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# Effect of *Eucalyptus camaldulensis* Terpens, Alkaloids and Phenols Agnaist *Fusarium oxysporum*

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**Abstract**

This study includes isolation, purification, and identification of *Fusarium oxysporum* from chili pepper infected plants. *Eucalyptus camaldulensis* were collected and air dried at room temperature, then ground to semi powdered state. Phenols, alkaloids and terpens were extracted from *Eucalyptus camaldulensis*. The antifungal activity, type of extracts was evaluated at different concentrations 5 and 10 mg / ml of these compounds were prepared and their antagonistic activity was studied. The Percentage of radial growth inhibition of fungi by plant extracts was measured after 7 day incubation. Results showed that terpens extract was the most active against fungi and alkaloids extract had less antifungal activity and the percentage of mycelia radial growth were 99.55 % – 72.44 % respectively.

**Keywords:** *Eucalyptus*, *Fusarium*, phenols, alkaloids, terpens and extracts.

# تاثير المستخلص التربيني والفينولي والقلويدي لنبات *Eucalyptus camaldulensis* على فطر *Fusarium oxysporum*

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**الخلاصة**

تضمنت الدراسة الحالية عزل وتنقية وتشخيص *Fusarium oxysporum* من اوراق الفلفل الحار المصاب . بالإضافة الى ذلك فقد تم جمع الأجزاء الهوائية (الاوراق) لنبات *Eucalyptus camaldulensis* واستخلصت كل من الفينولات والتربينات والقلويدات من تلك الاجزاء الهوائية ثم اختبرت فعاليتها المضادة للفطر المعزول وصولا الى استعمالها في السيطرة على نمو الفطريات. أستعملت تراكيز مختلفة من تلك المستخلصات (5، 10) ملغم / مل وأضيفت إلى الفطر بطريقة تسمم الوسط . ثم قياس أقطار النمو الشعاعي بعد 7 ايام من حضانة الفطر اوضحت النتائج إن المستخلص التربيني كان الأكثر فعالية تجاه الفطر من المستخلصات الأخرى وكان المستخلص القلويدي الأقل تأثيرا حيث بلغت النسبه المؤية لتثبيط نمو المايسلا 99.55 %في تركيز 10 % و 72.44 % في تركيز 5 % على التوالي.

## Introduction:

Plant diseases play a direct role in the destruction of natural resources in agriculture. In particular, soil-borne pathogens cause important losses, fungi being the most aggressive [1]. The problems caused by synthetic pesticides and their residues have increased and the need for effective biodegradable pesticides with greater selectivity. Alternative strategies have included the search for new types of pesticides, which are often effective against a limited number of specific target species, are

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biodegradable into non-toxic products, and suitable for use in integrated pest management programmers [2]. Applied chemical pesticides are one of the effective and fast means for reducing the loss of post-harvest diseases [3]. This damage increased significantly with improper use and randomly left to grow, in order to reduce the use of these chemicals that accumulate in fruits and vegetables. As persistent hazardous chemicals, that use by governments and international organizations of many of these pollutants, previously used to control a wide range of fungi, led to an imbalance in the natural enemies in the environment, the low rate of which helps to maintain the pathogen [4]. The natural plant products derived from plants effectively meet this criterion and have enormous potential to influence modern research. When extracted from plants, these chemicals are referred to as botanicals. The use of botanical pesticides is now emerging as one of the prime means to protect crops and their products and the environment from pesticides [5]. Botanicals degrade more rapidly than most chemical pesticides, and therefore are considered to be eco-friendly and less likely to kill beneficial pests than synthetic pesticides with longer environmental retention [6].

**Materials and Methods:**

**Isolation and identification:** *F. oxysporum* from the infected and died seedlings oF chili pepper was carried out by taking it and washed by flowing water for one hour, then parts of the roots and crown region were cut to small pieces (5mm) and were surface sterilized by immersion in sodium hypochlorite (1% free chlorine) for 2min, rinsed with sterile distilled water, transferred to PDA plates and incubated for 4 days at 27±2 Cº

**Collection of the studied plants:** *Eucalyptus camaldulensis* leaves were collected from the gardens of Baghdad University and air dried at room temperature then ground to semi powdered state by using grinder then stored in labeled and clean containers into refrigerator until use.

**Preparation of plant extracts:** phenols: 200 g of dried materials were divided into 2 equal quantities, one was mixed with 300 ml of D.W. and the other was mixed with 300 ml of 1% HCl. Then samples were homogenized in electrical shaker for 5 min., and warmed by using centrifuge [7], alkaloids: were considered. A quantity of 100g dried materials was homogenized in electrical shaker with (350ml) of (4:1) ethanol: D.W., then filtered through muslin. Then through a filter paper in Bouknner funnel, the filtrate was concentrated to quarter of original volume, then acidified by drops of 2% $H_2SO_4$ until the pH became between 1 and 2, then it was extracted with chlorophorm 3 times in the separating funnel; alkaloids were precipitated by the addition of drops of concentrated $NH_4OH$, pH became 9 and 10, then extracted with chloroform-methanol (1:3) twice and with chloroform once, 2 layers appeared, lower layer was dried by electrical oven, and the residue was containing weak alkaloids. Upper layer, aqueous layer was dried by electrical oven; dried residue was extracted with methanol. Then kept in refrigerator until use [8], terpens: successively extracted in a soxhlet extractor for 24 hrs. With 200 ml chloroform. The solvent was removed by electrical oven at 40°C. Then scarp and storage in refrigerator until use [7].

**Evaluation of anti-fungal activity of the plant extracts:** Various volumes of the phenols, terpenes and alkaloids were prepared and each of these volumes was mixed apart with 100 ml of PDA (Potato Dextrose Agar) to prepare the required concentrations of this extracts (5, 10%). The blend of both extract and PDA shake well and poured in petri dishes and left to solidify in a sterile conditions. Piece of 5mm from the mycelial growth of mold culture of 5 days was plated in the center of each plate. The inoculated plates were incubated at 27∓2 C˚ for 7 days. Three replicates were made for each treatment with control. Diameters of fungal growth were measured after 7 days, and then the anti-fungal activity of each concentration was calculated by measuring the growth inhibition using the following formula [9].

$$\textbf{Growth inhibition}\% = \frac{[\textbf{Control Growth} - \textbf{Treatment Growth}]}{\textbf{Control Growth}} \textbf{ x 100}$$

**Statistical Analysis:**

The statistical test which used in this study was the ANOVA test, p (<0.05). For the 1st experiment, statistical analysis had shown significant difference at a level of probability P (< 0.05) between different degrees of extracts which were used to measure the diameter of radial growth of fungal colonies.

**Results and Discussion:**

**Table 1-** Percentage of growth inhibition of *Fusarium oxysporum* by using phenols, alkaloids and terpens extracted from *Eucalyptus camaldulensis* at 5 and 10 % concentration.

| Fungus | *E. camaldulensis* | | | | | |
|---|---|---|---|---|---|---|
| | Phe. | | Alk. | | Ter. | |
| | 5% | 10% | 5% | 10% | 5% | 10% |
| *F. oxysporum* | 81.66 | 100 | 88.33 | 63.66 | 94 | 100 |

Phe. = Phenol; Alk. = Alkaloid; Ter. = Terpene

Results showed that percentage of growth inhibition varied according to type of extract, and their concentrations which were used in this study. Plant extracts (phenol, alkaloid and terpens) showed inhibitory action against *F. oxysporum* at all concentrations that used in this study.

Results in the table-1 revealed that phe. and ter. at 10% con. was significantly better than others in reduction growth of *F. oxysporum* which recorded 100 %, while alk. at 10 % was significantly less than others recorded 63.66 % only.


**Figure 1-** Effect of plant extract on Fungi at 5 % con.
**E=*Eucalyptus*, F=*Fusarium*, C= control and t= terpen.**

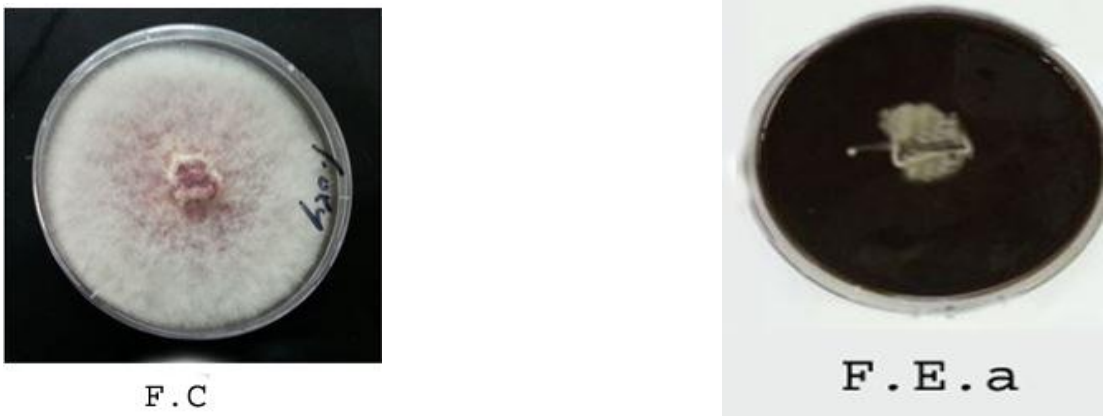


**Figure 2-** Effect of plant extract on Fungi at 10 % con.
**E=*Eucalyptus*, F=*Fusarium*, C= control and t= terpen.**

In other study the effect of *Eucalptus* phenol at 10 % con. on growth of *F. oxysporum* found that inhibition percentage was 33.34 % that mean incompatible with my results [10].

The antifungal action of terpens generally depends upon their hydrophilic or lipophilic character [11]. Also, terpens affect the activities of membrane enzymes and interfere with respiratory pathways [12]. These oils also caused degeneration of fungal hyphae, and hyphae appeared empty of cytoplasmic content [13]. Phenols effect on fungi is according to concentration. At low concentrations, phenols affect the enzymatic activity but at higher concentrations, they cause protein denaturation [14].

Phenols effect on fungal cells because these compounds sensitize the phospholipid bilayer of the fungal cytoplasmic membrane increasing permeability and unavailability of vital intracellular constituents [15]. Many studies emphasized that the antifungal effect of phenols are depended on their hydrophobicity and partition in the fungal plasmatic membrane [16].

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