Research Article **ISSN: 2349 – 4492**

Asian Journal of Research in Biological and Pharmaceutical Sciences

Journal home page: www.ajrbps.com

https://doi.org/10.36673/AJRBPS.2020.v08.i03.A15

ISOLATION AND IDENTIFICATION OF GREEN MOULD PENICILLIUM SPECIES ON HARVESTED CITRUS FRUIT

Saleh H. M. El. Majberi*[1], Nagwa S. A Alraaydi[1] and Mona E. Elyass[2]

[1*]Department of Botany, Sciences Faculty, Benghazi University, Libya.
[2]Department Lab Medicine and Medical Microbiology, Faculty of Medical Technology, Omar Al-Mukhtar University, Al-Bayda, Libya.

ABSTRACT

Green mould disease is one of the most dangerous and common disease infect many fruit types, specially citrus fruits. Infection may be start from the field and lately develop in storage. This disease is more obvious on orange fruits. Four citrus varieties were selected as follow: Orange, Mandarine, Lemon and Grape fruits.10kg from the previous citrus varieties were randomly collected from the local markets in Benghazi city in winter 2018. Immediately the collected fruit samples were kept in sealed sterile plastic bags and placed at room temperature 27 ± 1. Four different selective media were chosen: (Sabroid Agar "SDA", Potato Dextrose Agar "PDA", Molar Agar and Nutrient Agar) and isolated the causal agent from rots and cultivated at 27 ± 1 for one week and identified by morphological and cultural characteristic of growing colonies conidiophores conidia of *penicillium sp*. Which it is identified as *penicillium digitatum.*

KEYWORDS

Citrus fruits, *Penicillium sp* and Postharvest diseases.

Author for Correspondence:

Saleh H. M. El. Majberi,

Department of Botany,

Sciences Faculty, Benghazi University, Libya.

Email: idress.hamad@omu.edu.ly

INTRODUCTON

Commonly cultivated citrus belongs to three genera; Citrus spp, Fortunella spp. and Poncirus spp. of the family Rutaceae (Cohn, 1972)[1]. 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)[2].

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)[3]. The genus citrus contain all the species widely cultivated in West Africa including Nigeria (Nasiru *et al,* 2015)[4]. 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 66 mg of vitamin C a 100 percent of the daily dietary requirement for adults (Alfred and Patrick, 1985)[5]. According to (Pitt, 1979)[6]. 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)[7].

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)[8]. 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. Pummelous (Grape fruit) and the common acid group such as lemon and lime (Swingle, 1967)[2].

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)[3]. The genus citrus contain all the species widely cultivated in West Africa including Nigeria (Nasiru, *et al,* 2015)[4]. 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.

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)[9].

Aim of study

The aim of this research is to isolate and cultivate the causal agent of green mould disease on local and imported citrus fruits and to identify the penicillium to species level.

MATERIAL AND METHODS

Sample collection and Screening of orange fruit samples

At the begnin total 40kg from different citrus varieties have been randomly collected, starting from first of December 2016 until the end of May 2017. 10kg of each citrus varity have been taken including Orange (Sweet, Navel, Sour and Blood), Mandarine, Lemon and Grapefruits. Immediately the collect fruit samples were kept in sterile plastic bags and sealed to prevent contamination and loss

of moisture. All collect fruits were storage at room temperature 27 ±1 until running the experiments.

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

Isolation and cultivation of causal agent

Spores of growing Penicillium species was isolated from the surface of infected citrus fruits which collected previously by using sterilized needle few spores have been transferred from infected site (blue and green colored) and placed in the center of the 9 cm of petridish. 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 sp.* growth. The used media was supplemented with chloramphenicol (250mg per liter) as a bacteriostatic agent (Dawson, *et al,* 2001)[10].

Three replicates were used from each previous media after inoculated with *Penicillium sp*. spores. All plates were covered with cellophane to prevent contamination with another microorganism and kept at 27ºC for one week after that the petri dishes were transferred to the reifreidge to stop fungal growth (Kreuawab, *et al,* 2007)[11].

Identification of penicillium species

Temporary slides were prepared from pure growing moulds colonies then examined with light microscope. Morphological characters of mycelia and spores of *penicillium sp.* structures were study carefully. Photographing the observed fungus structures were Identified it was done by based on text book and special reference (Domsch K. H, *et al,* 1980)[12].

RESULTS AND DISCUSSION

Results

Visual screening of tested citrus fruit samples

The total number of four citrus fruit varities was 70 which including: Orange varities (Blood, Sweet, Sour and Navel), mandarine, lemon and grapefruits.

From macroscopic investigation of the previous citrus varities Table No.1 and Figure No.3 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.

Result of cultivation of *Penicillium* species from four citrus varities

The best growth for isolated agent on the sabroid agar Figure No.4.

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

On Sabroid agar the fungus formed radial colonies of white mycelia reaching 1cm dim in10-14 days at 24-27ºC with greenish color and the substrate mycelium was olive green with a clear zone observed (Figure No.5 to Figure No.9). 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 to 5 x 3-3.35µm. 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.*

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)[13]. The result of the current research was to study the identification of Penicillium species which cause green mold on local and exported citrus fruit varities in vitro. The first experiment in this study was to 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 27ºC. Typical colonies reaching 1.2cm 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)[12].

The results come similar with that reached by (Raper, *et al,* 1965)[14] 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 of all citrus growing on the regions of the world and no anymore a new isolates of strain are found.

These finding are in agreement with the results reported by (Tarabih, *et al,* 2013)[15]. 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 27ºC, no growth occur optimum temperature for mould growth is 27ºC, (Fu-Wen Liu, 2010)[16].

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)[17]. 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 - 25ºC. The germination of conidia of *P. digitatum* inside rind wounds requires free water and nutrients (Eckert and Eaks, 1989)[18].

Table No.1: Citrus varity, number of non infected and number of infected fruits

S.No	Citrus varities	Number of Non infected fruits	Number of Infected fruits
1	Orange (*Citrus Sinensis*)	-	-
2	Blood orange (*Citrus Sinensis*)	65	5
3	Sweet orange (*Citrus Sinensis*)	69	1
4	Sour orange (*Citrus Aurantium*)	68	2
5	Navel orange (*Citrus Sinensis*)	65	5
6	Mandarin (*Citrus Reticulata*)	67	3
7	Lemon (*Citrus Limon*)	66	4
8	Grapefruits (Citrus Paradisi)	66	4

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

Table No.2: Four types of used media and resulted *Penicillium* growth

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

Figure No.1: Grapefruits infected with green mould

Figure No.2: The growth of *Penicillium spp.* after 5 days in Grapefruits

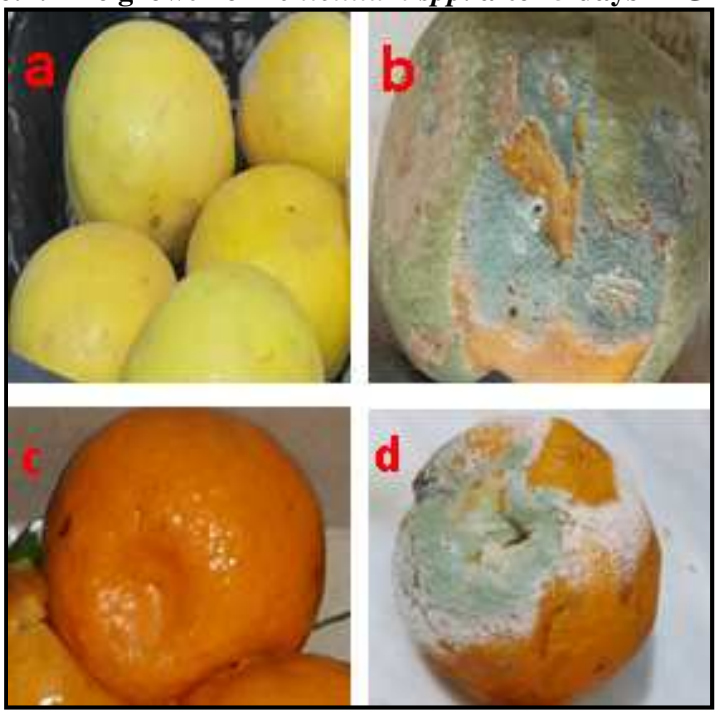

Figure No.3: Macroscopic investigation of the citrus varities

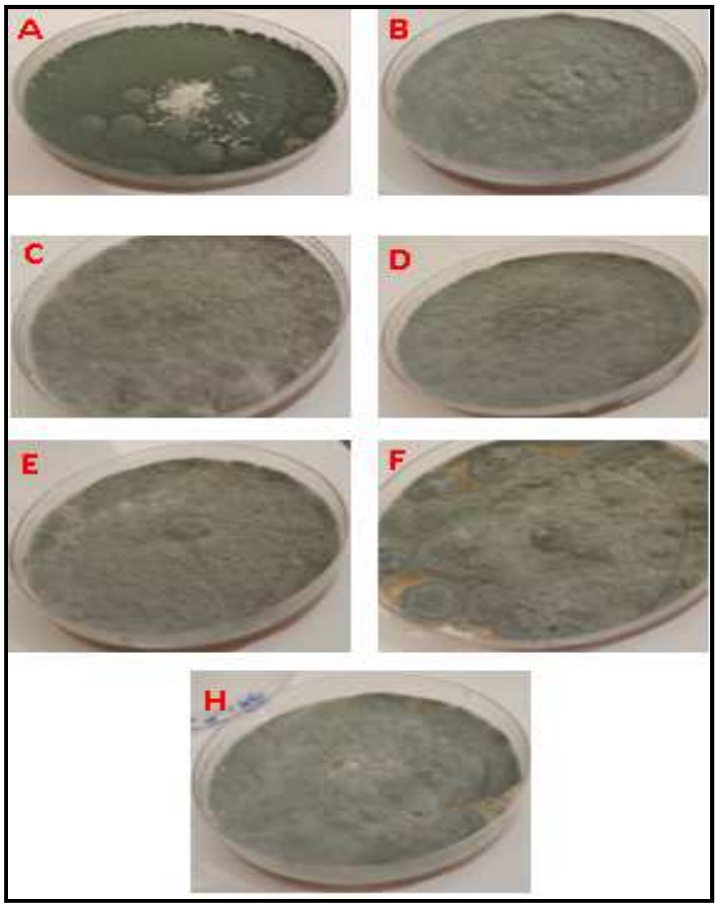

Figure No.4: The best growth for isolated agent on the sabroid agar

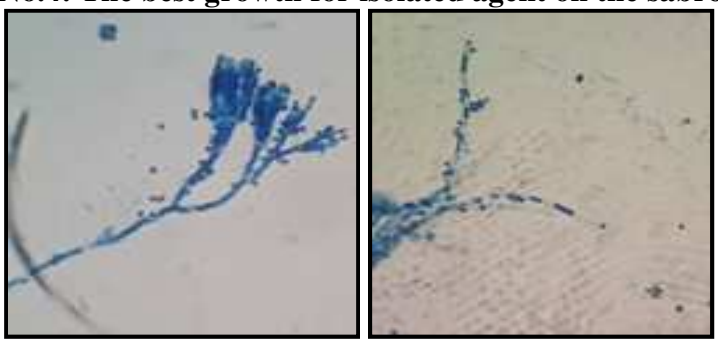

Figure No.5: (a) *Penicillium digitatum* stained with methyl cotton blue (b) *Penicillium digitatum.* The hypha and nucleii present inside it and septa between cells of hypha

Figure No.6: (a) *Penicillium digitatum.* The hypha branching and vertical phialides
(b) *Penicillium digitatum.* Conidial chains and Conidia

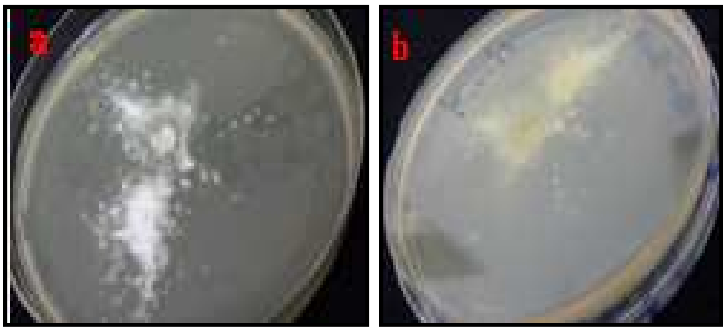

Figure No.7: (a) The growth of *Penicillium spp.* after 24 hours isolated from Grapefruits and (b) color of pigment Produced by *Penicillium spp*

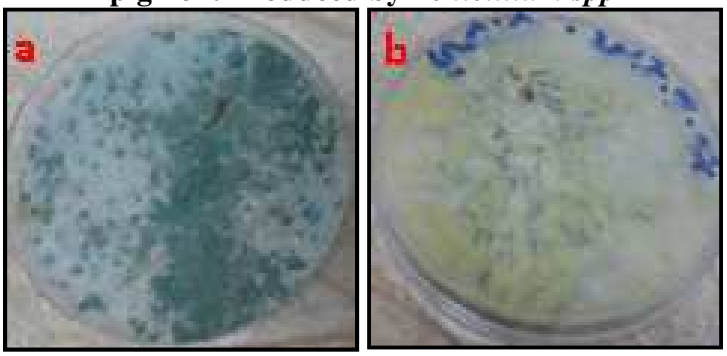

Figure No.8: (a) The growth of *Penicillium spp.* after 5 days in Grapefruits (b) color of pigment Produced by *Penicillium spp.*

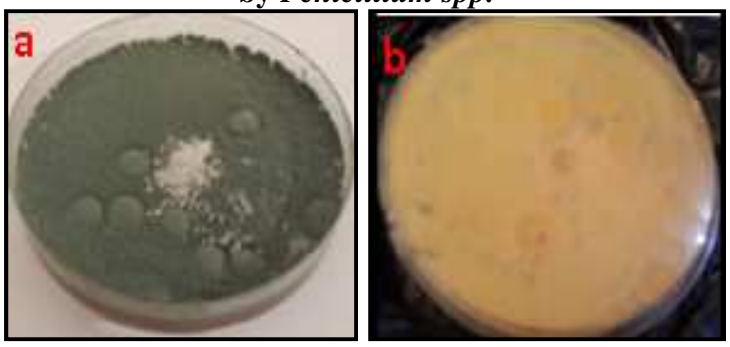

Figure No.9: (a) The growth of *Penicillium spp.* after 8 days in Grapefruits (b) color of pigment Produced by *Penicillium spp*

CONCLUSION

In this study, showed that the most common fungus causing green molds on studied citrus fruits, Orange varities, Mandarine, Lemon and grapefruits in eastern Libya is *Penicillium digitatum.*

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Botany, Sciences Faculty, Benghazi University, Libya for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Cohn E. Nematode diseases of citrus, (Economic Nematology), *Academic Press,* 1972, 215-244.
2. W. Reuther. H. J. Webber and L. D. Batchelor, Swingle W T and P C Reece. In the citrus industry, *Univ. Calif. Press,* 1, 1967, 190-430.
3. FAO. Food and agricultural organization FAOSTA agricultural data of world citrus area harvested and production statistics, *FAO,* 2004, 42-64.

4. Nasiru A M, Salau A I and Yakubu M. Fungi Associated With Spoilage of Citrus Sinensis in Fruits and Vegetables Market, Sokoto, Nigeria, *Global Advanced Research Journal of Agricultural Science, Special Anniversary Review,* 4(12), 2015, 919-922.
5. Alfred I I, Patrick O N P. Food values of some tropical fruits and vegetables: In the integrated food science and technology for tropics, *Pantry Citrus,* 2nd Edition, 1985, 305-306.
6. Pitt J I. The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces, *Academic Press, New York,* 1979.
7. Holmes G J, Eckert J W, Pitt J I. Revised description of penicillium ulaiense and its role as a pathogen of citrus fruit, *Phytopathology,* 84(7), 1994, 719-727.
8. Oadi N, Matny and Faiza I. Al-Rawi. Use of Antimicrobial and Biological Agent to Control Green Mold on Orange Fruit, *International Journal of Applied Agricultural Research, Research India Publications,* 7(1), 2012, 45-54.
9. El-Gali, Zahra Ibrahim. Control of penicillium digitatum on orange fruits with calcium chloride dipping, department of plant protection, faculty of agriculture, Omer Al-Mukhtar University, El-Beida Libya, *Journal of Microbiology Research and Review,* 2(6), 2014, 54-61.
10. Downes F P and Ito K. Compendium of methods for the microbiological examination of foods, *American Public Health Association, Washington, D.C,* 4th Edition, 2001.
11. Kreuawan Thonglem, Abhinya Plikomol and Wasu Pathom-aree. Growth inhibition of Penicillium digitatum by antagonistic microorganisms isolated from various parts of orange tree, *Maejo International Journal of Science and Technology,* 1(02), 2007, 208-215.
12. Domsch K H, Games W and Anderson T. Compendium of Soil Fungi, *London Academic Press,* 1, 1980, 8-860.
13. Palou L, Smilanick J L, Usall J and Vinas I. Control of postharvest decay blue and green molds of oranges by hot water, Sodium carbonate and sodium bicarbonate, *Plant Dis,* 85(4), 2001, 371-376.
14. Raper K B and Fennell D J. The genus Aspergillus Williams and Wikins, *Baltimore USA,* 1965, 9-686.
15. Tarabih M E and El-Metwally M A. Effect of jojoba oil and boric acid as postharvest treatments on the shelf life of Washington navel orange fruits, *International Journal of Agricultural Research,* 9(1), 2014, 1-16.
16. Fu-Wen Liu. Development and application of citrus storage technologies with concurrent consideration of fruit quality preservation, energy use and costs, *Technology on Reducing Post-Harvest Losses and Maintaining Quality of Fruits and Vegetables,* 2010, 26-47.
17. Kanetis L, Forster H and Adaskaveg J E. Comparative efficacy of the new postharvest fungicides azoxystrobin, fludioxonil and pyrimethanil for managing citrus green mold, *Plant Dis,* 91(11), 2007, 1502-1511.
18. Eckert J W and Eaks I L. Postharvest disorders and diseases of citrus fruits, In: The citrus industry: Crop protection, Postharvest technology and early history of citrus research in University of California Press, *Berkeley,* 5, 1989, 179-269.

Please cite this article in press as: Saleh H. M. EL. Majberi *et al. In* Isolation and identification of green mould penicillium species on harvested citrus fruit, *Asian Journal of Research in Biological and Pharmaceutical Sciences,* 8(3), 2020, 120-127.