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THE EFFECTS OF SOME PLANT EXTRACTS IN INHIBITING THE GROWTH OF THE FUNGI PENICILLIUM DIGITATUM AND P. ITALICUM

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Abstract

The study aimed to investigate the spread of green and blue mold disease in stores and local markets, isolate and diagnose the causes of this disease, and study the efficiency of using plant extracts against pathogens under laboratory conditions. The results showed isolating the pathogen from the infected orange fruits, which were collected randomly from the local markets of Babylon Governorate / Al-Musayyab district. Four isolates of *Pencillium digitatum* and three isolates of *Pencillium italicum* were obtained. One isolate of *Aspergillus fumigatus* was discovered, and this is the first record in Iraq. The test results showed that all isolates obtained from local markets were pathogenic to orange fruits. The results of this study showed the superiority of tea extract with all concentrations used to inhibit the growth of *P. digitatum* and *P. italicum*, which gave an average inhibition rate of 85.18, 87.77 and 90.73%, respectively, for concentrations 5, 10 and 15%, respectively, for *P. digitatum*. The average inhibition percentage was 86.85, 88.88 and 91.1% for the concentrations 5, 10 and 15% respectively for *P. italicum*.

Key words: Orange, ***Penicillium*****,** green mold, Blue mold, Plant extracts

1.Introduction

The Orange, *Citrus sinensis* (L.) Osbeck, is one of the citrus varieties that is classified among the important fruit trees, and its fruits are at the forefront in terms of global consumption because they are one of the rich sources of vitamin C in addition to simple sugars, organic acids and some important minerals such as potassium (Palou et al., 2015; FAO, 2016). In Iraq, orange production was estimated at 142717 tons for the winter season 2020 (Central Statistics Organization, 2020). Ripe citrus fruits such as oranges, whether in the field on trees or during storage or shipment for export, are exposed to infection with many fungi that cause fruit rot, including green mold caused by *Penicillium digitatum* and blue mold caused by *P. italicum* (Wang et al., 2022). These pathogens are the most economically important in citrus, which leads to large losses after harvest that may reach 30-80%, respectively (El-Otmani et al., 2011). Both green and blue mold pathogens produce large quantities of airborne conidia that infect oranges through insect wounds, twigs, or through collection and handling (Kellerman et al., 2016). Several methods have been used to control green and blue rot before and after harvest, including the use of chemical pesticides such as (IMZ) Imazalil and (TBZ) Thiabendazoie (Berk, 2016). However, the extensive use of chemical pesticides on citrus causes the emergence of resistant strains of fungi, in addition to the contamination of fruits with fungicides, which poses very great risks to human health and the environment in general (Hao et al., 2011; Piccirillo et al., 2018). Because of these negative effects, there has been a need to develop alternatives to fungicides to control post-harvest diseases, including biological resistance, by adopting antimicrobial organisms as a good and safe alternative

in the environment, and adopting natural products, including pomegranate peels, seed powders, water and alcoholic extracts of a number of plants instead of using fungicides. These materials are also characterized as being natural products that do not pollute the environment and are characterized by their rapid decomposition in the environment. They are non-toxic to humans and contain effective compounds that inhibit the growth of many plant pathogens (Davari et al., 2017; Yang et al., 2021 ; Kumar et al., 2021; Galicia-Campos et al., 2020 ; Wang et al., 2022). Given the importance of the disease and the attempt to find alternative control methods for the use of chemical pesticides, the study aimed to investigate the spread of green and blue mold disease in stores and local markets, isolate and diagnose the causes of this disease, and study the efficiency of using some plant extracts, and some Nano compounds against pathogens under laboratory conditions.

2.Materials and methods

2-1 . Isolation and identification of the *Pencillium digitatum* and *Pencillium italicum* from infected orange fruits.

Fungi P. digitatum and *P. italicum* were isolated from local orange fruits that showed symptoms of green and blue rot, which were brought from the local markets of the center of Babylon Governorate and the local markets of Al-Mussaib district. Round cuts were taken from the affected area, 3 - 5 pieces, with a diameter of 0.5 cm. The cuttings were planted in Petri dishes containing the P.D.A culture medium, four pieces per plate. The plates were incubated in the incubator at a temperature of 25 ± 1 °C for 2-3 days, after which the fungi growing on the medium were purified by taking a small piece with a needle from the edge of the colony and placed in the middle of a plate. Medium P.D.A. The dishes were placed in the incubator at a temperature of 25 ± 1 °C for 5-7 days, after which the isolated fungi were diagnosed morphologically and microscopically. The process was repeated several times to obtain pure cultures for both fungi. There is also another method by taking a smear of the pathogenic fungi spores using a needle and hitting them in the container dish On the medium of P.D.A. Also, the dishes were placed in the incubator at a temperature of 25 ± 1 ° C for 5-7 days, after which the isolated fungi were diagnosed morphologically and microscopically by Prof. Dr. Ahed Abd Ali Hadi, Al Furat Al Awsat Technical University - Technical College / Al Mussaib, based on phenotypic indicators and microscopic examination and depending on the taxonomic characteristics of mushrooms reported by Pitt, (1988) and Moubasher (1993).

2-2 Test the pathogenicity of the fungi *Pencillium digitatum* and *Pencillium italicum*

The fruits of healthy and homogeneous oranges were selected in terms of maturity, color and size as much as possible, then the fruits of the local orange were sterilized with 1% sodium hypochlorite solution by immersing them for 3-5 minutes in a 1000 ml beaker, after which they were removed from the minor solution and rinsed with sterile water three times and dried on sterile filter paper Then, a wound was made in the form of a (+) sign, with a length of 4 cm and a width of 4 cm using sterile medical blades. The injured areas were inoculated with a disc of *P. digitatum* and *P. italicum* colonies separately. The age of the colony was 7 days, with three replicates for each treatment, then incubated. For 8 days in laboratory conditions at a temperature of 25±1°C in

single-use cork containers, leaving a comparison treatment by inoculating oranges with a disc of PDA culture medium without the presence of the fungus. The fruits were examined every two days, and the development of infection was calculated using a transparent ruler by measuring two perpendicular diameters of the fungal colony developing on the fruits. The incidence of green rot and blue rot on orange fruits was estimated by calculating the number of fruits showing symptoms of the disease from the total fruits according to the following equation:

Percentage of infestation = (the number of infected fruits)/(examined for the total number of fruits) *100

The severity of infestation was also calculated by dividing the infested fruits into four degrees of infestation based on the surface area of the infestation and using the scale shown as follows: 0 = no symptoms. 1 = 1-25% of the infected fruit show rot. 2 = more than 25-50% of the fruit have symptoms of rot. 3 = more than 50-75% of the fruit show symptoms of rot. 4 = rot more than 75-100% of the fruit .

The percentage of severity of injury was calculated according to the Mckinney formula (1923) as follows:

The severity of the infection=[(No. of plants per degree 0×0)+…(No. of plants per degree 5×5)\ Total Plants ×5]=×100.

2-3 Plant extracts used:

Three plants were selected to study their effect against *P. digitatum* and *P. italicum* which included tea, moringa and fenugreek (Table 1).

Table (1) Plants used in plant extracts

Common name	Scientific name	Family	Used part
Tea	*Camellia sinensis*	Theaceae	Leaves
Moringa	*Moringa oleifera*	Moringaceae	Leaves and seed
Fenugreek	*Trigonella foenum – graecum* L.	Fabaceae	Leaves

2-3-1 Preparation of the aqueous extract of plants:

I followed the method of Seema et al. (2011) in preparing aqueous extracts by mixing 150 g of plant powder for each plant sample separately with 1000 ml of distilled water in a flask of 2000 ml capacity, leaving the mixture in a vibrating water bath at a temperature of 30 ° C for half an hour, then filtering the mixture using several layers. From the medical gauze, the filtrate was placed in the oven at a temperature of 45 °C until a thick liquid was obtained. The liquid was kept in sealed containers in the refrigerator at a temperature of 4 °C until use.

2.3.2 Testing the effect of aqueous extracts of tea, moringa and fenugreek on the growth of Pencillium digitatum and Pencillium italicum on PDA culture medium.

The method of Al-Quraishi (2011) was followed by mixing the aqueous extract of the selected plants with the dissolved PDA nutrient medium, after being sterilized and cooled to 45°C. Concentrations 5, 10 and 15% of each extract, while only distilled water was added to the control treatment at a rate of three replicates for each concentration. The dish containing one of the

concentrations mentioned previously, the dishes were incubated at a temperature of 25 ± 1 °C. The experiment was carried out using a complete random design C.R.D, and after the diameter of the fungal culture for the control treatment (without extract) reached the edge of the dish, the results were taken by calculating the average of two perpendicular diameters from the growth of each colony as well as calculating The percentage of inhibition according to the equation mentioned in Tomlin (1998).

3. Results and discussion

3.1 Isolation and diagnosis

The results of Table (2) showed the isolation of the pathogen from the infected orange fruits, which were collected randomly from the local markets of Babylon Governorate / Al-Musayyab district. Four isolates of *Pencillium digitatum* and three isolates of *Pencillium italicum* were obtained. One isolate of *Aspergillus fumigatus* was discovered, and this record is considered The first in Iraq for this isolate and it was grown on PDA culture medium. The isolates varied in terms of growth speed and colony color, where the symptoms of green mold infection are in the form of a soft water area and it is easy to remove the affected part of the finger, then white growth appears on it, which is the fungus that causes the disease, This is followed by the appearance of a green powder, which is the spores of the fungus, and there is an irregular white area of the mushrooms between the green part and the healthy part of the fruit and the infection intensifies until the whole fruit becomes soft and covered with a layer of green mushroom spores and ends with the drying of the fruit, but in mold Most of the symptoms of infection are common with green mold, but they differ in the color of the fungal spores, where the blue mold has blue color and the white area between the middle part The glaucoma of the affected and the healthy part are narrower and more regular than in the green mold (Al-Haba and Mahmoud Shaker Mustafa, 2016).

Table (2) shows some types of fungi isolated from infected orange fruits

No.	Fungi	Isolate
1	*Pencillium digitatum*	Pd1
2	*Pencillium digitatum*	Pd2
3	*Pencillium digitatum*	Pd3
4	*Pencillium digitatum*	Pd4
5	*Pencillium italicum*	Pi1
6	*Pencillium italicum*	Pi2
7	*Pencillium italicum*	Pi3
8	*Aspergillus fumigatus*	A.f

3-2 Test the pathogenicity of the isolated fungi

The test results showed that all isolates obtained from local markets were pathogenic to orange fruits, and isolate P.d 4 was more ferocious on orange fruits compared to the rest of the isolates, as isolate P.d 4 gave an average percentage of infection 91.66% and an infection severity rate of

70.83%, while isolate P.i2 gave The rate of infection percentage was 66.66%, and the rate of infection severity was 47.91 %. As for isolate P.d 1 it gave the lowest rate of infection, which amounted to 33.33%, while the rate of infection severity was 20.83% compared with isolate P.d 4 As for isolate A.f it gave an average percentage of infection The infection is also high, reaching 83.33%, and the average injury severity is 64.58% after five days of conducting the experiment, as shown in Table (3). The reason for the high incidence and severity of injury in general may be due to the lack of interest in the operations of harvesting, packaging, storage and transportation and may result in large losses If health conditions are not observed during the collection, storage and shipment of the crop, because the infection often occurs in the fruits that were injured or scratched during the handling of the fruits, especially when placed in a damp place. The mold can spread from the infected to the healthy during packing (Al-Haba and Mustafa, 2016).

Table. (3) The pathogenicity of fungi isolated from infected orange fruits

No.	Isolate	% disease incidence	% severity
1	Pd1	33.33	20.83
2	Pd2	41.66	25.00
3	Pd3	50.00	43.75
4	Pd4	91.66	70.83
5	Pi1	58.33	39.58
6	Pi2	66.66	47.91
7	Pi3	50.00	35.41
8	A.f	83.33	64.58
L.S.D. (*P* 0.05)		13.37	7.67

Pd= *Penicillium digitatum* and Pi=*Penecillium italicum* and A.f= *Aspergillus fumigatus*

3-3 Effect of tea, moringa and fenugreek extracts on growth of *Pencillium digitatum* and *Pencillium italicum* in PDA medium

The results of Table 4 showed that there were significant differences between these extracts and their concentrations used on the growth of pathogenic fungi on the medium to which the above-mentioned plant extracts were added. The results of this study indicated the superiority of tea extract with all concentrations used to inhibit the growth of *P. digitatum* and *P. italicum*, which gave an average inhibition rate of 85.18, 87.77 and 90.73%, respectively, for concentrations 5, 10 and 15%, respectively, for *P. digitatum*, and it gave an average inhibition rate of 86.85, 88.88, and 91.1% for concentrations 5, 10 and 15%, respectively, for *P. digitatum*. *italicum*, and this study agreed with a study conducted by Abdel-Sada (2020) on the possibility of inhibiting the growth of toxic mushrooms by the commercial nanocomposite Zno, and the study of the integration between green tea extract and zinc oxide nanoparticles in reducing the toxicity of the fungus *Pencillium* spp. In vivo, green tea contains a group of active compounds that have a role in inhibiting the growth of the fungi studied, such as phenols, coumarins, etc., and then Moringa seed extract, which

gave an average inhibition rate of *P. digitatum* 75.85, 78.7 and 81.47% for concentrations 5, 10 and 15%, respectively, and it gave an average inhibition rate of *P. italicum* 71.85, 75.84, and 81.29% for concentrations 5, 10 and 15%, respectively. These results agreed with what Basahi (2019) indicated to the use of moringa plant extracts in the inhibition of pathogens. The green and blue mold *P. italicum* and *P. digitatum* on orange and lemon fruits achieved high rates of inhibition compared to the control treatment because they contain compounds such as saponins, tannins, flavonoids and glycosides, which have been proven to kill fungi, and Moringa seeds contain Terygospermin, which has a fatal action For fungi by destroying the cell membrane of the fungus in order to interfere with the existing lipid bilayer, which leads to cell swelling and then its explosion and death (Wu et al., 2018). Moringa leaf extract also recorded the effectiveness of Inhibitory for the growth of *P. digitatum* at the concentration 5, 10 and 15%, as the percentage of inhibition was 75.48, 75.48 and 77.77%, respectively. Also, it recorded an inhibitory activity for the growth of *P. italicum*, where the percentage of inhibition was 72.59, 75.47 and 77.77% for the following concentrations 5 and 10 and 15%, respectively, followed by the extract of fenugreek leaves, which achieved an average inhibition rate of 67.58, 70.36 and 74.92% for each of the concentrations 5, 10 and 15% for *P. digitatum*, as well as the rate of inhibition of 68.51, 71.4 and 75.92% for each of the concentrations. 5, 10 and 15%, respectively, of *P. italicum* compared with the control treatment. Several studies and research have confirmed the effectiveness of many plant extracts in inhibiting the growth of plant pathogens due to the presence of some active substances in these extracts, such as tannins, glycosides, phenols, alkaloids, resins, sterols and saponins. They are non-toxic to humans and animals (Galicia-Campos et al., 2020 and Wang et al., 2022.(

Table. 4 The effect of adding plant extracts to the PDA medium on the growth of pathogenic *P. digitatum* and *P. italicum*.

Treatment	Concentration %	Colony diameter (cm)	Inhibition (%)
Control	-	9	0
Tea +Pd	5	1.33	85.18
	10	1.1	87.77
	15	0.83	90.73
Tea +Pi	5	1.18	86.85
	10	1	88.88
	15	0.8	91.1
Moringa leaf +Pd	5	2.65	75.48
	10	2.2	75.48
	15	2	77.77
Moringa leaf +Pi	5	2.46	72.59

	10	2.41	75.47
	15	2	77.77
Moringa seed +Pd	5	2.17	75.85
	10	1.91	78.7
	15	1.66	81.47
Moringa seed +Pi	5	2.53	71.85
	10	2.03	75.84
	15	1.68	81.29
Pi+Fenugreek	5	2.91	67.58
	10	2.66	70.36
	15	2.25	74.92
Pd+Fenugreek	5	2.83	68.51
	10	2.57	71.4
	15	2	77.77
L.S.D (p=0.05)	0	0.33	3.7

Pd= *Penicillium digitatum* and *Penecillium italicum*.

4. Conclusion

The pathogen from the infected orange fruits was *Pencillium digitatum* and *Pencillium italicum*. One isolate of *Aspergillus fumigatus* was discovered, and this is the first record in Iraq. the superiority of tea extract with all concentrations used to inhibit the growth of *P. digitatum* and *P. italicum*.

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