Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 13 ~ Issue 2 (February 2026) pp: 01-04
ISSN(Online): 2321-9459
www.questjournals.org

Research Paper

The Effect of Aqueous Leaf Extract of *Eucalyptus leucoxylon* F. Muell on the Growth of *Aspergillus niger* Van Tieghem isolated from onion

Youssef Mohammed Azzu[1], Safaa Mostafa El-Deeb[2], Mawadda Abduljawad Erhouma[2]

[1] *Department of Botany, Faculty of Science, University of Tripoli*
[2] *Department of Botany, Faculty of Science, University of Gharyan*

Abstract
The study was conducted to examine the effect of the aqueous extract of Eucalyptus leaves on the growth of Aspergillus niger *using three different concentrations 20%, 50%, and 80%. The study results showed that the aqueous extract of Eucalyptus leaves had an inhibitory effect on the fungus, with the inhibition percentages being 11.11% at 20%, 22.00% at 50%, and the highest inhibition of 77.78% at 80% concentration.*

*Received 12 Feb., 2026; **R**evised 20 Feb., 2026; **A**ccepted 23 Feb., 2026 © The author(s) 2026.*
P**ublished with open access at **www.questjournals.org

I. Introduction

Several studies have reported that medicinal plants contain diverse secondary metabolites such as alkaloids, saponins, carbohydrates, resins, steroids, lipids, volatile oils, glycosides and tannins, that possess antimicrobial properties (Al-Ghazali, 2012; Al-Saeedi, 2012) One of the greatest challenges facing humans is environmental pollution, particularly fungal contamination. Saprophytic fungi can cause contamination of many food products consumed by humans (Al-Eidani, 2014). Many stored grains and food products are accompanied by microscopic organisms such as yeasts and bacteria. Fungi play a dangerous role, especially during storage, where favorable conditions for growth lead to spoilage and a reduction in the value of stored materials. Scientists have identified around one hundred thousand fungal species, and a large number of them are responsible for various plant infections. Some fungi are found inside seeds or on their surfaces, in water, air, and soil, and can infect crops, reducing seed vitality and causing economic losses (Al-Saeedi, 2012). Such infections lead to major agricultural losses both in the field and in storage, reducing crop yield (Sadoon, 2008). Among the soil-dwelling fungi are *Fusarium spp.* and *Rhizoctonia solani*, which cause root and stem base rot (Al-Himyari, 2016). Some of these fungi such as *Aspergillus niger* also produce decay and secrete toxins, posing significant health risks to consumers. The primary reason for this is the ability of some fungi to produce mycotoxins (Al-Khafaji, 2013), with the most dangerous being aflatoxins**,** which are secondary metabolites. However, most chemical fungicides pose numerous risks. They are toxic environmental pollutants and carcinogenic to humans and animals, especially when plant seeds are used as a food source (Al-Eidani, 2014). Scientific studies have also proven that fungicides build up in the soil, disturbing its microbial balance and harming beneficial organisms. Fungicide residues can enter water systems, where they persist and gradually accumulate within aquatic food chains. Their high cost, significant environmental impact, and the possibility of pests developing resistance to pesticides make it necessary to seek alternatives to chemical pesticides. Today, food security has become a major issue worldwide, and alternatives to chemical pesticides must be sought to combat plant pests and diseases—particularly fungi (Sadoon, 2008). With the increasing dangers of plant diseases on one hand, and the confirmed harmful effects of chemical pesticides on the other, many scientific studies have shown the high efficiency of using plant extracts as safe, eco-friendly alternatives. Plant-based extracts are generally safer and eco-friendlier options for suppressing microbial growth (Al Khero & Al Obaidy, 2020). Studies have also discovered several compounds within these extracts that inhibit fungal growth, especially in plant-pathogenic fungi, or reduce the severity of fungal diseases (Fayyad & Jaafar, 2013). One such promising extract is the aqueous extract of Eucalyptus leaves**.**

Thus, this study aimed to test the potential effect of different concentrations of the aqueous extract of Eucalyptus leaves (*Eucalyptus leucoxylon*) on the growth of *Aspergillus niger*, that isolated from onion.

II. Materials and Methods:

Extract Clarification and Storage

The aqueous extract was prepared by soaking 50 grams of finely ground, dried Eucalyptus leaves in one liter of distilled water. The mixture was stirred thoroughly, left to stand for 24 hours at room temperature, and then filtered through Whatman paper to remove large particles. The filtrate was centrifuged at 3000 rpm for ten minutes to obtain a clear solution, which was stored in a sealed container under refrigeration until use. This filtrate represented the 100% stock extract (Al-Okayshi et al., 2011)

Preparation of Growth Medium (Potato Dextrose Agar - PDA)

The medium was prepared by weighing 39 grams of PDA powder and dissolving it in 1000 mL of distilled water in a flask. The mixture was then heated to boiling, covered tightly, and sterilized in an autoclave at 121°C for 20 minutes. After cooling, the medium was poured into Petri dishes in a laminar flow hood at a rate of 25–30 mL per plate. (*Abo Ghania & Qashira, 1988*).

Fungal Isolation and Identification

The fungus used in this study was isolated from onion. The infected sample was washed with sterile water, dried on a filter paper, cut into small pieces, and placed in sterile Petri dishes containing PDA medium (9 cm in diameter). The dishes were then incubated in an incubator at 25°C, and the fungal growth was observed and monitored for several days. The isolated fungus was prepared and examined under a light microscope. The fungus was identified as *Aspergillus niger*.

The identification was confirmed by Plant Protection Department, Faculty of Agriculture, University of Tripoli.

Effect of Eucalyptus Leaf Aqueous Extract on *Aspergillus niger*

To determine the effectiveness of the aqueous extract of Eucalyptus leaves, three concentrations of the extract were prepared: 20%, 50%, and 80%. These were obtained by diluting the stock extract with sterile distilled water. For example, to obtain a 20% concentration, 20 mL of the stock extract was mixed with 80 mL of sterile distilled water. The same method was used to prepare the other concentrations. Three 200 mL glass flasks were prepared, each containing 100 mL of sterile PDA medium in liquid form. To each flask, 5 mL of the respective extract concentration was added, and the flasks were shaken to ensure thorough mixing and homogeneity between the extract and the medium. For each concentration, four replicates were prepared and poured into Petri dishes (9 cm diameter). As for the control group, PDA medium without any extract was poured into Petri dishes. After the medium solidified, a 1 cm disc of *Aspergillus niger* from a pure culture was transferred to the center of each plate using a sterile cork borer. The plates were incubated at room temperature and observed daily for several days. After 7 days, the diameter of fungal colonies in the control plates (where fungal growth reached the plate edge) and in the treatment plates (with different extract concentrations) was measured using a ruler. The average diameter of four replicates per treatment was calculated. The percentage of inhibition was then calculated using the following formula (Al-Saeedi, 2012):

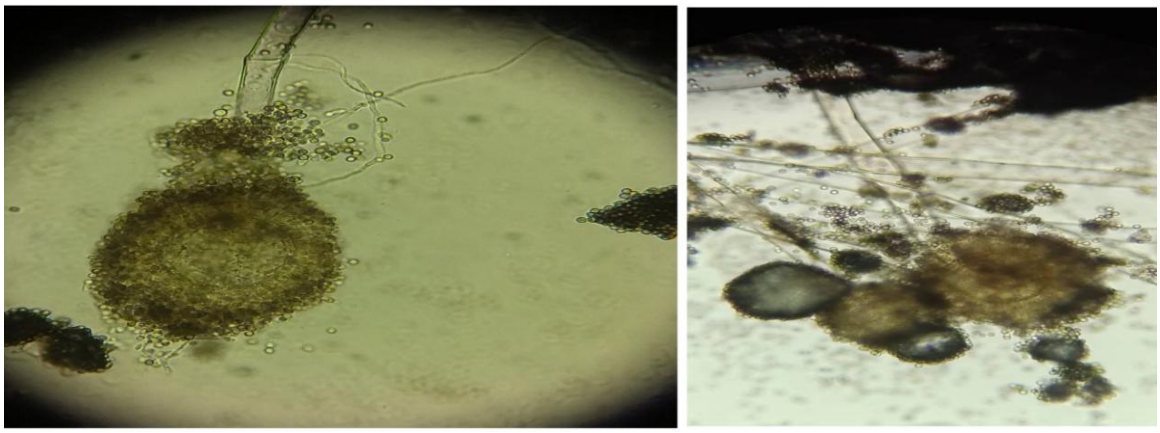
$$\textbf{Inhibition \%} = \frac{\text{Mean Diameter (Control)} - \text{Mean Diameter (Treatment)}}{\text{Mean Diameter (Control)}} \times 100$$

Statistical Analysis

Statistical analysis was performed using the SPSS software (version 21) through the Statistics Department, Faculty of Science, Gharyan. One-way ANOVA was used to determine the effect of extract concentrations on fungal colony diameter.

III. Results and Discussion

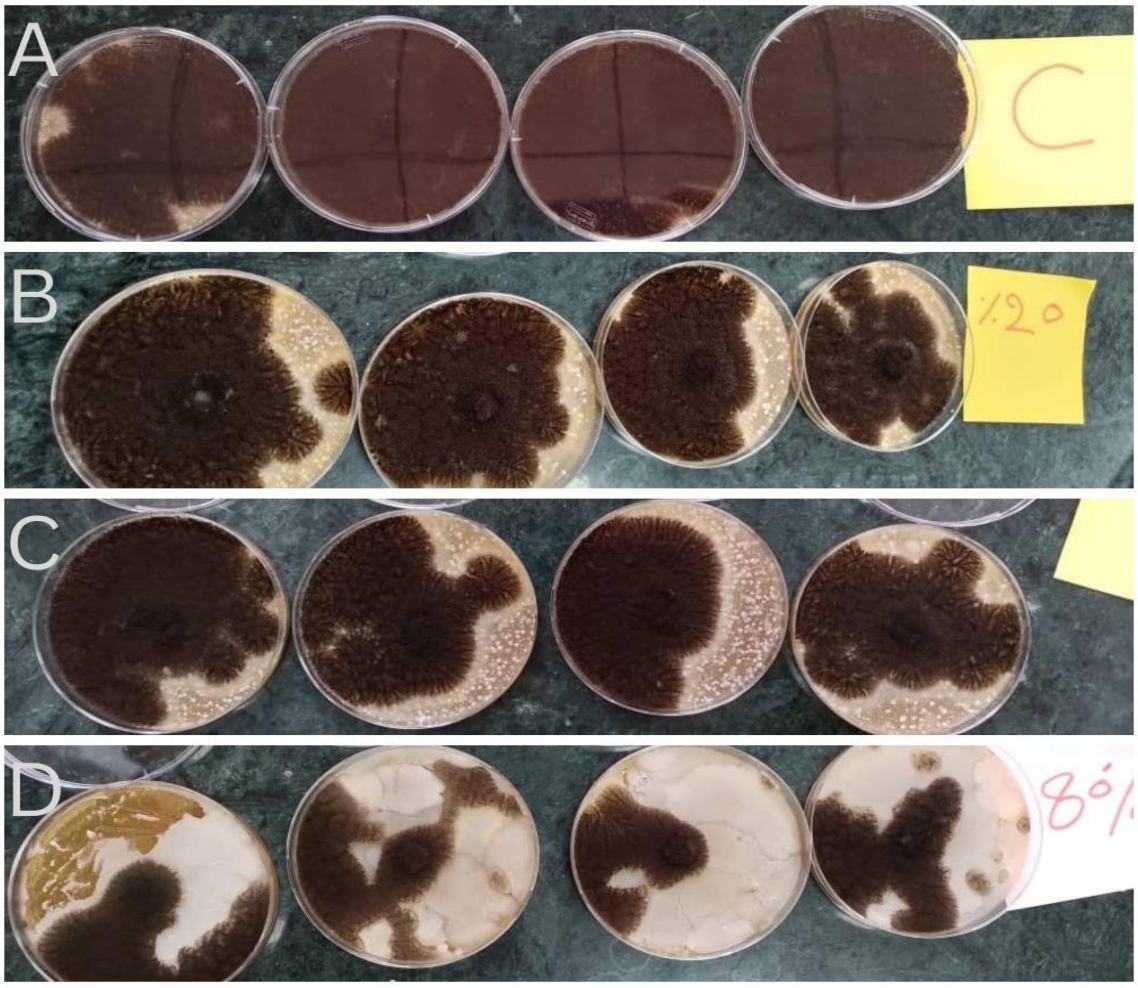
The study results showed that the aqueous extract of *Eucalyptus* leaves had an inhibitory effect on the growth of *Aspergillus niger*. There was an inverse relationship between extract concentration and fungal colony diameter on PDA solid medium.



(**Figure 1**: *Aspergillus niger* under the microscope)

As the concentration of the extract increased, the average colony diameter decreased, and the percentage of inhibition increased. For example:

- At 20% concentration, the average colony diameter was 8 cm, with 11.11% inhibition (Figure 3).
- At 50% concentration, the average colony diameter was 7 cm, with 22.22% inhibition (Figure 4).
- At 80% concentration, fungal growth was significantly inhibited, with an average colony diameter of 2 cm and 77.78% inhibition (Figure 5).
- In the control plates, the fungal colony reached a diameter of 9 cm with 0% inhibition (Figure 2).



(**Figure 2:** A(Control dishes), Growth of *Aspergillus niger* in the treatment dishes with different concentration of the aqueous extract of *Eucalyptus* leaves), B (with 20%), C (with 50%), D (with 80%)

The aqueous extract of eucalyptus leaves contains phenolic acids such as chlorogenic acid, caffeic acid, and ferulic acid. and iso-chlorogenic acid**.** These compounds are known to inhibit the growth and germination of certain weeds and plants growing under *Eucalyptus* trees. They also possess antifungal activity against plant-pathogenic fungi or can reduce the severity of diseases caused by such fungi (Fayyad & Jaafar, 2013). The inhibitory effect of the Eucalyptus leaf extract may be attributed to its impact on the permeability of the fungal cell membrane and the activity of transport enzymes. These compounds accumulate outside the cell membrane, leading to growth retardation or complete inhibition of the fungus. As a result, the fungal colony surface area decreases, which reflects the inhibition of fungal growth. The results of this study align with the findings of Jabbar (2016), and Sadoon & Ali (2008), which demonstrated that both aqueous and alcoholic extracts of Eucalyptus showed inhibitory effects on the growth of *Aspergillus niger*.

The results also indicated that the concentration of the cold aqueous extract of *Eucalyptus* leaves significantly affects the diameter of fungal colonies. The differences in diameter between the 80% and 50% concentrations, and between the 80% and control concentrations, were notable. However, there were no significant differences between the 50%, 20%, and control concentrations (Table 1).

Table 1: Shows the percentages of inhibition growth of *A. niger* at different concentrations of the aqueous extract of Euclyptus leaves

Extract Concentration	Fungal Colony Diameter (cm)	Inhibition Percentage (%)
Control (0%)	9 cm	0%
20%	8 cm	11.11%
50%	7 cm	22.22%
80%	2.8 cm	77.78%

IV. Conclusion

This study was conducted to determine the effect of the aqueous extract of *Eucalyptus* leaves on the inhibition of *Aspergillus niger*. Three different concentrations of the aqueous extract (20%, 50%, and 80%) were used.

The fungus was isolated from onions and cultured on Potato Dextrose Agar (PDA)**.** The study results showed that the aqueous extract of Eucalyptus leaves had an inhibitory effect on the growth of *Aspergillus niger*, with the highest inhibition rate of 77.78% at the 80% concentration of the aqueous extract.

References

[1]. Abu Ghania, Abd Al-Nabi & Qashira, Bashir (1988). *Practical Plant Diseases*. Publications of the University of Tripoli, p.141.

[2]. Al Khero, A., & Al Obaidy, M. (2020). *Effect of Aqueous and Alcoholic Extract of Eucalyptus camaldolehsis Dehn Leaves on Growth of Fungal Root Rot of Pinus brutia Ten In Vitro*. Journal of Education and Science, 29(1), 75–92.

[3]. Al-Eidani, Intidhar (2014). *Testing the Efficiency of Some Plant Extracts and Baker's Yeast (Saccharomyces cerevisiae) and Physical Conditions in Inhibiting the Growth of Aspergillus flavus and Aspergillus parasiticus, which Produce Aflatoxin B1*, Master's Thesis, College of Education for Pure Sciences, University of Karbala.

[4]. Al-Ghazali, Linda (2012). *Study of the Inhibitory Effect of Some Plant Extracts Against Certain Dermatophytes*, Master's Thesis, College of Science, University of Karbala.

[5]. Al-Himyari, Yasser (2016). *Integrated Management of Root and Stem Rot Pathogens of Potatoes*, Journal of Karbala for Agricultural Sciences, Central Iraq, 3(4), pp.104–122.

[6]. Al-Khafaji, Hamed (2013). *Effect of Aqueous Extracts of Leaves of Sidr (Ziziphus spina-christi) and Eucalyptus camaldolehsis on the Growth of Fungi Isolated from Wheat Seeds*, Euphrates Journal of Agricultural Sciences, 5(1), pp.155–160.

[7]. Al-Okayshi, Zainab Hussein & Thamer Khudair (2011). *Effect of Aqueous Extracts of Plant Leaves on the Percentage of Inhibition in Wheat, Accompanying Weeds, Eucalyptus, and Nerium oleander*, Euphrates Journal of Agricultural Sciences, 3(3), pp. 31–37.

[8]. Al-Saeedi, Walaa (2012). *Evaluation of the Efficiency of Aqueous and Alcoholic Extracts of Oak Fruits and Fenugreek Seeds Compared to Some Fungal Pesticides in Controlling Fungi Associated with Broad Bean and Spinach Seeds*, Master's Thesis, College of Science, Al-Qadisiyah University.

[9]. Fatima, S., & Kadam, V. (2013). *In-vitro Phytopathology (Laboratory Manual of Plant Pathology)*. LAMBERT Academic Publishing, 347 pages.

[10]. Fayyad, Mohammed & Jaafar, Mohammed Amer (2013). *Effect of Some Plant Extracts and Residues on the Infection of Cowpea (Vigna unguiculata) with Charcoal Rot Disease Caused by Macrophomina phaseolina*, College of Agriculture, University of Basra.

[11]. Ismail, Eid (2014). *Fungal Toxins: Theoretical Concept and General Overview*. Dar Al-Kutub wa Al-Watha'iq, Baghdad, p.285.

[12]. Jabbar, Barj (2016). *Effect of Castor, Eucalyptus, and Trichoderma harzianum Extracts on Soil-Borne Fungi Fusarium sp. and Rhizoctonia sp., In Vitro and In Field*, Al-Qadisiyah Journal for Agricultural Sciences, 1(6), pp. 144–157.

[13]. Sadoon, Abdulamir & Ali Mahood (2008). *Evaluation of the Efficiency of Some Crude Plant Extracts in Inhibiting Storage Fungi in Wheat in Diwaniyah Silos*, Master's Thesis, College of Science, University of Al-Qadisiyah.