The Sensitivity of some Types of Citrus Fruits, the role of the Propolis and the Color of Storage Bags in the Development of Green Mold Caused by Fungi *Penicillium Digitatium*

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Abstract

The results of the study indicated that green mold caused by *Penicillium digitatium* was noticed in all types of citrus fruits (orange, mandarin, bitter orange, lemon). The most sensitive among these fruits was the mandarin with 56.12% infection, followed by orange with 40.18%. On the other hand, the bitter orange and the lemon showed more resistant to the disease, and the their infection percentages were (32.14% and 30.55% respectively. The results indicated that the color of the bags had a great effect on the development of the disease where the infection percentages when using light green bags and light yellow bags were (19.68%, 17.24%) respectively. While for the dark black bags, the infection percentage was 13.73%. The use of porpolis in all concentrations (6, 8, 10, 12) g/l did not lessen the size of the infected area compared with contaminated treatment by pathogen.

Keywords: *food; nutrition; toxicity; disease; humidity*

Introduction

Citrus crops suffer from many diseases, whether before or after the harvest, which cause losses in the quantity and quality of production. Perhaps the most important of these diseases are fungal diseases, especially the green mold disease, as the losses resulting from this disease in 2017 were estimated at 50 million metric tons in cultivation areas of all citrus fruits [(15)]. This disease is spread in most citrus cultivation areas in the world, especially in warm areas with high humidity [(1,2,3)]. The risk this disease can cause increases when appropriate conditions are available such as a temperature ranging between 5 - 25 ° C and relative humidity of more than 80% with the availability of lighting required for the production of spores [(4,5)]. Scratches formed during harvesting and the presence of insects also help in the development of the disease. Moreover, P. digitatium has the ability of producing some cellulose and pectin enzymes that increase the risk of this disease [(6)]. Several methods were used to fight this disease, such as the use of paraffin wax, evaporation inhibitors [(7)] and chemical pesticides [(8)]. In order to know the importance causes of the disease, its severity on citrus fruits and how to reduce it, the study was conducted according to the following axes:

1- Investigating the sensitivity of several citrus to the infection of the green mold disease.

2-The role of storage bags and role of propolis in the development of the disease.

Materials and Methods

This experiment was carried out at the University Of Anbar/ College of Agriculture/ Plant Protection Department/ Plant Pathology Laboratory for the winter season 2017-2018. Fruits of different types of citrus were collected (orange, bitter orange, lemon, and mandarine) from the orchards of the district of Heet, Ramadi and Fallujah. These fruits showed symptoms of infection with the disease [(9)]. These infected fruits were placed in sterile polyethylene bags and brought to the laboratory, then pieces from the infected area were extracted from the fruits located in the collection areas. The pieces

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taken were 1 cm in length. They were later placed in petri dishes containing PDA media. They were later transferred to the incubator at a temperature of 25 ° C for a period of 7 days. After that, the Pathogenic fungus were identified according to the species key [10], and the pathogen was diagnosed to the type level depending on the type key [11].

Sporophobia Preparation

Sporophytes were prepared according to [12], 1 ml sterile distilled water was added to each growing petri dish in which the *Penicillium digitatium* was observed, and the water was moved stirred the plate by using a sterile L-shaped glass rod. Then the water containing the sporophyte of the fungus was extracted and put into a test tube A 9 ml container of sterile distilled water. After that, a series of dilutions were added until the concentration reached is 10-7 spores/ml. This concentration is determined using a Haemocytometor slice.

The sensitivity of some Citrus Fruits to Green Mold Caused by the Fungus *Penicillium digitatium*

This experiment was carried out according to the randomized complete block design (RCBD). The treatments were divided as follows

1. T1 = treatment of orange fruit
2. T2 = treatment of mandarin fruits
3. T3 = treatment of (bitter orange) fruits
4. T4 = treatment of lemon fruits
5. T5 = contaminated pathogen treatment
6. T6 = sound comparison treatment

Each treatment included 4 replicates. In each iteration, there were 3 healthy homogeneous fruits from all types of citrus used. Then all fruits were scratched with superficial scratches orthogonally by a sterile knife of 1 cm length, then all treatments were contaminated, except for the T6 treatment, with the pathogenic fungus of the concentration of 10-7 spores/ ml, and then the fruits of each iteration were placed in a sterile polyethylene bag perforated by a cork drill 5 ml. Then, it was left under the natural conditions of the laboratory. Measurements were taken after (10) days by measuring the rate of fungal growth of the infected spot, and then calculating the percentage of the infected area according to the formula:

% infected area = (Fung growth rate)/(The peripheral growth rate of the fruit) × 100.

The effect of Citrus Storage Bags Color on the Development of Green Mold Caused by Fungus *Penicillium digitatium*

In this experiment, orange fruits, bitter orange, lemon, and local mandarin were used as homogeneous healthy fruits were selected. The treatments were distributed according to RCBD design. The treatments were conducted as follows:

1. T1 = dark black storage bags
2. T2 = Transparent green storage bags
3. T3 = transparent yellow storage bags
4. T4 = exposed orange fruits contaminated with the pathogen
5. T5 = healthy exposed orange fruits

Four replicates were used for each treatment at the rate of 3 orange fruits per replicate. These fruits were scratched superficially, as explained in the previous paragraph. After that all the treatments were contaminated, except for the T5 treatment, by spore concentration at 10-7 spores/ ml, then the bags were sealed tightly and left under laboratory conditions for 10 days. Then the fungal growth of the infected area caused by the fungus was measured. The Effect of Different Propolis Concentrations on % of the *P. digitatium* Infection Area

Propolis was obtained from the apiary section of the Plant Protection Department/ College of Agriculture/ University Of Anbar, then (6, 8, 10, 12) grams of propolis were weighed. Then each weight was placed in a glass beaker containing 1000 mL sterile distilled water. Then the water was heated to a temperature of 60-70 ° C with constant stirring for 5 minutes. The resulting solution was later sprayed on the scratched orange fruits, and then the fungus *P. digitatium* was sprayed at a concentration of 10-7 spores/ ml. The same process was repeated for all weights of the propolis In the same

way. Three replications were used for each treatment and three oranges for each iteration. The contaminated comparison treatment was treated with the same fungus solution on the scratched fruits. The treated fruits were left for 10 days under laboratory conditions, after which the fungal growth rate of the infected area was measured for each treatment, and the percentage of the infected area was calculated according to the following formula:

% for the infected area = (Fung growth rate)/ (The peripheral growth rate of the orange) × 100

Results and Discussion

Table (1) shows that green mold disease caused by *P. digitatium* is spread in all citrus cultivation areas and in all types of citrus fruits used in this study, and this confirms what is reported in [13, 14], that this disease is spread in most areas of citrus cultivation In the world. The table also shows that the severity of the disease increases on the fruits of these citrus after the harvest [15, 16]. The risk of this disease is found to be due to the ability of the pathogen (*P. digitatium*) to grow in a temperature range of 5-25 ° C when moisture is available at a rate ranging from 75 - 80% (1).

Table (2) shows that the fruits of the citrus fruits used (orange, bitter orange, lemon, mandarin) varied in their sensitivity to green mold disease, as mandarin fruits recorded the highest levels of sensitivity, and the percentage of infection area reached 56.12%, followed by orange fruits with a percentage of 40.18%, while the fruits of lemon and bitter orange were less sensitive to green mold, as the percentage of the infection area was (30.55%, 32.14%) respectively. This difference in the sensitivity of the citrus fruits used may be due to the difference in the thickness of the outer wall of the fruits and the difference in the cellular composition of the cell wall of these fruits, especially in the difference in the concentration of ethylene gas of the most important means of defense in citrus fruits [17,18].

Table (3) shows that the color of the storage bags used in preserving citrus fruits had an effect on the development of infection with green mold caused by the fungus *P. digitatium*. Particularly, transparent colors such as transparent yellow and transparent green recorded an infection rate of 17.24% and 19.68%, respectively, while the dark black color was the least affected in the development of the disease, as the percentage of the infected area was 13.73%. This effect is ascribed to the color of the storage bags which affects the amount of light passing through them, which is a key factor in the formation of the pathogen spores. The table also shows that the overlap between the fruits of different types of citrus fruits and the color of the storage bags has a significant impact on the development of the disease. As for the fruits of bitter orange placed in dark bags, it was the lowest in the infection rate with 2.38%. As illustrated in Table (4), the use of the propolis did not lead to any Significant reduction in the infected area, and the reason may be attributed to the fact that the heat used led to the decomposition of the substances has a role in resisting the disease.

Table (1) Results of isolating *P. digitatium* from different citrus fruits in some areas of Al Anbar Governorate

Type of Fung Isolated from the infected fruits	Fruit Type	Fruits Collection Areas
P. digitatium	Orange	Ramadi
P. digitatium	Bitter Orange	
P. digitatium	Mandarin	
P. digitatium	Lemon	
P. digitatium	Orange	Falluja
P. digitatium	Bitter Orange	
P. digitatium	Mandarin	
P. digitatium	Lemon	
P. digitatium	Orange	Heet
P. digitatium	Bitter Orange	
P. digitatium	Mandarin	
P. digitatium	Lemon	

Table (2) the sensitivity of some types of citrus fruits to green mold disease caused by *P. digitatium* under laboratory conditions

% Infected Area	Treatments
0.00 D	A1B1
56.12 A	A1B2
0.00 D	A2B1
40.18 B	A2B2
0.00 D	A3B1
32.14 C	A3B2
0.00 D	A4B1
30.55 C	A4B2

* A1 = Mandarin fruits, A2 = Orange fruits, A3 = bitter orange fruits, A4 = Lemon fruits, B1= Sound treatment, B2= Contaminated treatment.

** Each number represents an average of four iterations and in each iteration there are three fruits.

***Treatments that share the same letter show no significant differences among them according to the choice of Dunkin polynomial at the level of significance 5%.

Table (3) the effect of interference between different types of citrus fruits and the color of storage bags on the development of green mold disease (*P. digitatium*) under laboratory conditions

% The infection with the disease in different citrus types	effect of interference between different types of citrus fruits and the color of storage bags on the development of green mold disease					Treatments / Citrus Fruits
	B5	B4	B3	B2	B1	
A 16.49	0.00 H	11.92 EF	22.79 BC	29.30 A	18.43 CD	A1
A 16.25	0.00 H	14.08 DE	26.66 AB	AB 25.00	15.62 DE	A2
B 3.33	0.00 H	2.38 GH	2.38 GH	4.76 GH	7.14 FG	A3
	0.00 D	9.45 C	17.24 A	19.68 A	13.73 B	The effect of the color of the storage bags on the development of disease in fruits

* A1 = orange fruits, A2= mandarin fruits, A3= bitter orange fruits, B1= dark black, B2= transparent green, B3= transparent yellow, B4= contamination-free treatment without bags, B5= healthy comparison treatment without bags.

** Each number represents an average of four iterations and in each iteration there are three fruits.

*** Treatments that share the same letter show no significant differences between them, according to the choice of Dunkin polynomial at the level of significance 5%

Table (4) the effect of different concentrations of propolis on fruits' resistance against green mold disease caused by P. digitatium

% for the infected area	Equation Symbol	The Fruit
18.66 %A	T1	Orange Fruits
18.30 %A	T2	Orange Fruits
18.15 %A	T3	Orange Fruits
18 %A	T4	Orange Fruits
18.80 %A	T5	Orange Fruits

* A1= Concentration of 6g / L, A2= 8g / L, A3= 10g / L, T4= 12g / L T5= contaminated treatment with fungus *P. digitatium*

** 3 replicates were used for each treatment with 3 fruits in each replicate

*** Results were analyzed according to Dunkin polynomial results under the probability of 5%.

*** Treatments that share the same letter show no significant differences among them.

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