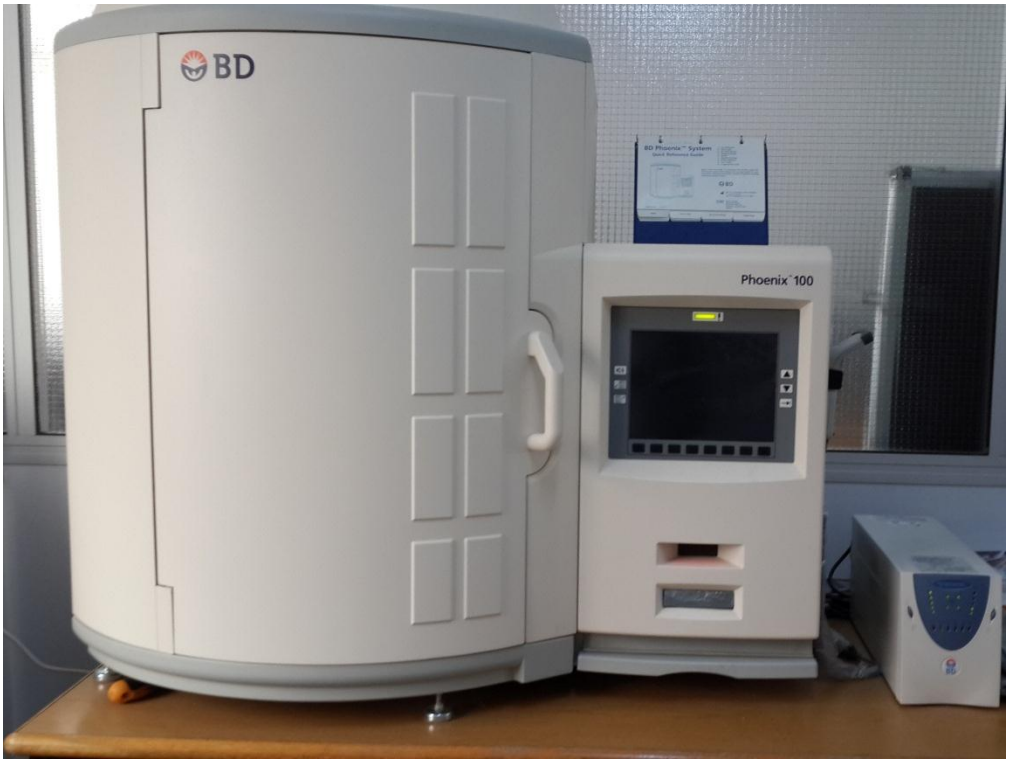
Source: Gutherie and Picciano, 1995.

4. The Anatomy of citrus fruits e.g (Orange and Lemon) :





5. Identification of unknown microflora on the surface of studied fruits by Phoenix system ™ 100 .



الـــــخـــــــلاصـــــــــة

تعتبر الأمراض الفطرية ما بعد الحصاد التي تصيب ثمار الفاكهة من أهم وأخطر الكائنات الدقيقة الممرضة التي تسبب خسائر إقتصادية تصل إلي 50% والتي قد تتطور وتنتشر أثناء النقل والتخزين وتعتبر ثمار الحمضيات (الموالح) من أهم المنتجات الزراعية في ليبيا خاصةً في المنطقة الغربية وقد تتعرض هذه الثمار للإصابة بالعديد من الكائنات الممرضة أهمها الفطريات والبكتيريا .

في هذه الدراسة والتي سلطت الضوء على العفن الأخضر المتسبب عن البنسليوم (*Penicillium digitatum*) والذي تم تأكيده بعد عزله وتعريفه كمسبب لهذا المرض من خلال انتشار هذا المرض بشكل كبير على ثمار الحمضيات (الموالح) سواءً كانت المحلية أو المستوردة وقد تم اختيار هذه الدراسة لمعرفة سلوك وطبيعة الإصابة وآليتها على ثمار الحمضيات ما بعد القطف حيث بدأنا بدراسة الإمراضية على 4 أصناف وهي : البرتقال بأنواعه الأربع (الدمي ، الحامض ، الحلو ، بوصرة) ، اليوسفي ، الليمون ، الشفشي ، ومن خلال النتائج المتحصل عليها تبين أن الفطر المسبب لهذا المرض بعد تطبيق فرضيات كوخ في منطقة الدراسة هو (*Penicillium digitatum*) ومن خلال دراسة القدرة الإمراضية (Pathogenicity test) تبين أن الفطر قادر على إصابة الأصناف الأربعة المذكورة بنفس الشراسة بعد استخدام ثلاثة طرق من العدوى الصناعية في المختبر .

من خلال تجربة البرودة تبين أن درجة الحرارة المثلى لنمو الفطر 25–27م° ±1، أما من خلال دراسة تأثير عمق وموقع الجروح تبين أنه ليس لها تأثير فعال على حدوث الإصابة في الأصناف المختارة ، وبعد دراسة الفلورا الطبيعية الموجودة على أسطح الثمار المدروسة تبين وجود *Staphylococcus hominis* على أسطح صنف البرتقال (الدمي ، الحامض و بوصرة) واليوسفي ، ووجد أيضاً *Staphylococcus haemolyticus* على أسطح صنف الليمون ، وكذلك وجد *Staphylococcus warneri* على أسطح صنف الشفشي ، وأن هناك فروق كبيرة في وجود أو عدم وجود الميكروفلورا على أسطح هذه الثمار حيث أن الثمار المزال عليها الفلورا

الطبيعية بعد غسلها أسرع في الإصابة مقارنةً بالثمار المتواجد عليها هذه الفلورا بشكل طبيعي وبشكل عام ، أما اختبار فعالية ثلاثة أنواع لعصارة عينات قيد الدراسة (Peel Juice – Sac Juice – Whole fruit) تبين أن نمو الفطر في عصارة القشرة أسرع منه في باقي العصارات وأن الفطر قيد الدراسة تابع للفطريات الهوائية الإجبارية .

وعند تفسير التدخل لتغيير الوسط الحامضي لأنسجة الثمار المدروسة باستعمال (خل التفاح المركز 4 – 5 % ، محلول خل التفاح ، محلول بيكربونات الصوديوم ، محلول ملح الطعام ، ماء معقم " كنترول ") اتضح أن هذه المواد ليس لها دور في منع الإصابة ولكنها ساهمت في تأخيرها بعض الوقت باستخدام (محلول بيكربونات الصوديوم) لمدة خمسة أيام .

ومن اختبار دراسة تكوين اللجنين في قشرة عينات الدراسة بعد جرحها وتركها لمدة 12 ساعة ومن ثم حقنها تبين أنها ساهمت في تأخير الإصابة لمدة ستة أيام ولكن لم تمنعها من الإصابة بهذا الفطر ، أما اختبار دراسة تأثير حركة جراثيم الفطر في الهواء داخل الغرفة تبين أن هذه الجراثيم خفيفة الوزن فهي من الجراثيم الجافة لذلك تظل عالقة في الهواء لبضعة أيام وحركاتها سقوطها وترسيبها على أسطح الثمار عمودياً أسرع منه أفقياً وأن تخزين الثمار قيد الدراسة على مستويات مرتفعة من مستوى سطح الأرض لا يمنعها من الإصابة بالفطر . ومن دراسة تخصص العزلات المختلفة اتضح أن كل نوع من فطريات الدراسة متخصص لإصابة ثمار محددة دون غيرها .

ومن دراسة تأثير نضج ثمار الليمون على تقديم أو تأخير الإصابة بفطر البنسليوم تبين أن ثمار الليمون الناضجة أصيبت بشكل أسرع خلال أربعة أيام مقارنة بثمار الليمون الغير ناضجة والتي أصيبت بالعدوى بعد ستة أيام ، ومن دراسة تفاعل العزلات المختلفة في المعمل على الوسط البيئي (Sabroid Agar) تبين أن عزلات الدراسة تابعة لجنس واحد وهو البنسليوم .

وأخيراً فإن النتائج المتحصل عليها من برنامج مكافحة الفطر على الثمار المدروسة المحقونة والغير محقونة بالفطر باختبار طريقتين فيزيائيتين وهما : الغطس في الماء الساخن لمدة 5 دقائق عند درجة 45 – 50 م° ، والغمر في ماء البحر لمدة 3 ساعات ونوعان من الزيوت

الطبيعية (زيت الزيتون – زيت الكافور) وزيت معدني (زيت بيبي جونسون) وأخيراً الشمع تبين أنها ليس لها دور في منع الإصابة بالفطر ولكنها ساهمت في تأخير موعد الإصابة لمدة ثلاثة أيام بواسطة الشمع و خمسة أيام لباقي المواد الأخرى .

دراسة استراتيجيات الإصابة ما بعد الحصاد لأنواع البنسليوم على ثمار الحمضيات المصابة بالعفن

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علم النبات

جامعة بنغازي

كلية العلوم

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