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Evaluation the efficiency of different concentrations of Chitosan, regular and Nano urea fertilizer in inhibiting the growth of the fungi *Penicillium digitatum* and *P. italicum* that cause Green and Blue mold on oranges.

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Abstract: 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. The study showed the superiority of Nano urea fertilizer over regular urea fertilizer, but with a small percentage in inhibiting both *P. digitatum* and *P. italicum*. The percentage of inhibition achieved 88.88, 100 and 100% for each of the concentrations 5, 10 and 15%, respectively, for *P. digitatum*, and the percentage of inhibition of 89.25, 100 and 100% for each of the concentrations 5, 10 and 15% for *P. italicum*, respectively, compared to With regular urea fertilizer. The results showed that there were significant differences between the regular and Nano chitosan treatments and the comparison treatment with its effect on the growth rate of P. *digitatum* and *P. italicum* for both normal and Nano chitosan, each of which achieved a high rate of inhibition of the two pathogenic fungi.

Keywords: Pathogenic fungi, green mold, Blue mold, Orange, chitosan.

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 [1]. Citrus belongs to the family Rutaceae, and includes four genera, the most important of which is the genus Citrus, as it includes most of the economically important varieties and species. The world's citrus fruit is about 140 million tons, according to the Food and Agriculture Organization of the United Nations [2]. In Iraq, orange production was estimated at 142717 tons for the winter season 2020 [3]. 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 rot caused by *Penicillium digitatum* and blue rot caused by *P. italicum* [4]. These pathogens are the most economically important in citrus, which leads to large losses after harvest that may reach 30-80%, respectively [5]. Both green and blue mold pathogens produce large quantities of airborne conidia that infect oranges through insect wounds, twigs, or through collection and handling [6]. 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 [7]. 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 [8, 9]. 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 [10, 11]. Recently, Nano-composites have also been used in combating plant pathogens, as this Nanotechnology is one of the most promising methods in combating various plant diseases due to its action as antimicrobials [12, 13, 14]. 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 [15] and [16].

2-2. Evaluation of the efficiency of different concentrations of normal and Nano urea fertilizer in inhibiting the growth of *P. digitatum* and *P. italicum* on PDA culture media

Urea was obtained from the local markets and then sent to the workshops of the Ministry of Science and Technology Department of Materials to be crushed and converted to Nanoscale using special mills to reach a minute size of approximately 51.53 nm. % for each of the normal and Nano, and the sterile PDA culture medium was prepared and the concentrations were prepared by adding 5, 10 and 15 g of each of the regular and Nano urea fertilizers, each separately, to 95, 90 and 85 ml of the PDA culture medium, respectively, then the medium was shaken for the purpose of homogeneity The media were poured into sterilized dishes with a diameter of 9 cm. After the solidification of the medium, the dishes were

inoculated with a 0.5 cm disc for each of the two fungi separately and placed in the middle of the dish. Three replicates were used for each treatment of urea fertilizer regular and Nano, with the implementation of comparison dishes containing the culture medium alone and inoculated with a disc of both fungi. Then the dishes were incubated at a temperature of 25 ± 1 °C, then the diagonal growth of the fungi was measured by taking the average of two perpendicular diameters passing through the center of the plate after the growth of the fungi in the comparison treatment reached the edge of the plate [17], and the percentage of inhibition were calculated according to the following equation: % Inhibition = [(R – r)/ R] × 100.

Where, r is the radius of the fungal colony against the Urea and R is the radius of the fungal colony without the Urea.

2-3. Evaluation of the efficiency of different concentrations of normal chitosan and Nano-chitosan in inhibiting the growth of the fungi *Pencillium digitatum* and *Pencillium italicum* on PDA culture medium.

Ordinary and Nano chitosan were obtained from Dr. Zuhair's office for medical and laboratory supplies in Baghdad in the form of a fine white powder. 20 gm of normal and Nano chitosan were dissolved separately in 50 ml of acetic acid and the volume was completed to 1000 ml sterile distilled water and then mixed well to obtain The stock solution) The effectiveness of both normal and Nano chitosan against the growth of the two previously mentioned fungi was evaluated separately. Three concentrations of each of the regular and Nano chitosan 1, 2, 3 and 5% were prepared by adding 1, 2, and 3 And 5 ml of the base solution to 99, 98, 97 and 95 ml of sterile PDA medium, respectively. Then the medium was shaken for the purpose of homogenization. The media was poured into sterile dishes with a diameter of 9 cm. After the solidification of the medium, the dishes were inoculated with 0.5 cm discs taken from the colonies of the two fungi previously mentioned and placed in The center of each dish, and three dishes were used for each treatment, in addition to the comparison treatment. The culture medium without chitosan was then incubated at a temperature of 25 ± 1 °C for a period of seven days. Then the national growth of the fungi was measured and the percentage of inhibition was calculated according to what was mentioned in paragraph 2 - 2.

3- RESULTS AND DISCUSSION:

3-1. Isolation and diagnosis.

The results of Table (1) showed the isolation of the pathogen from the infected orange fruits (Figure 1), 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.

Table (1) 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

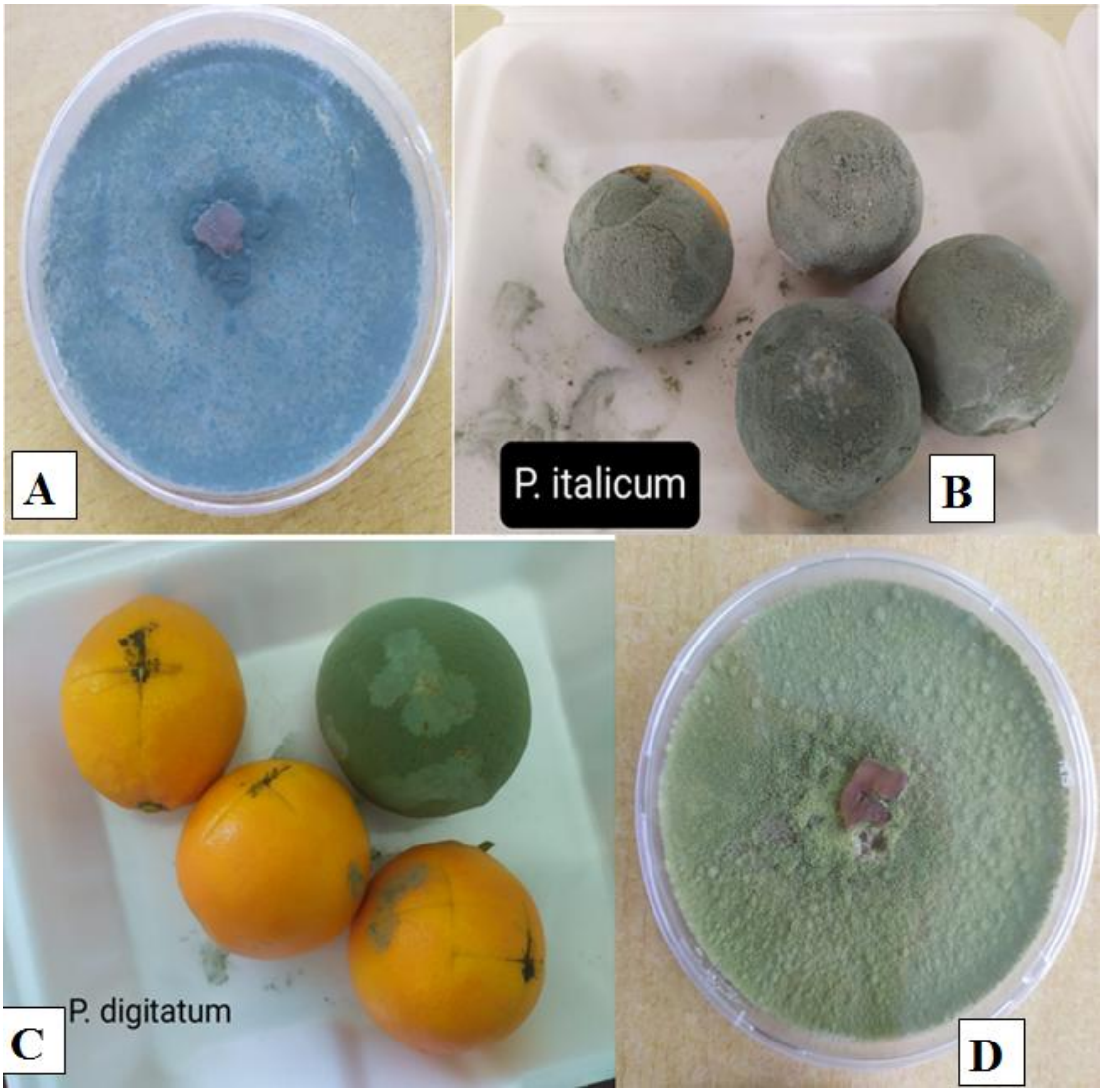

Figure 1. A= The growth of the fungi *Pencillium italicum* on PDA culture medium, B= Blue mold on oranges. C= Green mold on oranges. D= *Pencillium digitatum* on PDA culture medium 7 Days old.

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.

3-2. Evaluation of the efficiency of different concentrations of normal and Nano urea fertilizer in inhibiting the growth of the fungi Pencillium digitatum and Pencillium italicum on PDA culture medium.

The study showed the superiority of Nano urea fertilizer over regular urea fertilizer, but with a small percentage in inhibiting both *P. digitatum* and *P. italicum*, where the percentage of inhibition was 88.88, 100 and 100% for each of the concentrations of 5, 10 and 15%, respectively for *P. italicum, P. digitatum* and the average percentage of inhibition of 89.25, 100 and 100% for each of the concentrations 5, 10 and 15% respectively for P. italicum compared with the normal urea fertilizer, which in turn achieved the rate of inhibition of 87.4, 100 and 100% for each of the concentrations 5, 10 and 15% For the fungus *P. digitatum*, for the fungus *P. italicum*, it achieved an average inhibition rate of 87.95, 100 and 100% for concentrations 5, 10 and 15%, respectively, and as shown in Table (2).

Table 2. evaluating the efficiency of different concentrations of regular and Nano urea fertilizer in inhibiting the growth of the fungi *Pencillium digitatum* and *Pencillium italicum* on PDA culture medium.

Treatment	% Concentration	Colony diameter (cm)	Inhibition (%)
Control	0	9.00	0.00
Pd+Urea	5	1.13	87.40
	10	0.00	100.0
	15	0.00	100.0
Pi+Urea	5	1.08	87.90
	10	0.00	100.0
	15	0.00	100.0
Pd+Nano- Urea	5	1.00	88.80
	10	0.00	100.0
	15	0.00	100.0
Pi+ Nano- Urea	5	0.96	89.20
	10	0.00	100.0
	15	0.00	100.0
L.S.D (p=0.05)	-	0.11	1.40

Each number represents an average of three replicates, pd = *Pencillium digitatum*, Pi = *P. italicum*,.

3-3 Evaluation of the efficiency of different concentrations of normal chitosan and Nano-chitosan in inhibiting the growth of the fungus *Pencillium digitatum* and *Pencillium italicum* on PDA culture medium

The results showed, as in Table (3), that there were significant differences between the regular and Nano chitosan treatments and the comparison treatment, due to its effect on the national growth rate of *P. digitatum* and *P. italicum*, for both normal and Nano chitosan, each of which achieved a high rate of inhibition of the two pathogenic fungi. The percentage of inhibition of Nano-chitosan of *P. digitatum* was

77.77, 80.36 and 86.29% for each of the concentrations 1, 2, and 3%, respectively, and the rate of inhibition was achieved 100% for each of the concentrations 5, 10, and 15%, while the inhibition rate of the fungus *P. italicum* was 80.36, 85.18 and 86.48% for concentrations 1, 2 and 3%, respectively, and also achieved an average inhibition rate of 100% when using it at concentrations 5, 10 and 15%. As for regular chitosan, it also achieved a high rate of inhibition that reached 75.85, 77.77 and 82.4% at concentrations 1, 2, and 3%, and the rate of inhibition was 100% at concentrations 5, 10 and 15% for *P. digitatum*, while the average inhibition rate for *P. italicum* was 74.4, 77.77 and 85.18% for concentrations 1, 2 and 3%, respectively.

Table 3. evaluating the efficiency of different concentrations of normal chitosan and Nano-chitosan in inhibiting the growth of the fungi Pencillium digitatum and Pencillium italicum on PDA culture medium

Treatment	% Concentration	Colony diameter (cm)	Inhibition (%)
Control	0	9.00	0.00
Pd+Chitosan	1	2.17	75.85
	2	2.00	77.77
	3	1.58	82.40
	5	0.00	100
	10	0.00	100
	15	0.00	100
Pi+Chitosan	1	2.30	74.40
	2	2.00	77.77
	3	1.33	85.18
	5	0.00	100
	10	0.00	100
	15	0.00	100
Pd+ Nano- Chitosan	1	2.00	77.77
	2	1.76	80.36
	3	1.23	86.29
	5	0.00	100
	10	0.00	100
	15	0.00	100
Pi+ Nano- Chitosan	1	1.76	80.36
	2	1.33	85.18
	3	1.21	86.48

	5	0.00	100
	10	0.00	100
	15	0.00	100
L.S.D (p=0.05)	-	0.06	0.92

Each number represents an average of three replicates, pd = *Pencillium digitatum*, Pi = *P. italicum*,.

At concentrations 5, 10 and 15%, the rate of inhibition was 100%,. In monocotyledonous and dicotyledonous plants, these responses include increased callus formation, inhibition of protease enzyme, lacnin formation, increased activity of chitinase and glycinase, production of phytoaloxins, stimulation of jasmonic acid production, and stimulation of defense genes in addition to its action as an antioxidant. Of the nitrogen, which is estimated at about 6.89%, which makes it a useful chelating agent for many nutrients. Chitosan can be easily converted into emulsions, films, hydrogels, edible dressings and films. The first study on chitosan as a fungicide was published in 1979 by researchers Allan and Hadwiger, where they mentioned that It prevents the formation of cell walls of fungi, and this attracted the attention of industries interested in The agricultural sector to avoid losses resulting from the spread of fungal diseases that lead to a reduction in yield and quality [12].

4-CONCLUSIONS

The pathogen from the infected orange fruits was *Pencillium digitatum and Pencillium italicum*. The regular and Nano chitosan and Urea treatments effect on the fungi growth.

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