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EFFECT OF GINGER AND CINNAMOMUM EXTRACTS IN PROTECT ORANGE FRUITS FROM INFECTION *PENICILLIUM DIGITATUM* THE PATHOGEN OF GREEN MOULD DISEASE

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Abstract

Study aimed to evaluate the efficacy of some plant extracts of Ginger plant *(Zingibar officinale*) and Cinnamomum plant *(Cinnamomum zylanicum*) to protect orange fruits from *Penicillium digitatum*, that causes green mold disease, isolated from infected orange fruits located in local markets in Najaf Governorate, Iraq. The concentrations of Cinnamomum plant extract showed efficacy against pathogenic fungus compared to concentrations of ginger extract, the Cinnamomum plant extract in the concentrations of (10, 15 and 20 mg/ ml) inhibition of the fungi on Potato Dextrose Agar medium in Petri dishes, the diametrical growth rate of pathogen after 3 days of growth were (3.34, 3.74 and 3.6), respectively, compared to the concentration rate of 5 mg / ml and control treatment which gave (5.31 and 4.75) cm respectively. Cinnamomum plant extract also showed high inhibition efficiency in the growth of pathogenic *P. digitatum*, while the diametrical growth rate was 2.10 compared to the diametrical growth rate of fungus with the Ginger extract, which gave 6.16 cm. Results indicated all concentration of Cinnamomum extracts had led protection of orange fruits from infect with *P. digitatum*. Treatment of orange fruits soaked with Cinnamomum extracts (20 mg/ml) inoculated with sporulation suspension of *P. digitatum* (T6) was significantly affected by inhibiting the growth regions and the depth of infected fruits, it gave (1.60 and 0.73) cm respectively, compared to the treatment of T2 which gave the highest rate of growth regions and growth depth of the fungus *P. digitatum* reached (6.10 and 5.96) cm respectively.

Key words: Cinnamomum, Ginger, Plant extracts, Orange fruits, Green mould disease.

Introduction

Orange *Citrus sinensis* (L.) Osbeck is one of the citrus varieties that are classified among the important fruit trees, which is important after grapes in international trade in terms of global consumption as one of the sources rich in vitamin C in addition to simple sugars, organic acids and some important mineral elements such as potassium (Adel *et al*., 1985; Muhamad and Mussa, 2003). Citrus fruits are susceptible to diseases caused by many fungal pathogens in field and storage, mechanical damage, injuries and abrasion may be helpful during harvesting, transport and storage like *Penicillium, Aspergillus, Rhizpous* and *Pythium*, to infect with diseases like green mold disease which causes *Penicillium digitatum*, it is one of the economic problems that lead to the damage of fruits at the Arab and global level and it is a disease of storage that cause great economic losses and health damage caused by Mycotoxin produced by the fungus is not limited to orange disease but the disease affects other types of citrus (Ogawa, 1988 and Smith *et al*., 1994 and Al-Haidery and Dewan, 2015). There is many ways to control *P. digitatum* including chemical control (Obagwu and Korsten, 2003) various biologic control and use of natural products in disease control (Pal and Gardener, 2006). Research has tended to use natural products including water and alcohol extracts for many of various plants instead of using fungicides, as well as the natural products are non-polluting and rapidly degradable in the environment and are non-toxic to humans because it contains effective compounds inhibiting the growth of many pathogens. Ginger (*Zingibar officinale*) and Cinnamomum (*Cinnamomum zylanicum*) which belong to the Ginger

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family, are used in the control against many plant pathogens, because they have highly desirable environmental traits such as rapid degradation, low toxicity and high specialization (Schutte *et al*., 2003). This study aimed to use the extracts of Ginger and Cinnamomum plants to reduce the incidence of green mold disease on the orange fruits that caused by *Penicillium digitatum.* The study included the following topics: Test the effect of the extract of Ginger and cinnamomum plants in the growth and sporulation of *P. digitatum.* Test the effect of the extract of Ginger and cinnamomum in protection of orange fruits from infected by green mold diseases caused by *P. digitatum.*

Materials and Methods

Isolation of *Penicillium digitatum* from orange fruit

Samples of infected orange fruits were brought from local markets to the plant pathology Laboratory / plant protection department- college of Agriculture / University of Kufa, which showed signs of rot and took small pieces of white growth by sterilized needle and then planted in petri dishes containing PDA medium and then placed in the incubator and after 4 days examined the dishes, fungal growth diagnosed in laboratory of plant pathology based on specific taxonomic keys (Pitt, 1988, Hocking and Pitt, 1997).

Preparation of the sporulation suspension of *P. digitatum*

The spores suspension of *P. digitatum* was prepared by plating the fungus in onto potato dextrose agar, the petri dishes contain pure culture were incubated in the incubator at a temperature of 25 ± 2°C. After 5-7 days 10 ml sterile distilled water was added to each dish, then harvesting the inoculum source by small sterile brush by scaling the fungal growth from surface of PDA for purified cultured. Spores suspension were collected by sterilized beaker and measured the concentration of spores in the suspension (Dewan, 1989) by taking 1 ml of spores suspension and diluted in tubes containing 9 ml distilled water until get dilution 10^8, the spores suspension was obtained at a concentration of 1.2×10^8 by a dilution method with distilled water and measured by Haemocytometer slide.

Pathogenicity test of *P. digitatum*

The pathogenicity test was carried out on homogeneous orange fruits in terms of maturity, color and size brought from local markets. Surfaces fruits were sterilized with hypochlorite 10% then washed with sterile water and dried on sterile filter paper and make injuries on fruits surface by sterilized knife then inoculated with 0.5 ml of sporulation suspension and replicated three fruits per treatment and incubated for 5 days in laboratory conditions at 25 ± 2°C in containers (capacity of each 8-10 fruits) and control treatment which had fruits without adding spores suspension. After incubation period measured damages occurred for fruits.

Preparing of cold water extract of Ginger and Cinnamomum plants

Samples of Ginger and Cinnamomum plants were collected in powder form used in the study from local markets in Najaf province and were placed in polythene bags and kept for use. To prepare cold water extract by adding 5, 10, 15 and 20 g of Ginger and Cinnamomum powder and each separately to 1000 ml sterile distilled water and left for 24 hours, the mixture was filtered into another flask by filter paper and sterilized by using 0.22µm Millipore filters to obtain pure extract (Harborne, 1984) and add to the filtrate 39 g of the P.D.A medium and complete the volume to 1 liter that sterilized in autoclave each separately to obtain the concentrations of (0, 5, 10, 15, 20) mg/ ml and pour it in Petri dishes according to the requirements of the experiment.

Effect of cold water extract of Ginger and Cinnamomum extracts in diameter growth of *P. digitatum* in Petri dishes

Petri dishes contain PDA medium with all concentrations of the cold water extracts for both studied plants inoculated with *P. digitatum* in center of Petri dishes and each alone according to the method described in previous paragraph and every concentration replicated three times and incubated at temperature of 25±2°C then measured diameter growth for pathogen after 72 hours (Sha'ban and Al-Mallah, 1993) and then count Colony Form Unit (CFU) for *P.digitatum* by take a disk 0.5 cm by cork borer of *P.digitatum* recent culture that PDA treated with concentration of Ginger and Cinnamomum extracts and diluted until 7^{th} dilution then transferred 1 ml of last dilution to a petri dish and pour PDA and replicated for three times for all concentration then incubated all petri dishes at temperature of 25±2°C after 1-3 days counted the colonies developing in every petri dish as following equation:

Number of Colony Formation Units (CFU) = number of colonies × inverted dilution

Effect of cold water extract of Ginger and Cinnamomum in protection of orange fruits from the infection *P. digitatum*

This experiment was conducted by soaked the fruits of oranges for 2 minutes for all concentrations of cold water extract for cinnamomum and ginger (0, 5, 10, 15,

20) mg/ml then it was inoculated with 0.5 ml of spore suspension of *P. digitatum*, as following treatments T1: Orange fruits are only injured. T2: Orange fruits injured + spore suspension of *P. digitatum*. T3: Orange fruits injured+ Spore suspension of *P. digitatum* + Cinnamomum extract (5 mg/ ml). T4: Orange fruits injured + Spore suspension of *P. digitatum* + Cinnamomum extract (10 mg/ ml). T5: Orange fruits injured + Spore suspension of *P. digitatum* + Cinnamomum extract (15 mg/ ml). T6: Orange fruits injured + Spore suspension of *P. digitatum* +Cinnamomum extract (20 mg/ ml). T7: Orange fruits injured + Spore suspension of *P. digitatum* + Ginger extract (5 mg/ ml). T8: Orange fruits injured + Spore suspension of *P. digitatum* + Ginger extract (10 mg/ ml). T9: Orange fruits injured + Spore suspension of *P. digitatum* + Ginger extract (15 mg/ ml). T10: Orange fruits injured + Spore suspension of *P. digitatum* + Ginger extract (20 mg/ ml).

Each treatment was repeated with three replicates. After 7 days the damage evaluated by measuring the area of the spot (growth of *P. digitatum* on the fruit surface), the infection depth, the presence of rot and the formation of spores.

Design and analysis of experiments statistically

Laboratory experiments were conducted according to the complete randomization design (C.R.D). The means were compared by using the least significant difference (L.S.D.) and at probability level 0.05 (Al-Rawi and Khalaf Allah, 2000).

Results and Discussion

Pathogenicity test of *P. digitatum*

Results showed *P. digitatum* which isolated from infected oranges fruits had high capacity pathogenicity to healthy orange fruits, inoculation with *P. digitatum* to healthy orange fruits showed high appearance of symptoms after 3-4 days of inoculation, appearance of the water layer and then colored the inoculated fruits in olive green. The reason for colored surface of orange fruits belong to *P. digitatum* that penetrates through fine wounds, it can be infect fruits and reproduce spores very speed everywhere in the atmosphere and on the surfaces of fruits, it is rapidly spread by wind (Kanetis *et al*., 2007). It has ability to produce various external enzymes that break down complex sugars into simple sugars and these metabolic processes enable it to growth and reproduction, one of these enzymes is pectinase haydrolyzes pectins, responsible for the analysis of pectin, it is important component is the middle layer and the cell wall of the higher plants (Patil and Chaudhari, 2010). As shown in fig. 1.

Fig. 1: Pathogenicity test of *P. digitatum.*

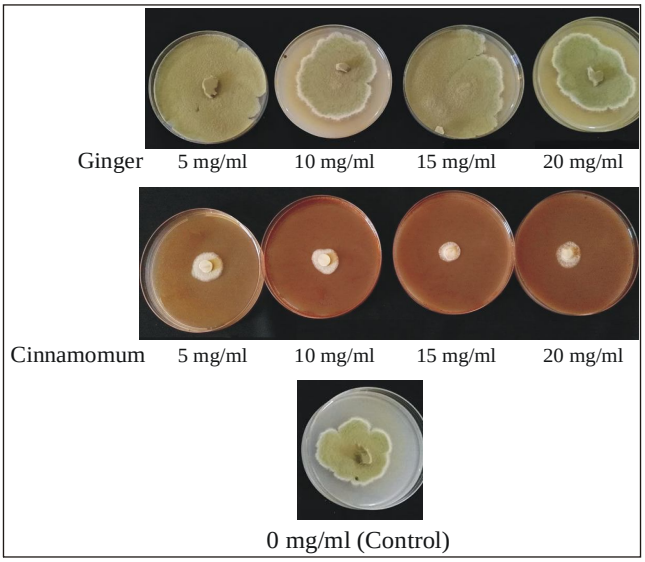

Fig. 2: Effect of Ginger and Cinnamomum extracts in diameter growth of *P. digitatum* in Petri dishes after 72 hours.

Effect of cold water extract of Ginger and Cinnamomum extracts in diameter growth of *P. digitatum* in Petri dishes after 72 hours of incubation

Results of table 1 showed that concentration rates of extracts (10, 15 and 20 mg/ ml) reduced the diameter

Table 1: Effect of cold water extract of Ginger and Cinnamomum extracts in diameter growth of *P. digitatum* in Petri dishes after 72 hours of incubation.

Extract concentration (mg/ ml)	Diameter growth of *P. digitatum* (cm)		Mean
	Ginger	Cinnamomum	
0 Control	4.75	4.75	4.75
5	8.5	2.13	5.31
10	5.55	1.65	3.60
15	6.85	0.63	3.74
20	5.33	1.35	3.34
Mean	6.19	2.10	
$L.S.D_{0.05}$	Concentrations=0.6407 Extracts=0.4052 Interaction =0.9060		

Table 2: Effect of cold water extract of Ginger and Cinnamomum extracts in Colony forming units (CFU) of *P. digitatum* in Petri dishes after 72 hours of incubation.

Extract concentration (mg/ ml)	Number of Colony forming units *P. digitatum* × 10^7		Mean
	Ginger	Cinnamomum	
0 Control	7	7.33	7.16
5	17.66	4.33	10.99
10	13.33	3	8.16
15	14.33	2.66	8.49
20	12.66	4.33	8.49
Mean	12.99	4.33	
$L.S.D_{0.05}$	Concentrations=1.898 Extracts=1.200 Interaction =2.684		

growth of *P. digitatum* in Petri dishes after 3 days of growth were (3.60, 3.74 and 3.34 cm) respectively compared with the concentration rate (0 and 5 mg/ml) which gave (4.75 and 5.31 cm) respectively (Fig. 2) which indicates that increasing the concentration of plants extracts increases the inhibitory capacity for the growth of fungus, there was also a difference in the effectiveness of the extract type. Same results show that the cinnamomum extract had led to inhibition the growth of *P. digitatum*, were diametrical growth rate was 2.10 compared with diameter growth rate with Ginger extract which gave 6.16 cm (Pelczar *et al.*, 1986; Farage *et al.*, 1989) they indicated that phenolic compounds have the ability to change the nature of proteins and damage the cellular membranes of fungi cells by binding them to the active sites of cellular enzymes and inhibiting their action. In the study of the effect of the interaction between the extract concentration and the type of plant extract, it was found that the concentration of 15 g of the cinnamomum gave the highest rate of inhibition of the growth of *P. digitatum*, It was 0.63 cm compared to other treatments, while the concentration gave 5 g of ginger with the lowest inhibitory rate of 8.5 cm. The cold water extraction method followed was appropriate in extracting the active ingredients of the cinnamomum plant extract and thus affected the growth of fungi and inhibited it (He and others, 2005).

Effect of cold water extract of Ginger and Cinnamomum extracts in Colony forming units (CFU) of *P. digitatum* in Petri dishes after 72 hours of incubation

Results of table 2, showed that control treatment that Colony forming units of *P. digitatum* in Petri dishes after 3 days of growth was 7.16 CFU, while the concentrations (0, 10, 15, 20 mg/ ml) there is no significant difference to control treatment, it gave the number rate of Colony forming units were (8.16, 8.49, 8.49) CFU respectively, while the concentration rate (5) mg /ml showed the highest rate of Colony forming units was 10.99 CFU of compared to other concentrations, results of same table also showed that the rate of extract of the Cinnamomum led to a reduction in the number of Colony forming units *P. digitatum* compared to the rate of Ginger extract where the number rate of Colony forming units were (4.33 and 12.99) CFU respectively. Effect of the interaction between the extract concentration and the type of plant extract, it was found that the concentration (15 mg/ ml) of the Cinnamomum had reduced the number of colony forming units *P. digitatum* reached 2.66 CFU compared with the other the interaction treatments, which gave the concentration (5 mg/ ml) of the Ginger highest rate of colony forming units was 17.66 CFU.

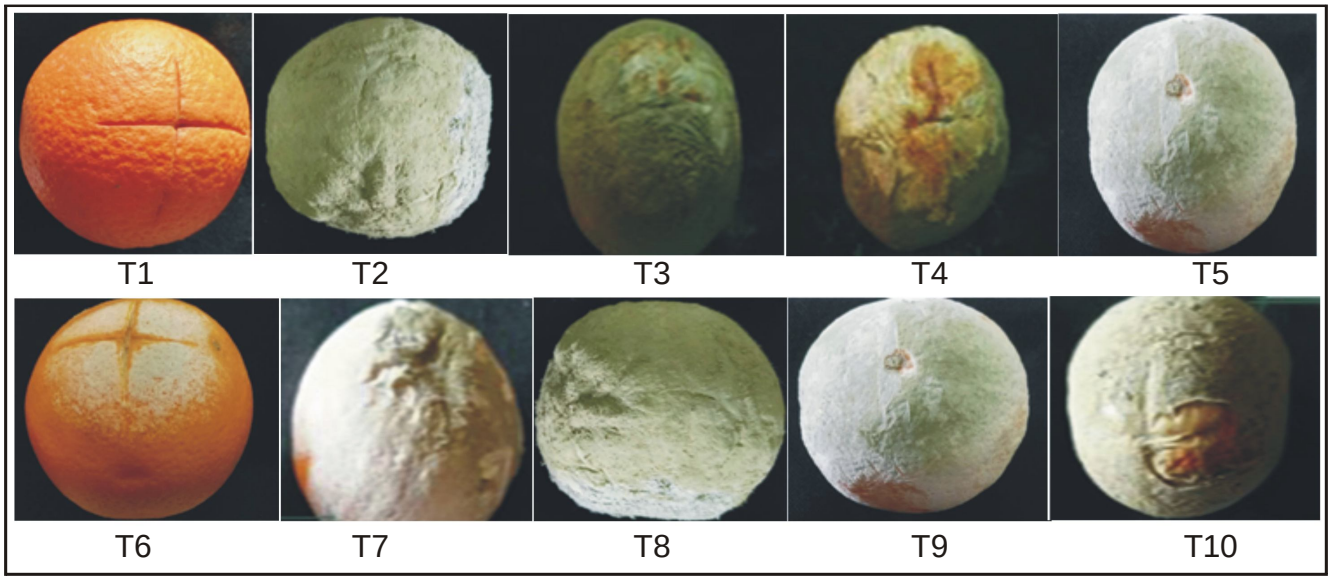

Fig. 3: Effect of concentrations of the cold water extract of the Cinnamomum and Ginger in the protection of orange fruits from infection of *P. digitatum.*

Table 3: Effect the concentrations of the cold water extract of the Ginger and Cinnamomum in the protection of orange fruits from infection of *P. digitatum*.

Spore formation	Mold	Infection depth	Growth area	Treatments
-	Non	0.00	0.00	T1
+	Total	5.96	6.10	T2
+	Total	5.30	5.96	T3
+	Total	4.70	5.86	T4
+	Partial	3.83	3.86	T5
+	Partial	0.73	1.60	T6
+	Total	5.93	6.03	T7
+	Total	5.46	5.93	T8
+	Total	5.80	5.90	T9
+	Total	4.93	5.60	T10
		0.606	0.446	L.S.D=0.05

Effect the concentrations of the cold water extract of the Ginger and Cinnamomum in the protection of orange fruits from infection of *P. digitatum*

Results of table 3, indicate that all concentrations of the cinnamomum extract led to the protection of orange fruits from the infection of *P. digitatum*. Results of the same table showed that all concentrations of Ginger have increased growth of *P. digitatum* by increasing mold of orange fruits (Fig. 3), where treatment of the cinnamomum extract T6 was superior significant in inhibiting growth area and growth depth for fruits infected it were (1.60 and 0.73) cm respectively compared with T2 which gave the highest growth area and growth depth of *P. digitatum* were (6.10 and 5.96) cm respectively. While the concentration treatments of Ginger extract (T7, T8, T9 and T10) showed no significant inhibitory role in the growth area and growth depth of fruit treated with spore suspension of *P. digitatum*. The reason for inhibition of the cinnamomum extracts of *P. digitatum* may be due to contain many active compounds like Glycosides, Tannins, Resins, Saponins and Phenols, which have an inhibitory role in the growth of pathogenic fungi (Hashim *et al*., 2008) or because of the increased concentration of inhibitory substances in the extract lead to inhibition of fungi such as substance Eugenol which is a strong antifungal against many pathogens (Park *et al*., 2007).

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