The inhibitory effect of the aqueous and acetonic plant extracts of Eugenia caryophyllata and Cinnamomum zeylnicum against some phytopathogenic fungi

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ABSTRACT

This study included the efficacy of the aqueous and acetonic extracts of *E. caryophyllata* and *C. zeylnicum* and the fungicide Top tapsin 70% in the growth of some phytopathogenic fungi which include: *A. niger*, *A. flavus* and *N. crassa* that are isolated from infected fruits and vegetables , through their effect in mycelial growth. The phytochemical screening of the active ingredients showed that the aqueous and acetonic plant extracts of *E. caryophyllata* and *C. zeylnicum* contained tannins, saponins, resins, flavonoids, alkaloids, fuocoumarins and terpenes. The results showed that the aqueous and acetonic extracts of *E.caryophyllata* and C.zeylnicum have significant inhibitory effect for growth of phytopathogenic fungi on solid culture medium (PDA) , in measuring with controlled treatment at level of probability 5% , where the aqueous and acetonic extracts have high inhibition percentages at concentration (30ml) and these extracts gave higher inhibition percentages than Top tapsin 70 % at a various concentrations.

Keywords: Aqueous extract, Acetonic extract, Phytopathogenic fungi.

Introduction

Fungi are the most crucial and common pathogens and the basic reason of crop diseases . It infects a wide range of fruits and vegetables during storage and transporting [1]. Many kinds of food including meats , fruits and cereals are extremely susceptible to fungal spoilage . Fungal growth has been reported to be responsible for food spoilage , which leads to significant economic losses [2] . *Penicillium* , *Aspergillus* and yeasts are common fungi which can be easily isolated from spoiled food , especially fresh fruits , vegetables and grains , produce many toxins [3] . Fungi are not limited to spoiling food , feed and grains , but they also have healthy damages for humans and animals, as well as changing the color , taste and smell of these foods , as well as their secretions of toxic metabolites and therefore it is necessary to search for safe methods like biological methods which are one of most important methods to combat molds [4] , compared with fungicides which have negative environmental effects for mammalian toxicity and high costs are making their use unattractive therefore searching for alternatives such as natural plant based chemicals [5]. One of the biological methods is the use of the plants , plants have ability to synthesize aromatic secondary metabolites , like phenols , phenolic acids , quinones , flavones , flavonols , tannins and coumarins [6]. These groups of compounds show antimicrobial effect

and serves as plant defense mechanisms against pathogenic microorganisms [7]. Many research workers have tried to find out safe and economical control plant diseases by using plant extracts of different plant parts [8] , for example , using plant extracts of *Eugenia caryophyllata(flowers) and Cinnamomum zeylnicum (bark)* to combat some pathogenic fungi. *Eugenia caryophyllata is* one of medicinal plants known for their use in the medical field, as they contain volatile oils, that are used as an antifungal and antibacterial [9] and has ability to inhibit and block the growth of many microorganisms that cause skin diseases [10]. Chemical analyzes refer that *Eugenia caryophyllata* contain effective compounds such as alkaloids, triterpenoids, coumarins and cyanogenic glycoside according to tests performed by [11]. *Cinnamomum zeylanicum* also known for its medicinal use and effective against parasites, insects and pathogenic fungi, also it is a disinfectant against microbes such as bacteria and fungi [12]. Chemical analyzes indicated that *Cinnamomum zeylanicum* contained volatile oils and resinous compounds, cinnamic acid, eugenol [13]. The researchers indicated that the effect of plant extracts and volatile oils extracted from them on the two species *Aspergillus flavus* and *Aspergillus parasiticus* confirmed the presence of an inhibitory effect on the growth of the two types of fungi and their production of aflatoxins using *Cinnamomum cassia* extract and volatile oils extracted from *Eugenia caryophyllata* [14]. This study aimed to use the aqueous and acetonic plant extracts of *Eugenia caryophyllata* and *Cinnamomum zeylnicum* to inhibit some phytopathogenic fungi.

2. Materials and Methods

Experimental Site and Source of Materials

Experiments were carried in Department of Biology , College of Science , University of Anbar. The infected fruits and vegetables were collected from different markets in Al-Anbar Government , exactly, Al Ramadi , Al Fulluja , Al Rutba and Heet markets. The infected fruits and vegetables are carried quickly to the laboratory for experiment.

Preparation of culture media

Potato Dextrose Agar medium (PDA) (BAM Media M127) was prepared by dissolving 39 grams in 1000 ml of distilled water. The medium was autoclaved at 121 C for 20 minutes at 15 lb. The sterilized medium was allowed to cool to 45 C before supplemented with streptomycin sulphate (3grams) and aseptically dispensed into sterilized 9cm diameter Petri dishes.

Isolation and Identification of fungal pathogens

Fungi were isolated from infected fruits and vegetables after washing them by transferring 1cm from the area that is infected with fungi and sterilized with 1% Sodium hypochlorate solution , then washed with distilled water , sterilized and dried between two sterile filter papers and cultured on Petri dishes that contain PDA medium containing the antibiotic Streptomycin Sulphate and incubated for 7 days at 28 °C [15]. After growth of fungi in Petri dishes, fungi were identified according to morphological features like shape, color, colony diameter and its height, also according to microscopic features like shape, size, color,

composition of conidiophores and other compositions according to the aid of books [16]. Some Isolates were identified by PCR Technique (Molecular Identification).

Phytochemical Screening of active ingredients in plant extracts

Several chemical tests were carried out on the main active ingredients in the aquatic and acetonic extracts for *Eugenia caryophyllata* and *Cinnamomum zeylanicum* by using Dragendroff reagent to detect alkaloids [17] , Fehling reagent to detect glycosides [18] , Ferric chloride 1% to detect tannins [19] , Ninhydrine reagent to detect amino acids [20] , Alcoholic potassium hydroxide 50% to detect flavonoids [21] , the appearance of foam to detect saponins [22] , the appearance of turbidity to detect resins [23] , the greenish yellow precipitate to detect fuocoumarins and the brown precipitate to detect terpenes [24].

Detection of PH of plant extracts

50 ml of distilled water or acetone 70% was mixed with 10 grams of plant powder by magnetic stirrer for 10 minutes , then the solution was filtered and the PH value was estimated using the PH-meter device [19].

Preparation of aquatic plant extracts

According to [25] , aquatic plant extracts were prepared by mixing 20 grams of plant powder for each plant sample separately with 400ml of distilled water in a volumetric flask with a capacity of 1000ml , then leaving the suspension in a magnetic stirrer for 24 hours , then the suspension filtered using several layers of medical gauze and then sterilized through a Millipore filter 0.22um , then the clear liquid of extract was kept in sealed containers in the refrigerator at 4C until use [26].

Preparation of acetonic plant extracts

The same previous method was followed to prepare the acetonic extracts , replacing the distilled water with acetone 70% [27].

Effect of aquatic and acetonic plant extracts in mycelial growth inhibition of fungal pathogens

According to [26] method, the aquatic and acetonic plant extracts were mixed with PDA medium separately after being sterilized and cooled to temperature of 50C , at concentrations : 10ml , 20ml , and 30ml of extracts /100ml of PDA medium at a rate of three replicates for each concentration and after solidification of extract-media mixtures , a disc with a diameter 5mm of a 5-7 day-old cultures of each fungus was placed in the center of petri dishes . The control plates of aquatic extracts consisted PDA. The control plates of acetonic extracts consisted PDA with acetone 70% at concentrations: 10ml , 20ml , and 30ml. The fungicide plates consisted Top tapsin 70% at concentration 2mg with PDA. All treatments were in three replicates. All plates of control and treatments incubated at (25-28)°C for 5-7 days, except *N.crassa* isolates which incubated for 3days . Radical growth in treatments and control was measured by using a ruler . The percentage inhibition of mycelial growth by each extract was computed using formula : $I = 100 \times (C-T) / C$, Where :

I = percentage inhibition of mycelial growth

C= mycelial growth of fungus in control plate

T = mycelial growth of fungus in the treatment [28].

STATISTICAL ANALYSIS

The data were subjected to the SPSS System to determine the significant differences between the studied factors at a probability level of 5% , as an analysis of variance (ANOVA) was conducted and the LSD value was calculated.

3. Results

Isolation and Identification of pathogenic fungi

The results of isolation from infected fruits and vegetables showed the emergence of fungal isolates: *Aspergillus niger, Aspergillus flavus* and *Neurospora crassa.* Morphology of *Aspergillus niger* can identify by growing on substrate giving colonies of felt similar to yellow to white septate hyphae, converting black with the configuration of conidia. *Aspergillus niger* produces black or extremely dark brown spores of biseriate phialides [29]. The microscopic identification indicated that the conidiophore length is between 800-2200 micrometers and the diameter of rough round conidia is 3-4.5 micrometer[30] , *Aspergillus flavus* and *Neurospora crassa* identified by PCR Technique.

Phytochemical screening of active ingredients in plant extracts

The results of phytochemical screening of active ingredients in the aquatic and acetonic plant extracts of *Cinnamon zeylanicam* and *Eugenia caryophyllata* showed that the extracts contained a group of active ingredients: alkaloids, saponins, tannins, resins, terpenes, glycosides, fuocoumarins and flavonoids. The PH value of the aquatic and acetonic extracts was also identified (Table1 & Table 2).

Table.1 : Phytochemical screening of active ingredients in aquatic plant extracts.

Test	*Eugenia caryophyllata*	*Cinnamomum zeylanicum*	Expected result
Tannins	+	+	Bluish-green precipitate
Saponins	+	+	Foam
Glycosides	+	+	Red precipitate
Amino acids	-	-	Purple color
Resins	+	+	Turbidity
Alkaloids	+	+	Brown precipitate
Terpenes	+	+	Brown precipitate
Fuocoumarins	-	-	Greenish-yellow precipitate
Flavonoids	+	+	Yellow color
PH	**4.6**	**5**	

Table. 2 : Phytochemical screening of active ingredients in acetonic plant extracts

Test	*Eugenia caryophyllata*	*Cinnamomum zeylanicum*	Expected result
Tannins	+	+	Bluish-green precipitate
Saponins	+	+	Foam
Glycosides	+	+	Red precipitate
Amino acids	-	-	Purple color
Resins	+	+	Turbidity
Alkaloids	+	+	Brown precipitate
Terpenes	+	+	Brown precipitate
Fuocoumarins	+	-	Greenish-yellow precipitate
Flavonoids	+	+	Yellow color
PH	**4.7**	**4.6**	

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Inhibition of Mycelial growth of pathogenic fungi by aqueous plant extract of Eugenia caryophyllata

The aqueous extract of *Eugenia caryophyllata* reduced mycelial growth and inhibited *Aspergillus niger* at concentrations 20 ml and 30 ml, where the inhibition percentages were: 62.2 % , 85 .7 % respectively. As for *Aspergillus flavus* , the aqueous extract reduced mycelial growth at all concentartions : 10 ml , 20 ml and 30 ml , where the inhibition percentages were : 45% , 61.8% and 88% respectively . As for *Neurospora crassa* , the aqueous extract reduced mycelial growth at all concentrations , where the inhibition percentages were : 42.3 % , 93.4% and 92.5% . As for the fungicide Top tapsin 70% , it has a high effectiveness against *Neurospora crassa* , where the inhibition percentage was 71.7% at concentration 2mg , while its effectiveness against *Aspergillus flavus* was 37% with the same concentration.Top tapsin 70% did not have any effectiveness against *A.niger* , However , the inhibitory effect of plant extract and Top tapsin 70% solution in the mycelial growth of *A.niger* , *A.flavus* and *N.crassa* was significant different $P< 0.05$ at a various concentrations. Fungitoxicity of aqueous extract against *A.niger*, *A. flavus* and *N.crassa* increased as the concentration increased (Table 3) and Figures (1,2 and3).

Table. 3 : - Effect of Aqueous Plant Extract of Eugenia caryophyllata in mycelial growth of pathogenic Fungi

Aqueous Extract	**Colonies Diameters (mm)**			
E.caryophyllata	**Conc.**	***A. niger***	***A. flavus***	***N.crassa***
	10 ml	42	27.5	49
	20 ml	14	19.1	5.6
	30 ml	6	6	6.3

Top tapsin 70 %	2 mg	42	31.5	24
Control	Cont.	42	50	85
L.S.D (0.05)		1.88	2.64	2.49

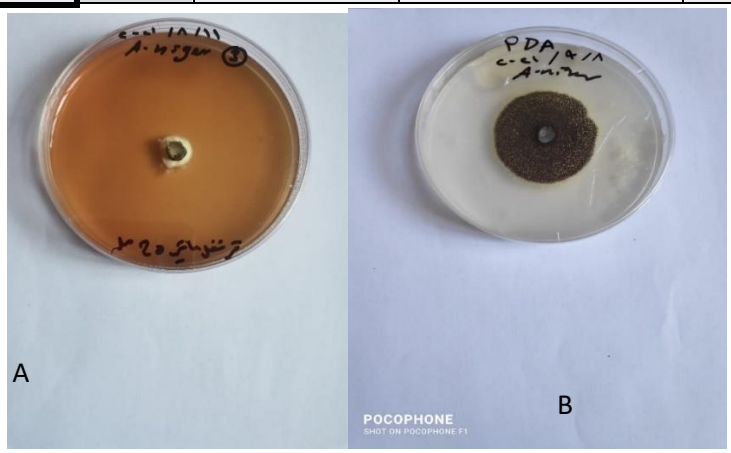

Figure (1) : Inhibition of Mycelial growth of A.niger by aqueous extract of Eugenia caryophyllata. A-Control B-Treatment

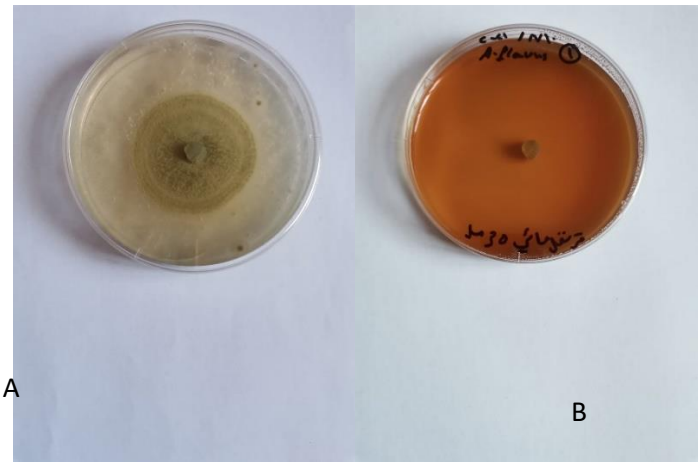

Figure (2) : Inhibition of Mycelial growth of A.flavus by aqueous extract of Eugenia caryophyllata. A-Control B-Treatment

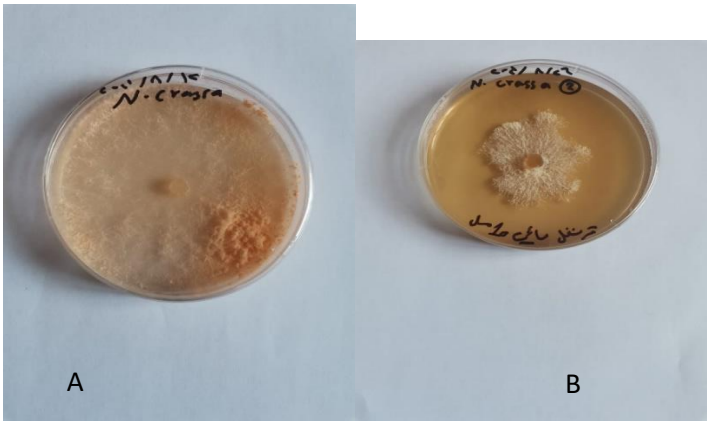

Figure (3) : Inhibition of Mycelial growth of N.crassa by aqueous extract of Eugenia caryophyllata. A-Control B-Treatment

Inhibition of Mycelial growth of pathogenic fungi by acetonic plant extract of Eugenia caryophyllata

The acetonic plant extract of *Eugenia caryophyllata* reduced mycelial growth and inhibited *A.niger* at all concentrations , where the inhibition percentages were: 31.1% , 77% , 88.5% respectively. As for *A.flavus* the acetonic extract also reduced mycelial growth at all concentrations , where the inhibition percentages were: 57.1% , 78.9% , 89.7 respectively. As for *N.crassa* the acetonic extract also reduced mycelial growth , where the inhibition percentages were: 85.5% , 90.5% , 94.1 respectively (Table 4) and Figures (1 ,2 and 3).

Table. 4: - Effect of Acetonic Plant Extract of Eugenia caryophyllata in redical growth of pathogenic Fungi.

Acetonic Extract	**Colonies Diameter**			
	Conc.	***A. niger***	***A. flavus***	***N.crassa***
E.caryophyllata	10 ml	30	21	12
	20 ml	10	10.3	8
	30 ml	5	5	5
Top tapsin 70 %	2 mg	42	31.5	24
Control	Cont.	43.6	49	85
L.S.D (0.05)		1.17	4.03	1.14

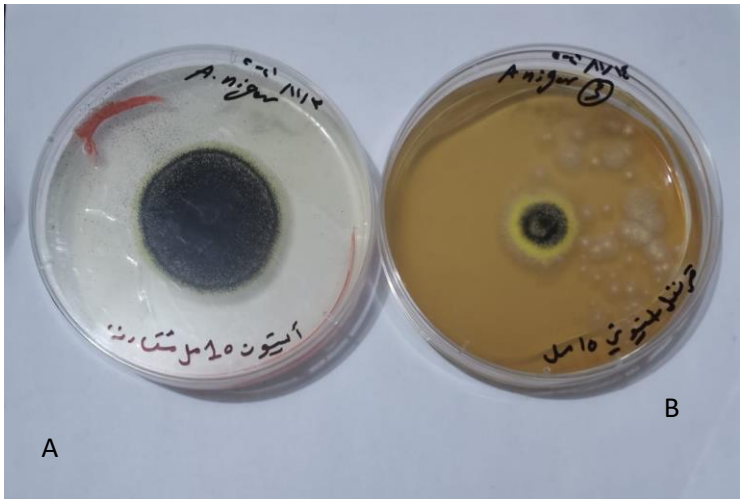

Figure (1) : Inhibition of Mycelial growth of A.niger by acetonic extract of Eugenia caryophyllata. A-Control B-Treatment

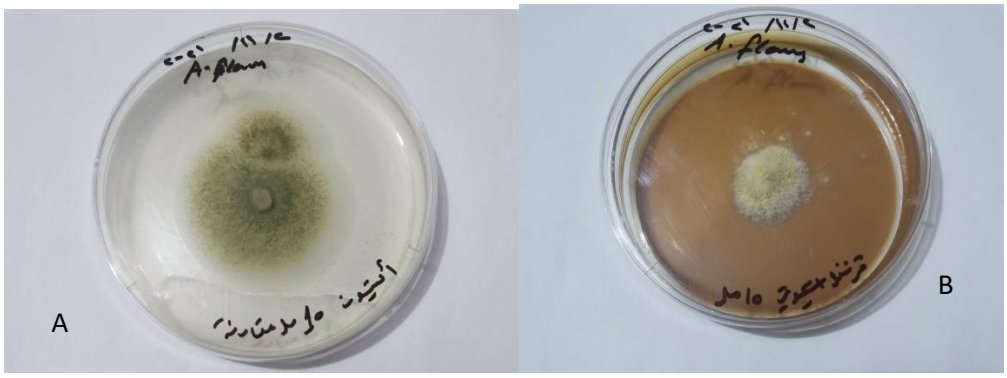

Figure (2) : Inhibition of Mycelial growth of A.flavus by acetonic extract of Eugenia caryophyllata. A-Control B-Treatment

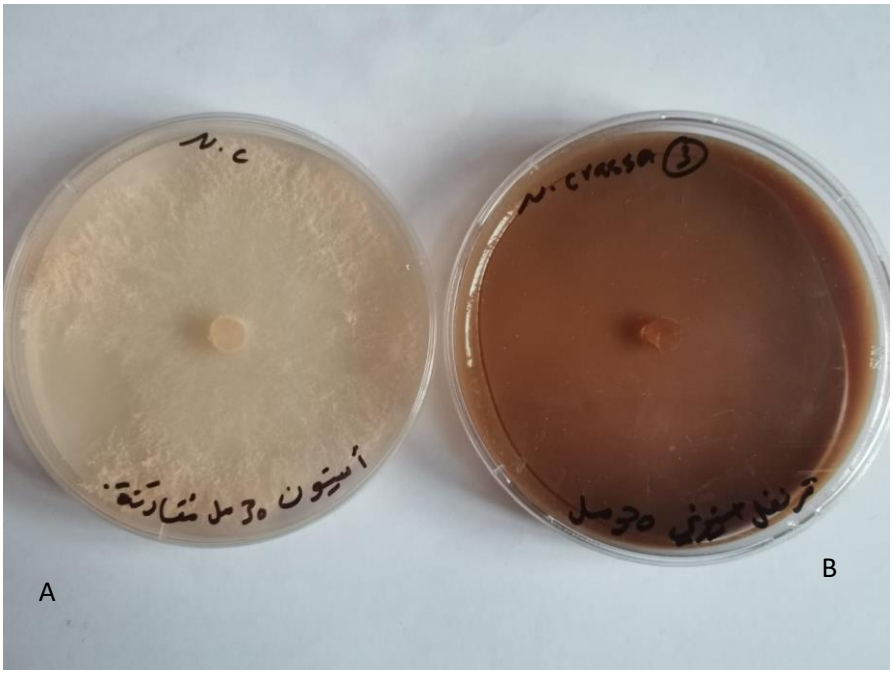

Figure (3) : Inhibition of Mycelial growth of N.crassa by acetonic extract of Eugenia caryophyllata . A-Control B-Treatment

Inhibition of Mycelial growth of pathogenic fungi by aqueous plant extract of Cinnamomum zeylnicum

The aqueous extract of *Cinnamomum zeylnicum* reduced mycelial growth of *A.niger* at concentrations : 20 ml and 30 ml ,where the inhibition percentages were : 3.3% , 7.1% respectively. As for *A.flavus* , the aqueous extract reduced mycelial growth at the same concentrations , where the inhibition percentages were : 4% , 16% respectively. Finally, aqueous extract of Cinnamomum zeylnicum did not have any inhibitory effect against *N.crassa* (Table 5).

Table.5: - Effect of Aqueous Plant Extract of Cinnamomum zelynicum in redical growth of pathogenic Fungi

Aqueous Extract	**Colonies Diameter**			
C.zelynicum	**Conc.**	***A. niger***	***A. flavus***	***N.crassa***
	10 ml	42	50	85
	20 ml	40.6	48	85
	30 ml	39	42	85
Top tapsin 70 %	2 mg	42	31.5	24
Control	Cont.	42	50	85
L.S.D (0.05)		1.35	1.30	0.00

Inhibition of Mycelial growth of pathogenic fungi by acetonic plant extract of Cinnamomum zeylnicum

The acetonic plant extract of *Cinnamomum zeylnicum* reduced mycelial growth of *A.niger* at all concentrations , where the inhibition percentages were: 1.3% , 8.2% , 39.2% respectively. As for *A.flavus*, the acetonic extract reduced mycelial growth at the same concentrations, where the inhibition percentages were: 23.2% , 26.5% , 45.9% respectively. As for *N.crassa* , the acetonic extracts gave a high inhibitory effect at concentrations : 20 ml , 30 ml , where the inhibition percentages were : 82.2% , 92.2 respectively. Table (6) and Figures (1 and 2).

Table.6 : Effect of Acetonic Plant Extract of Cinnamomum zelynicum in redical growth of pathogenic Fungi

Acetonic Extract	**Colonies Diameter**			
C.zelynicum	**Conc.**	***A. niger***	***A. flavus***	***N.crassa***
	10 ml	43	37.6	85
	20 ml	40	36	10
	30 ml	26.5	26.5	6.6
Top tapsin 70 %	2 mg	42	31.5	24
Control	Cont.	43.6	49	85
L.S.D (0.05)		1.14	2.75	1.19

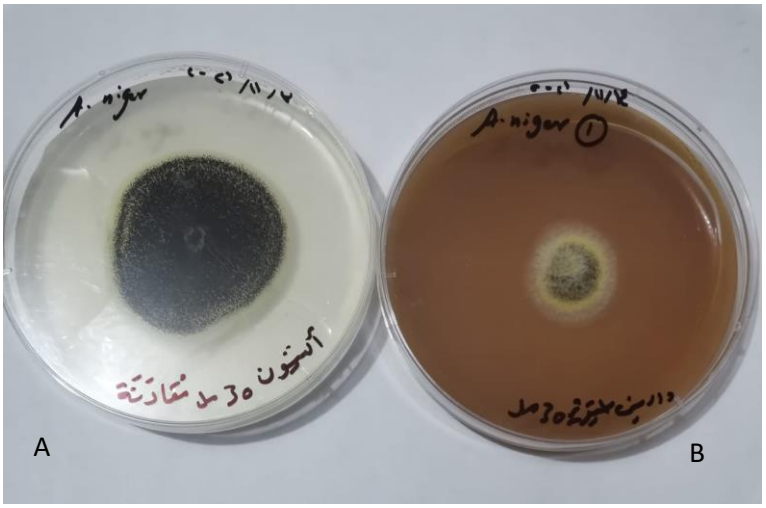

Figure (1) : Inhibition of Mycelial growth of A.niger by acetonic extract of Cinnamomum zeylnicum . A-Control B-Treatment

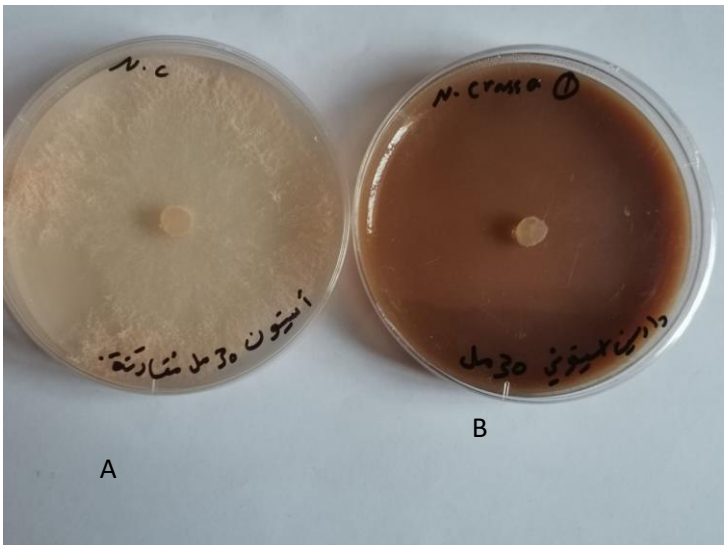

Figure (2) : Inhibition of Mycelial growth of N.crassa by acetonic extract of Cinnamomum zeylnicum. A-Control B-Treatment

5. DISCUSSION

The mycelial growth inhibition of the pathogenic fungi by the aqueous and acetonic plant extracts of *E.caryophllata* and *C.zeylnicum* investigated in this study indicated that the antifungal activity showed by the tested plant extracts had inhibitory effect on the growth of *A.niger* , *A.flavus* and *N.crassa* . The results further revealed that antifungal activities of the extracts were enhanced by increasing the concentration

from 10-30ml (v/v) ; hence the inhibition activities were concentration dependent. This is in agreement with [31] who indicated that increase in the antifungal activities had corresponding

increase in concentration of plant extracts. The aqueous and acetonic of *E.caryophyllata* and *C.zeylnicum* exhibited high inhibitory effect in mycelial growth against *A.niger* , *A.flavus* and *N.crassa*. The antifungal activity of *E.caryophyllata* extract conforms to the result of [32] who indicated that this extract is very effective in inhibition the growth of *F.solani* , *A.solani* , *R.solani* and *M.phaseolina*. The antifungal properties of *E.caryophyllata* attributed to the presence of Tannins , Glycosides and Terpenoids [33]. *C.zeylnicum* extract exhibited high inhibitory effect against pathogenic fungi conforms to the result of [34] who indicated that this extract is very effective in inhibition the growth of *Pythium aphanidermatum.* However, the differences in the efficacy of the extracts could be attributed to the differences of their active ingredients [35]. Major ingredients of plant extracts are phenols , flavonoids , alkaloids , quinones , tannins , saponins and sterols [36] and their antifungal properties against various plant pathogens have been established [37]. These products might either have direct inhibitory effects on pathogens , exhibiting fungicidal or fungistatic properties. They could help in the establishment of favorable conditions for antagonistic microbes [37].The fungicide Top tapsin 70% at 2mg/ml was less effectiveness than plant extracts at a various concentrations in inhibition the mycelial growth of the pathogens.

6. CONCLUSIONS

This study demonstrated that the aqueous and acetonic plant extracts of *E.caryophyllata* and *C.zeylnicum* were more effective than Top tapsin 70% and could be used as antifungals because they contained many active ingredients such as alkaloids , tannins , glycosides , flavonoids , fuocoumarins , resins , saponins and terpenes. The aqueous and acetonic extracts showed the highest inhibitory effect against pathogenic fungi at concentration 30ml (v/v). The utilization of plant extracts to control disease in vegetable field minimizes or eliminates the risks and hazard of toxic fungicides , especially on freshly consumed vegetables , So the plant extracts are safer than fungicides because they have few side effects. It is anticipated that further research into these extracts would identify the active ingredients responsible for their fungicidal activity.

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