P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2023,(17), (1):85– 96

Isolation and molecular identification of fungi contaminating fruits and vegetables in cold storages in Al – Anbar city and studying the effect of aqueous extract and dry powder of *Eugenia caryophyllata* on it.

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ARTICLE INFO

Received: 06 / 05 /2023
Accepted: 22 / 05/ 2023
Available online: 11 / 06 / 2023

DOI: 10.37652/juaps.2023.178867

Keywords:
molecular identification,
fungi, Eugenia,
cold storages,
aqueous extract,
dry powder,

ABSTRACT

The current study was conducted in the Fungi Research Laboratory, Department of biology, College of Sciences, University of Anbar , the study included collecting 93 samples of infected fruits (fruits and vegetables) in Cold stores for different regions of Al- Anbar province. The fungi that contaminated the fruits were isolated and characterized microscopically and molecularly, and aqueous plant extracts and dry powders were prepared of *Eugenia* flowers for the purpose of studying their effect on radial growth and pathogenicity of these contaminated fungi, *Aspergillus flavus* و *Aspergillus niger* ، *Neurospora crassa* This was done by using the method of poisoning the food medium PDA with concentration 10%, 20%,30% v/v for aqueous extract and 0.1, 0.2, 0.3 mg/ml for dry powder of *Eugenia* powder, to evaluate the efficacy of the aqueous extracts and dry powder against contaminated fungi. The fungicidal effect Topsin M 70% was also tested with concentration 0.02 mg/ml of PDA. The active chemical compounds of the plant extracts have been detected using several chemical reagents and by using GC MASS technology for aqueous extracts and the pH value was determined also.

The results showed that the aqueous extract of *Eugenia* flowers inhibited the radial growth of contaminated fungi *Aspergillus niger*, *Aspergillus flavus*, *Neurosporo crassa* Where the highest inhibition percentage was reached when the concentration was 30% v/v and gave 6, 6, 5.6 mm respectivily with inhibition ratio 85.7, 85.7, 93.4 % with significant differences from the control treatment at probability level 0.05.

Also the results showed, Eugenia dry powder, it gave an effective inhibition towards the isolated fungi, *A. niger, A.flavus, N. crassa.*

The results showed the ability of *Eugenia* dry powder to stimulate resistance in tomato fruits before and after infection with fungi, at an incubation temperature of 11º c. Pre-inoculated with these *Neurosporo crassa* and *Aspergillus flavus*, *Aspergillus niger* contaminated fungi and beyond. The superiority of *Eugenia* dry powder in the pre-inoculation treatment in reducing the severity of the infection with the contaminated fungus, as it gave the highest effectiveness in reducing the severity of the infection with *N.crassa*, amounting to 26.6%, compared to the control treatment, which gave an infiction severity 80%.

The results of the qualitative chemical detection indicated the active compounds in the plant extract, The extract contain many effective compounds with antifungal activity such as alkaloids, resins, reducing sugars, tannins, saponins, glycosides, Terpenes, flavonoids and fucoumarins.

GC-MS results showed that *Eugenia* extract contains many active chemical compounds that are attributed to antifungal activity, such as euginol and thymoquinone.

Introduction:

A lot of food, including fruits and vegetables It is highly sensitive to fungal contamination, It is known that fungi cause contamination of these Food leading to clear economic loss [1]. It is responsible for the spoilage of foodstuffs, as it causes putrefaction and the emission of unpleasant odors [2]. It alters the flavor of these foods. Fungi are the most common pathogens of crops, as they are a major cause of infection in many fruits and vegetables during storage and transportation [3]. Fungi are associated it is commonly contaminated with food in refrigerators, as it prevails in the spoilage of refrigerated food, especially when Low water activity, high acidity or due to packaging conditions, and Fungi commonly isolated from chilled fruits and vegetables are called psychrotrophic fungi [4], The genera of these fungi include several types, including: *Geotricum* ، *Fusarium* ، *Aspergillus* ، *Penicillium* ، *Mucor* ، *Rhizopus*, *Cladosporium* ، *Monascus* [5]. Some fungal species are characterized by their ability to produce mycotoxins, the most important of which are aflatoxins produced by some species of the genus *Aspergillus* spp. The damage of fungi is not limited to the spoilage of food, fodder and grains only, but also has health damage to humans and animals, as it secretes toxic metabolites; So, it is necessary to search for safer methods and ways as biological methods are among the most important methods used in mold control [6].So, the searches for antidotes became Microbes of plant origin represent the trend of most researchers, especially after pathogens have developed resistance to used antibiotics [7]. Several studies have indicated that medicinal plants contain a variety of classes of compounds biologically active as alkaloids, tannins, flavonoids, terpenoids, tannins, saponins and glycosides have antifungal activity [8]. Researchers in the field of natural antibiotics for microorganisms have indicated the use of plant extracts for several reasons, including their abundance, ease of access, low cost, and most of all, that they are safer due to their lack of side effects [9].

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Therefore, the current study aimed at the following:

1- Isolation of fungi that contaminating fruits and vegetables in refrigerated storages and their diagnosis visually and microscopically and by pcr.
2- Detection of the effective chemical compounds of Eugenia flowers, a qualitative chemical and technical detection by using a technique GC-MASS.
3- Study the effect of different concentrations of dry powder and aqueous extract of Eugenia flowers on the growth of fungi isolated from refrigerated stores laboratory.
4- Study the ability of dry powders and aqueous extracts of a plant extract in stimulation the resistance in tomato fruits before and after fungal infection.

Materials and Methods:

Site and Materials for the Experiment:

In Department of Biology, College of Science, University of Anbar conducted the experiments. The contaminated produce was gathered from various marketplaces in the Al-Anbar Government, specifically Al Ramadi, Al Fulluja, Al Rutba, and Heet markets. The contaminated fruits and vegetables are instantly transported to the lab for testing.

media preparation for culture:

39 grams of Potato Dextrose Agar medium (PDA) (BAM Media M127) were dissolved in 1000 cc of distilled water to create the medium. At 121 ºC for 20 minutes, the medium was autoclaved at 15 lb. Before adding streptomycin sulphate (3 grams) and aseptically dispensing the sterilized medium into sterilized 9 cm diameter Petri plates, the sterilized medium was allowed to reach to 45º C.

Isolation and Identification of fungal pathogens:

Fungi were isolated from contaminated fruits and vegetables by washing them, transferring 1 cm from the infected area, sterilizing with 1% sodium hypochlorate solution, washing and sterilizing and drying between two sterile filter papers, and then cultured on Petri dishes containing PDA medium containing the antibiotic streptomycin sulphate and incubated for 7 days at 28 °C [10]. Fungi were recognized after growing in Petri dishes based on morphological characteristics including shape, color, colony diameter, and height, as well as microscopic characteristics like shape, size, and color, Conidiophore composition and other compositions with the help of books [11], By using PCR, several isolates were detected (Molecular Identification).

Molecular identification:

Molecular diagnosis was carried out at the Scientific Progress Laboratory– Baghdad, by kits found in table1.

Table 1: kits used for molecular diagnostics.

	kits	company
1	Wizard Genomic DNA Purification Kit , 1 Agarose, Ethidium Bromide Solution (10mg/ml) , Go Tag Green Master Mix , Nuclease Free Water, TAE 40X, Quantifluor dsDNA System.	Promega
2	Absolute Ethanol , Isopropanol	ROMIL pure chemistry
3	Primers	Macrogen

Primers for the sequence of nitrogenous bases [12], were used in table 2.

Table 2: primers which used in the molecular diagnostics.

Primer name	Sequence	Annealing temp. (º C)	Product size (bp)
ITS1 forward	5`TCCGTAGGTGAACCTGCGG'3	55	550 – 600 bp
ITS4 revers	5`TCCTCCGCTT ATTGATATGC3`	55	550 – 600 bp

Molecular diagnosis of fungal isolates was carried out according to the following steps:

1- Genomic DNA Extraction: DNA was extracted from the two isolates *Neurospora crassa* و *Aspergillus flavus* by kit from promega company According to the manufacturer's instructions.
2- Stages of DNA replication by polymerase chain reaction [13].
3- Gel Electrophoresis. After amplification of the DNA by technique PCR Electrophoresis was carried out using an agarose gel.
4- The sample was sent to the Korean company macrogen for sequence.

Phytochemical screening of plant extracts' active components:

Several chemical analyses of the primary active components in the aquatic *Eugenia caryophyllata* preparation were done to detect alkaloids, glycosides, tannins, amino acides, flavonoids, saponins, resins and terpens.

Quantitative detection of active chemical compounds in plant extracts using a technique Gas Chromatography Spectrometry mass.

Detection of active chemical compounds using a technique GC.Mass In Ibn Al-Bitar Center, Ministry of Industry and Minerals, Where use the gas chromatography model JOEL in the examination, the components of the chemical plants were identified in a database of the spectrum of known compounds kept in a library GC- MS.

PH of plant extracts detection:

10 grams of plant powder and 50 ml of distilled water were combined using a magnetic stirrer for 10 minutes, after which the solution was filtered and the PH value was calculated using a PH-meter equipment [14].

Preparation of aqueous plant extract

According to [15] aquatic plant extracts were made 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, leaving the suspension in a magnetic stirrer for 24 hours, then filtering the suspension using several layers of medical gauze and sterilizing it through a Millipore filter 0.22um. After this process, the suspension was then filtered, and the clear liquid of extract was stored in sealed containers in the refrigerator at 4c until use [16].

Study the effect of aquatic extract of Eugenia (flowers) on the prevention of fungal mycelia growth:

After sterilization and cooling to 50C, the aquatic plant extract was mixed with PDA medium at concentrations of 10ml, 20ml, and 30ml of extracts/100ml of PDA medium at a rate of three replicates for each concentration. After the extract-media mixtures solidified, a disc with a diameter of 5 mm of a 5- to- 7- day-old culture of each fungus was placed in the center of the petri dishes. The PDA concentrations used in the control plates of aquatic extract were 10ml, 20ml, and 30ml. Topsin 70% at a concentration of 2 mg was used in the fungicide plates along with PDA. Three duplicates of each treatment were used. Except *for N. crassa* isolates (incubated for 3 days), all control and treatment plates were incubated at (25-28) ºC for 5-7 days. A ruler was used to measure the radical development in the treatment and control groups. The following formula was used to calculate the percentage of each

extract's inhibition of mycelial growth: I = 100 x (C-T) / C, Where:
I = percentage of mycelial growth inhibition
C= mycelial growth of fungus in control plate
T = mycelial growth of fungus in the treatment [17].

Effect of dry powder of *Eugenia* (flowers) on the radial growth of contaminated fungi:

This experiment was designed to investigate the effect of dry powder of *Eugenia* flowers on the radial growth of contaminated fungi, this experiment involved mixing the dry powder with PDA in ratio 0.1, 0.2, 0.3 mg / ml of PDA, the mixture is placed on thermal magnetic stirrer to mix well and then sterilize the media in an autoclave , after that, the media was poured into Petri dishes with three replicates for each concentration, and for each fungus, while the comparison dishes included medium without any addition.

Evaluation of the efficiency of dry powder and aqueous extract of *Eugenia* plants in reducing fungal infection of tomato fruits before and after pollination at an incubation temperature of 11ºC.

This experiment was designed to investigate the ability of aqueous plant extract and dry powder on Inducing resistance in tomato fruits towards the contaminated fungus, which included two methods:

The first method included treatment of tomato fruits with plant extract and dry powder separately before infection with fungus (preventive method), the tomato fruits were taken in the amount of 5 fruits and immersed in a 1% sodium hypochlorite solution for 5 minutes for sterilization, then they were dried and each fruit was pierced 5 holes with a sterile piercing needle and sprayed with aqueous extract at a rate of 2 ml for each fruit for aqueous extract and 10 mg for dry powder. The fruits were left to soak their holes with aqueous extract and dry powder for 24 hours. On the next day, the fruits were sprayed with 6 microliters of spore suspension at a concentration of 10^6 spore / ml of distilled water [18], spores suspended, attended according to the method of [19]. The fruits were then placed in sterile polyethylene bags and sealed, and then incubated in the incubator at a temperature of 11 C (Matches the temperature of the refrigerator) for 7 days.

The second method involved treating tomato fruits with aqueous plant extracts

And dry powders separately after being infected with pathogenic fungi (therapeutic method), The sterilized and perforated tomato fruits were sprayed with spore 6 microliters, and the fruits were incubated in the incubator for 24 hours, so that the fruits were absorbed by the spores, On the next day, the tomato fruits were sprayed with aqueous plant extracts at a rate of 2 ml and dry powders at a rate of 10 mg, and the fruits were placed in sterile polyethylene bags, It was incubated for 7 days in the incubator at 11°C.

The control treatment, it included spraying tomato fruits with spore suspension only, without any addition, and they were placed in sterile polyethylene bags, and incubated at a temperature of 11º C for 7 days.

After that, the severity of the injury was calculated according to the equation used by [20]. Where a special list for disease guide was used, which included four grades for the severity of the injury:
0 = healthy fruits
1 = Fruits covered by rot by 1 - 25% of their area.
2 = Fruits covered by rot by 26 – 50 % of their area.
3 = Fruits covered by rot mor than 50 % of their area.

The percentage of injury severity was calculated according to the following equation:

The injury severity (%) = No. of fruit in degree 0*0+……. +No.of fruit in degree 3*3/ the total number of fruits * highest degree of injury * 100.

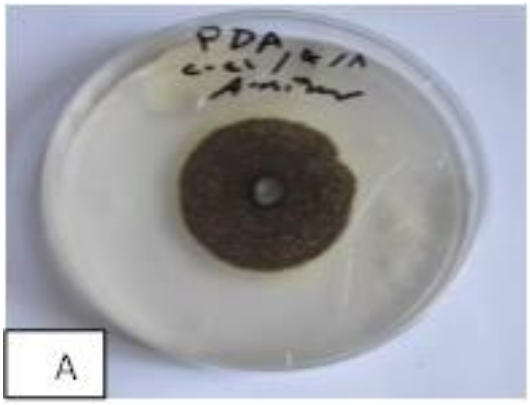
STATISTICAL ANALYSIS:

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.

The results:

Isolation and diagnosis:

The results of isolation and diagnosis of 93 samples of infected fruits (fruits and vegetables) in refrigerated stores for different regions of Anbar / Iraq, the presence of different species of fungi; *Aspergillus niger, Aspergillus flavus, Neurosporo crassa* , All fungi were diagnosed phenotypically and microscopically, based on the taxonomic keys for fungi classification, And moleculary with technique PCR.



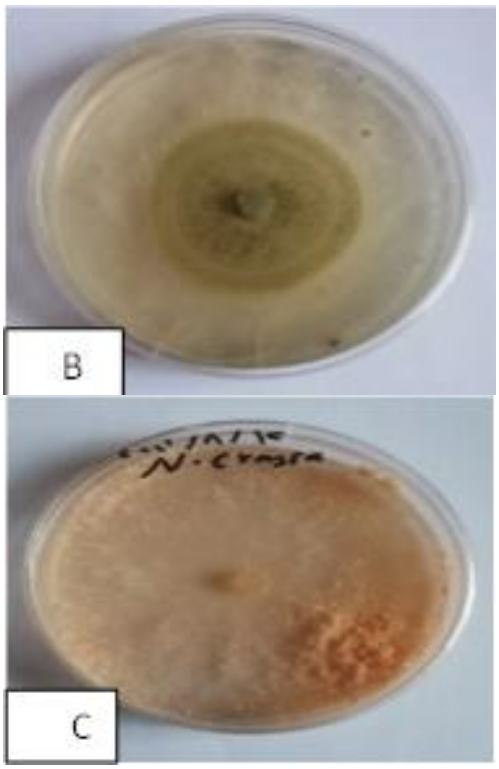
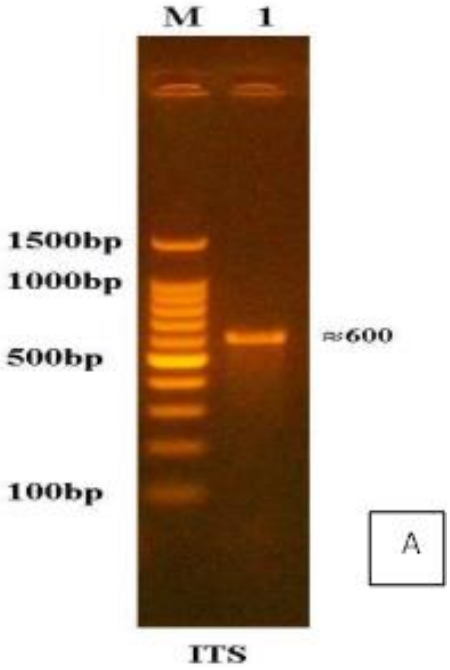


Figure 1: Fungi isolated from fruits and vegetables in refrigerated storage on PDA medium at pH 5.6 and incubation temperature 28ºC. A= *Aspergillus niger*, B= *Aspergillus flavus*, C= *Neurosporo crassa*.



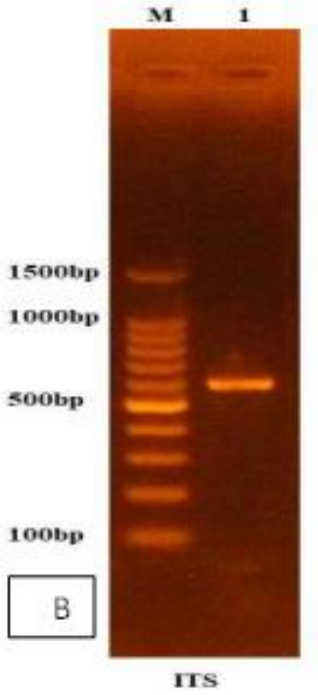


Figure 2: Shows the results of DNA replication on an agarose gel at a concentration of 1.2%. A= *Neurosporo crassa*, B= *Aspergillus flavus* . by primer ITS1, ITS4.

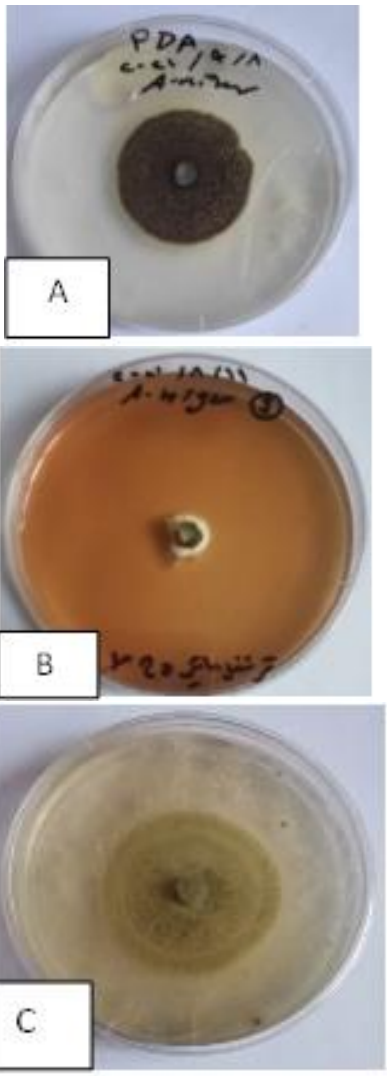
Effect of aqueous extract of *Eugenia* on the radial growth of contaminated fungi:

The results presented in Table 3 and Figure 3 showed the inhibitory effect of the aqueous extract of *Eugenia* against the infected fungal isolates, *Aspergillus niger*, *Aspergillus flavus*, *Neurosporo crassa*, the rates of diameters of fungal colonies 42, 27.5, 49 mm respectivily in concentration 10 % v/v. The differences were significant between the averages of the treatments and the control treatment at a probability level of 0.05 for the two fungi, *A. flavus* and *N. crassa* while the fungus *A. niger* The effect of the extract did not reach to Significant level at the same concentration mentioned above.

the concentration of 30% v/v, it was found that all diameters of fungal colonies decreased clearly, at rates of 6, 6, 5.6 mm, The differences were Between the averages of the treatments and the control treatment were significant for all fungal isolates previously mentioned. The results showed that the aqueous extract of cloves was superior to the fungicide Topsin M 70% In inhibiting the growth of contaminated fungi, especially at concentrations of 20% and 30% v/v

Table 3: Inhibitory effect of aqueous extract of *Eugenia* flowers on the radial growth of contaminated fungi on PDA medium at pH 5.6 and an incubation temperature of 28º for 5 days.

Aqueous Extract	Colonies Diameters (mm)			
E. caryophyllata	Conc.	*A.niger*	*A.flavus*	*N.crassa*
	10	42	27.5	49
	20	14	19.1	5.6
	30	6	6	6.3
Topsin70 %	2 mg	42	31.5	24
control	Cont.	42	50	85
L.S.D. (0.05)		1.88	2.64	2.49



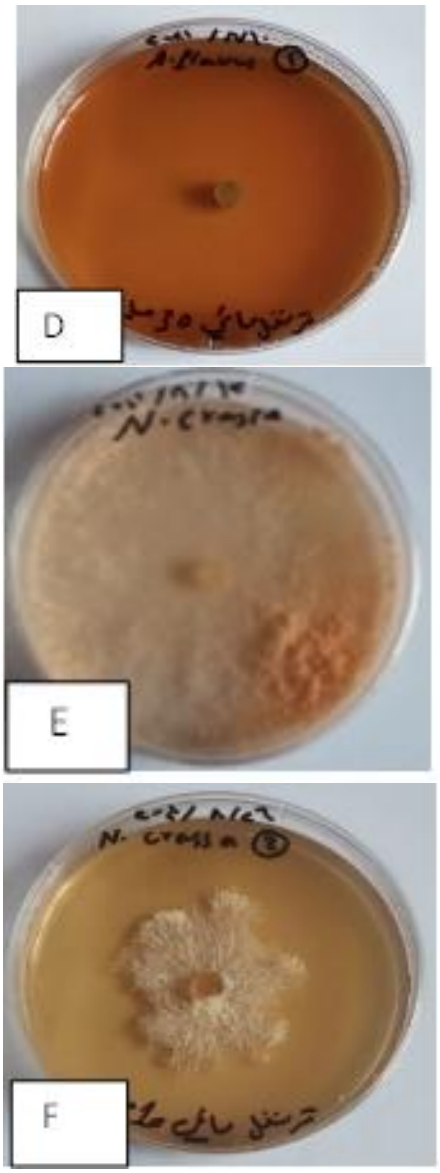


Fig. 3: Inhibitory effect of aqueous extract of *Eugenia* flowers on the radial growth of contaminated fungi on PDA medium. A: control of *A.niger* , B: A.niger + *Eugenia*, C: control of *A. flavus*, D: *A. flavus* + *Eugenia*, E: control of *N. crassa*, F*: N. crassa* + *Eugenia*.

Effect of dry powders of *Eugenia* on the radial growth of contaminated fungi:

The results of table 4 and figure 4 show the strong inhibitory activity shown by dry powder of *Eugenia* against fungi *A.niger, A. flavus, N. crassa* at all concentrations used with clear significant differences between the averages of the treatments and the control treatment at the level of probability 0.05, reached the diameters of the fungal colonies 10, 11.3, 11.6 mm in concentration 0.1 mg/ ml PDA . colony diameters decreased significantly at concentration 0.3mg/ ml PDA and gave 5, 5.2, 5.1(mm).

Table 4: the inhibitory effect of dry powder of *Eugenia* flowers on radial growth of contaminated fungi on the nutrient media at pH 5.6 and a degree of incubation 28°c.

Dry powder	Colonies Diameters (mm)			
E. caryophyllata	Conc. (mg/ml)	*A. niger*	*A.flavus*	*N.crassa*
	0.1	10	11..3	11.6
	0.2	6.3	6.1	7.5
	0.3	5	5.2	5.1
Topsin 70 %	2 mg	42	31.5	24
control	Cont.	42	50	85
L.S.D. (0.05)		1.716	1.052	1.953

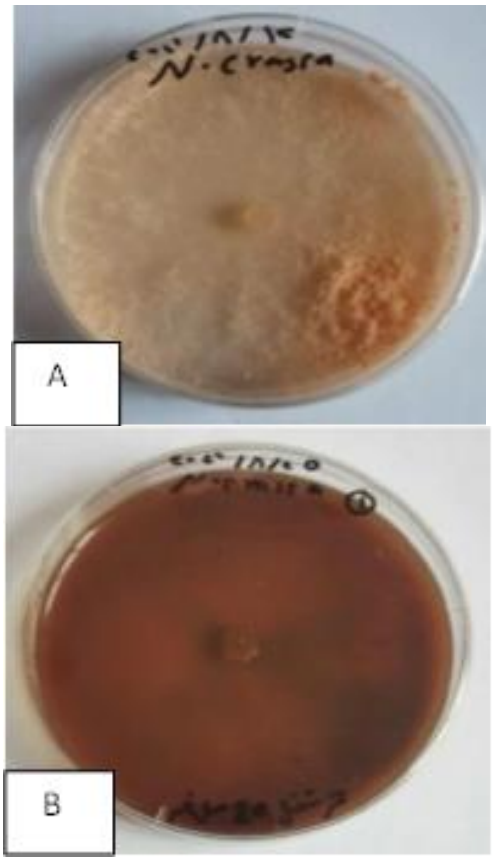


Fig. 4: The inhibitory effect of the dry powder of *Eugenia* flowers on the fungus *N.crassa* on PDA. A: control *of N. crassa*, B:*(N.crassa* + dry powder of *Eugenia*)

Evaluation of the efficiency of dry powders of *Eugenia* in reducing the infection of tomato fruits with fungi contaminated before and after pollination, with an incubation degree of 11 °c.

This experiment was designed to study the effect of *Eugenia* dry powder In reducing the severity of infection with fungi, *A. niger, A. flavus, N.crassa* Inside the fridge at a temperature of 11°c on tomato fruits through treating fruits with powder before and after inoculation with fungi, the results showed (table 5) , treating tomato fruits with *Eugenia* dry powder before inoculation with fungus *N.crassa* the best in reducing the severity of the infiction, It gave an infiction severity of 26.6% compared to control treatment Which gave an injury severity of 80% with a significant difference.

While the results showed, the treatment of tomato fruits with *Eugenia* dry powder after inoculation with the same fungus, the rate of infection reached 60% with a significant difference with the treatment of control, followed by the second degree effect of *Eugenia* dry powder in fungus *A. niger* then *A.flavus* In its ability to reduce the incidence of infection in tomato fruits, the severity of the infiction reached to 40 % and 50 % respectively and with significant differences with the control treatment.

Table 5: Evaluation of the efficiency of plant powders under study in reducing injury to tomato fruits with contaminated fungi before and after inoculation with an incubation degree of 11°c .

Dry powder	The severity of the infiction %			
E. caryofllata	The treatment	*A.niger*	*A.flavus*	*N.crassa*
	before inoculation	40	50	26.6
	After inoculation	60	65	60
control (tomato fruits spray with spore suspension)	control	73.3	80	80
L.S.D. (0.05)		3.165	7.089	1.103

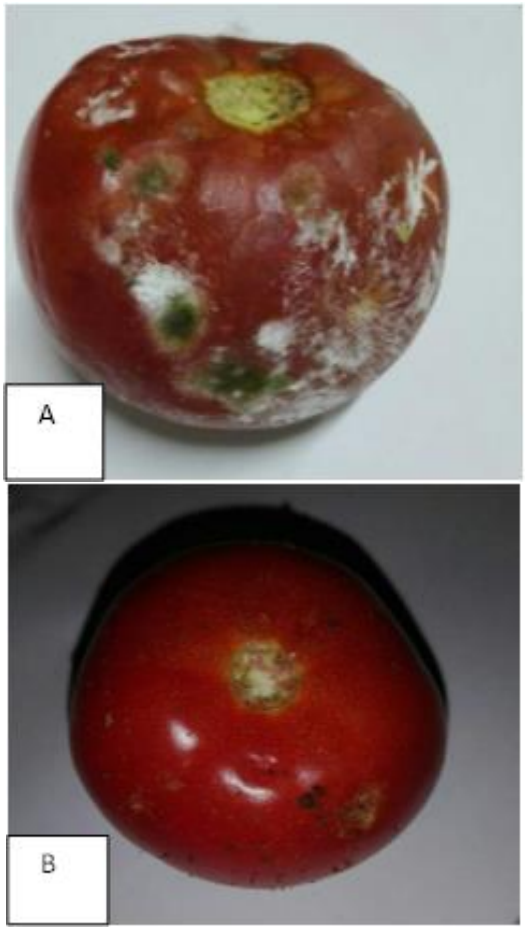


Fig. 4: Treatment of tomato fruits with dry powder of *Eugenia* after inoculation with fungus *A. niger* with incubation degree 11ºc for 9 days. A: Control treatment inoculated with fungus *A. niger* only., B: Treating tomato fruits with *Eugenia* dry powder after inoculation with fungus *A. niger*.

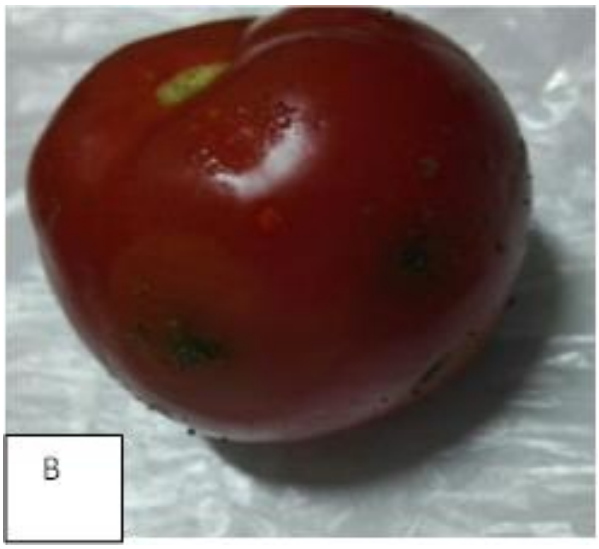


Fig. 5: Treating tomato fruits with clove powder before inoculation with fungus *N. crassa* with incubation degree 11ºc for 7 days., A: Control treatment inoculated with fungus *N. crassa* only., B: Treating tomato fruits with Eugenia dry powder before inoculation with fungus *N. crassa*.

Evaluation of the efficiency of aqueous plant extracts of *Eugenia* under study in reducing infection of tomato fruits with fungi before and after pollination at an incubation temperature of 11ºc.

This experiment was designed to study the effect of aqueous plant extracts of *Eugenia* In reducing the severity of infection with contaminated fungi, *A. niger, A. flavus, N. crassa* Inside the fridge at a temperature of 11ºc to the fruits of tomatoes that were treated with this extract before and after inoculation with fungi which mention above, The results in the table 6 showed, the aqueous extract of *Eugenia* is the most effective in reducing the severity of infection of tomato fruits before fungal inoculation *A. niger, A. flavus, N.crassa*, It gave the severity of infiction amounted to 56.6%, 58%, 60.6% respectively and with a significant difference from the control treatment, which gave a severe injury reached to 73.3%, 80%, 80% on respectively, while the infection severe after inoculation with fungi which mention above reached to 70%, 65%, 70.3% on respectively with a significant difference from the control treatment that gave the severity of the injury reached to 73.3%.

Table 6: Evaluation of the efficiency of aqueous plant extracts under study in reducing tomato fruit injury with contaminated fungi before and after inoculation 11ºc for 9 days.

Aqueous extracts	The severity of the infiction %			
	The treatment	*A,niger*	*A.flavus*	*N.crassa*
E. caryophyllata	before inoculation	56.6	58	60.6
	After inoculation	70	65	70.3
control (tomato fruits spray with spore suspension)	control	73.3	80	80
L.S.D. (0.05)		3.408	2.399	0.791

Quantitative detection of active compounds in plant extracts using GC.MS technology.

The results of the detection of effective antifungal compounds of *Eugenia* flowers using GC.MS technique, indicated the presence of many effective compounds (table 7).

Table 7: The most important effective chemical compounds of Eugenia plant.

	Effective chemical compounds	%	RT
1	Eugenol	58.95	9.342
2	Phenol,2-methyl-4-(2-propenyl)-acetate	2.69	11.835
3	Valeric acid hydrazide	0.33	14.219
4	Cyclopropane	27.44	9.768
5	Thymoquinone	0.55	13.548
6	Pentanoic acid	0.44	15.550
7	Butanoic acid	0.14	17.018
8	Propanamide	0.01	18.283
9	Carbamic acid	0.06	16.347
10	Acetic acid	1.89	12.452

Results of the qualitative chemical detection of the active compounds in aqueous extract of *Eugenia* flowers.

The preliminary chemical detection results for the active compounds in the aqueous extract showed that there are many active compounds. pH was also determined (table 8).

Table 8: Results of the qualitative chemical detection of the active compounds in aqueous extract of *Eugenia* flowers.

	Chemical disclosures	*Eugenia* flower
1	Alkaloids	+
2	Saponids	+
3	Turbines	+
4	amino acids	-
5	Resins	+
6	Flavonoids	+
7	Reducing sugars	+
8	Glycosides	+
9	Tannins	+
10	Fucoumarins	-
11	pH	4.6

Discussion:

The results of isolation, phenotypic and molecular identification of 93 samples of fruits and vegetables in refrigerated stores showed the appearance of the following fungal isolates: *A. niger*, *A. flavus*, *N. crassa*. The results showed that the aqueous extract of *Eugenia* had an inhibitory effect on the growth of fungi at all concentrations used, this agrees with [21] also,the results of our study showed that the aqueous extract of *Eugenia* was superior to the fungicide topsin 70% in inhibition of fungi The results also showed that the highest percentage of inhibition was at a concentration of 30% v/v.

The inhibitory activity of the aqueous extract of cloves is attributed to its containment of many active compounds such as alkaloids, resins, saponids, flavonoids, terpenes, and sugars and others, the physiological effects of the active compounds possessed by the plant extracts have fungal growth inhibitory effects as a result of interfering with one of the vital functions that it targets and works to invalidate it , Plant extracts inhibit the building of proteins and nucleic acids RNA and DNA .

[22] pointed that some active compounds in plants may increase the activity of some enzymes like, Fumarase, Dehydrogenase, Succinic dehydrogenase which leads to increased toxicity and thus reducing the growth rate of the fungus or destroying the cell wall. The plant extracts affect the metabolism of the compound Ergosterol, it is a type of lipid alcohol that is an essential component of the

cell wall of fungi this is due to its effect on enzyme activity 3- hydroxyl-3-methylglutase which responsible for building mevalonic acid which paves the way for building Ergosterol therefore, it is prevented the formation the compound, this leads to the inhibition of the action of ion channels and transporters and the destruction of the fungal cell membrane and the exit of the contents inside the cellular and its death [23].

The results in table 4 showed, the Clove powder had an inhibitory effect on the contaminated fungal isolates at a probability level of 0.05. and the best inhibition rate was in the treatment of 0.3 mg / ml. This result agrees with [24], who studied the effectiveness of clove powder against fungi that produce toxins and their ability to produce spores.

According to the results of tables 5 and 6, it is clear that the treatment of tomato fruits with dry powders and aqueous extracts reduced the severity of infection of these fruits with fungi *A. niger*, *A flavus*, *N. crassa*. The treatment with dry powders and aqueous extracts before inoculation recorded a higher effectiveness in reducing the severity of the fungi than in the treatment after inoculation.

Spraying fruits and vegetables in refrigerated warehouses with plant powders and aqueous extracts plays a major role in protecting these fruits from pathogenic and contaminated fungal infection, as well as reducing the severity of infection with these fungi. [25] efficacy of extracts of four plants, including cloves, as alternatives to the chemical pesticides Amazalil and Thiabenzadole against the fungus A. alternata that causes black spotting of mangoes in laboratory conditions. The results showed that the aqueous extract of cloves was superior to the rest of the aqueous extracts, as the inhibition rate reached 100%.

The results of quantitative detection using GC.MS technology showed that the clove extract contained effective antifungal compounds such as Eugenol at a ratio of 58.9. this is consistent with the results of GC.MS conducted by [26], where the Eugenole compound has anti-filamentous activity against fungi and yeasts, including fungi pathogenic to humans and fungal species contaminating food. [27].

Quality tests showed that the clove extract contains many active compounds, including alkaloids, tannins, polysaccharides, resins, flavonoids, and terpenes. This result agrees with [28], these compounds affect one of the vital functions that they target and work to nullify its effect, the plant extracts may inhibit the building of basic proteins and nucleic acids RNA and DNA.

The alkaloids affect the synthesis of the cell wall of fungi [29]. The action of flavonoids is due to the disruption of the plasma membrane, the creation of a dysfunction in the work of the mitochondria, the inhibition of cell division, and the inhibition of building DNA and RNA, which leads to the inhibition of proteins [30] and inhibiting the formation of fungal cell wall by inhibiting the construction of chitin and B-glucans, which are important in building the fungal cell wall.

As for the dysfunction caused by flavonoids in the work of mitochondria, it comes through their inhibition of proton pumps, which affects the electron transport chain, reduces the building of the energy complex ATP and thus the death of the fungal cell [31].

While the tannins [32] indicated that tannic acid inhibits the radial growth of the fungus *Penicillium digitatum* and its germination of spores in the laboratory. As the mechanism of action of tannins lies through the tearing of the cell wall and the plasma membrane of the pathogenic fungus, which leads to the exit and leakage of intracellular substances to the outside of the fungal cell, and thus the destruction of the fungal cell.

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P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2023,(17), (1):85– 96

العزل والتشخيص الجزيئي للفطريات الملوثة للفواكه والخضروات في المخازن المبردة في محافظة الانبار ودراسة تأثير المستخلص المائي والمسحوق الجاف لنبات القرنفل عليها.

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الخلاصة :

أجريت الدراسة في مختبر الفطريات ، قسم علوم الحياة ، كلية العلوم ، جامعة الأنبار، جمع 93 عينة من فواكه وخضروات من مخازن مبردة في محافظة الأنبار. عزلت الفطريات الملوثة للثمار وتشخيصها مجهريًا وجزيئيًا، حضرت مستخلصات مائية ومساحيق جافة من القرنفل لغرض دراسة تأثيرها على النمو والإمراضية لهذه الفطريات *Aspergillus flavus* و *Aspergillus niger* و *Neurospora crassa* بطريقة تسميم الوسط الغذائي PDA بتركيز 10٪، 20٪، 30٪ حجم / حجم للمستخلص المائي و 0.1 ، 0.2 ، 0.3 مجم / مل لمسحوق للقرنفل ، لتقييم فعالية المستخلصات المائية والمسحوق الجاف ضد الفطريات الملوثة. تم اختبار تأثير مبيد الفطريات توبسين 70 M٪ بتركيز 0.02 مجم / مل من PDA. كشف عن المركبات الفعالة للمستخلص النباتي باستخدام الكواشف الكيميائية و بتقنية GC– MS للمستخلصات المائية وحدد الأس الهيدروجيني أيضًا. أظهرت النتائج أن المستخلص المائي للقرنفل يثبط النمو الشعاعي للفطريات *Aspergillus niger* و*Aspergillus flavus* و *Neurosporo crassa* اعطى أعلى نسبة تثبيط عندما كان التركيز 30٪ وأعطى 6، 6، 5.6 ملم على التوالي. مع نسبة تثبيط 85.7، 85.7، 93.4٪ مع اختلافات معنوية عن السيطرة عند مستوى الاحتمال 0.05.أظهرت النتائج أن مسحوق القرنفل أعطى تثبيط فعال تجاه الفطريات*A. niger*، *A.flavus*، *N. crassa* .أظهرت النتائج قدرة مسحوق القرنفل الجاف على تحفيز المقاومة في الطماطم قبل وبعد الإصابة بالفطريات ، عند درجة حرارة 11م°. تم تلقيحها مسبقًا مع هذه الفطريات الملوثة وما بعدها. تفوق مسحوق القرنفل في المعاملة قبل التلقيح بالفطريات في تقليل شدة الإصابة بالفطريات الملوثة، حيث أعطى أعلى فعالية في التقليل من شدة الإصابة مقارنة مع السيطرة والذي أعطى شدة إصابة 80٪.دلت نتائج الفحص الكيميائي النوعي على المركبات الفعالة في المستخلص النباتي،اذ يحتوي المستخلص على المركبات الفعالة ذات الفعالية المضادة للفطريات مثل القلويدات والراتنجات والسكريات والصابونين والجليكوزيدات والتربين والفلافونويدات والفوكومارين .وأظهرت نتائج GC–MS أن القرنفل يحتوي على العديد من المركبات النشطة التي يعزى إليها النشاط المضاد للفطريات، مثل euginol و thymoquinone.

الكلمات المفتاحية: التشخيص الجزيئي، الفطريات، القرنفل، المخازن الباردة، المستخلص المائي، المسحوق الجاف.